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Influenza virus surveillance in Switzerland
Season 2019–2020

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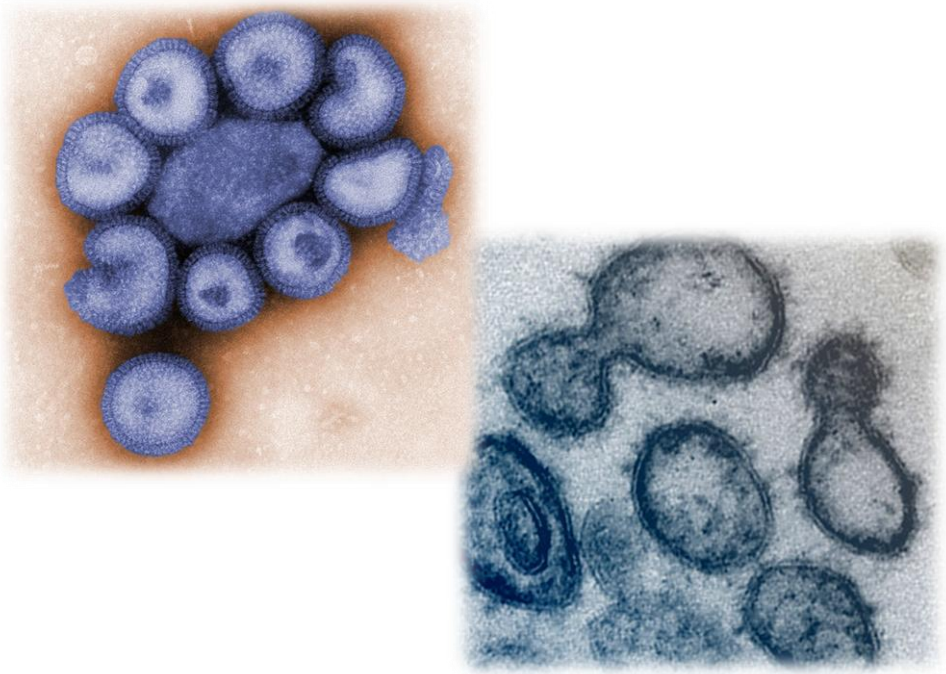

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Upper image: Ultrastructural details of influenza virus particles. Content providers: CDC/Dr. FA Murphy 1973. Photo credit: Dr. FA Murphy. Source: flickr.com

Lower image: cropped version of an electron microscope image showing SARS-CoV-2 isolated virus particles emerging from the surface of cells cultured in vitro. Image captured and colorized at NIAID's Rocky Mountain Laboratories (RML) in Hamilton, Montana. Credit: NIAID-RML. Source: flickr.com

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Abbreviations and Acronyms

CDC	Centers for Disease Control and Prevention
COVID-19	coronavirus 2019
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, PB	acidic protein, basic protein
RBC	red blood cells
RF:	relative fluorescent units
RNA	ribonucleic acids
RNP	ribonucleoprotein
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
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

Cette année la surveillance de l'influenza a débuté le 30 septembre 2019 (semaine 40/2019) mais, pour la première fois, s'est poursuivie après la semaine 16/2020. Cette situation est due à l'émergence du nouveau coronavirus pandémique SARS-CoV-2 responsable de la COVID-19. La surveillance de cette dernière se fera d'ailleurs dorénavant conjointement à celle de l'influenza au sein du système de surveillance Sentinella.

Un total de 1130 échantillons, pour 1118 individus, ont été testés pour la grippe entre les semaines 40/2019 et 16/2020, dont 43.1% se sont révélés positifs. Le screening systématique des virus SARS-CoV-2 a été initié en semaine 12/2020. Sur les 150 échantillons testés, 15 se sont révélés positifs dont 11 en semaine 12/2020. Seuls une cinquantaine de prélèvements ont été analysés dès la semaine 17/2020 ; tous se sont révélés négatifs pour l'influenza et le SARS-CoV-2.

Des virus de l'influenza A et B ont co-circulé cette saison avec un dominance de A(H1N1)pdm09 et de B de la lignée Victoria. Le sous-type A(H3N2) était minoritaire et la lignée B/Yamagata quasi inexistante en Suisse.

La majorité des virus A(H1N1)pdm09 isolés pendant la saison grippale 2019/20 étaient antigéniquement similaires à la souche vaccinale 2019/20, soit A/Brisbane/02/18 (6B.1A1). La majorité des isolats appartenaient au sous-groupe génétique 6B.1A5. Une sous-population de virus portant la substitution N156K dans le HA1 ont montré une reconnaissance réduite par les antisera ciblant la souche vaccinale de l'hémisphère nord 2019/2020.

Plus de la moitié des virus A(H3N2) étaient antigéniquement similaires la souche vaccinale 2019/2020 A/Kansas/14/17. Au niveau génétique, ces derniers étaient subdivisés en trois sous-groupes distincts ; les 3C.3a, contenant le A/Kansas/14/17; et les 3C.2a1b+T131K et 3C.2a1b+T135K. La souche vaccinale 2020/21 appartient au sous-groupe 3C.2a1b+T135K.

Tous les virus influenza B détectés au CNRI appartenaient à la lignée B/Victoria. La majorité appartenaient au sous-groupe génétique 1A(Δ162-164)B et étaient antigéniquement apparentés à la souche vaccinale 2020/2021 B/Washington/02/19.

Aucun virus présentant une sensibilité réduite aux inhibiteurs de la neuraminidase n'a été mis en évidence durant cette saison en Suisse.

Sans surprise, quelques cas d'infections humaines par des virus de l'influenza aviaire et porcine ont été rapportés par divers pays à l'OMS durant la saison 2019/2020; sans notion de transmissions interhumaines; mais aucun cas n'a été identifié en Suisse.

Zusammenfassung

Diesen Winter begann die Influenza Überwachung am 30. September 2019 (Woche 40/2019), und wurde jedoch erstmals nach Woche 16/2020 fortgesetzt. Diese Situation ist auf das Auftreten des neuen pandemischen SARS-CoV-2-Coronavirus zurückzuführen, das für COVID-19 verantwortlich ist. Gemeinsame SARS-CoV-2 und Influenza-Überwachung werden in der Zukunft im Sentinella-Überwachungssystem implementiert.

Zwischen den Wochen 40/2019 und 16/2020 wurden insgesamt 1130 Proben von 1118 Personen auf Influenza getestet, von denen 43,1% positiv getestet wurden. Das systematische Screening von SARS-CoV-2-Viren wurde in der Woche 12/2020 eingeleitet. Von den 150 getesteten Proben waren 15 positiv, 11 während der Woche 12/2020. Ab Woche 17/2020 wurden nur etwa fünfzig Proben analysiert; alle waren negativ auf Influenza und SARS-CoV-2.

Influenza A- und B-Viren haben sich in dieser Saison mit einer Dominanz von A(H1N1)pdm09 und B der Victoria-Linie gemeinsam verbreitet. Der Subtyp A(H3N2) war in der Minderheit und die B/Yamagata-Linie wurde in der Schweiz nicht nachgewiesen.

Die Mehrzahl der während der Influenza-Saison 2019/2020 isolierten A(H1N1)pdm09-Viren war dem Impfstoffstamm A/Brisbane/02/18 (6B.1A1) von 2019/2020 antigenisch ähnlich. Die Mehrzahl der Isolate gehörte zur genetischen Untergruppe 6B.1A5. Eine Subpopulation von Viren, die die N156K-Substitution in HA1 tragen, zeigte eine verringerte Erkennung durch Antiseren, die auf den Impfstoffstamm der nördlichen Hemisphäre 2019/2020 abzielen.

Mehr als die Hälfte der A(H3N2)-Viren war dem Impfstoffstamm A/Kansas/14/17 2019/2020 antigenisch ähnlich. Auf genetischer Ebene wurden A(H3N2)-Viren in drei verschiedene Untergruppen unterteilt; das 3C.3a, das A/Kansas/14/17 enthält; und

die 3C.2a1b+T131K und 3C.2a1b+T135K. Der Impfstoffstamm 2020/2021 gehört zur Untergruppe 3C.2a1b+T135K.

Alle am NRCI nachgewiesenen Influenza-B-Viren gehörten zur B/Victoria-Linie. Die Mehrheit fiel in die genetische Untergruppe 1A(Δ162-164) B und war antigenisch mit dem Impfstoffstamm B/Washington/02/19 2020/2021 verwandt.

In dieser Saison wurden in der Schweiz keine Viren mit verminderter Empfindlichkeit gegenüber Influenza Virus-Neuraminidase-Inhibitoren nachgewiesen.

Es überrascht nicht, dass der Weltgesundheitsorganisation in der Saison 2019/2020 mehrere Fälle von Infektionen beim Menschen mit Aviären und Schweinegrippeviren von mehreren Ländern gemeldet wurden, jedoch ohne Übertragung von Mensch zu Mensch. In der Schweiz wurde keine solche Infektion identifiziert.

Summary

The 2019/2020 influenza surveillance season began on September 30, 2019 (week 40/2019), but continued after week 16/2020 for the first time due to the emergence of the new pandemic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), responsible for coronavirus disease 2019 (COVID-19). Joint SARS-CoV-2 and influenza surveillance will be implemented within the Sentinel surveillance system in the future.

A total of 1130 samples from 1118 individuals were tested for influenza between weeks 40/2019 and 16/2020, of which 43.1% tested positive. The systematic screening of SARS-CoV-2 viruses was initiated during week 12/2020. Of the 150 samples tested, 15 were positive, 11 during the week 12/2020. Only approximately 50 samples were analyzed from week 17/2020. To date, all have tested negative for influenza and SARS-CoV-2.

Influenza A and B viruses have co-circulated this season with a dominance of A(H1N1)pdm09 and B of the Victoria lineage, respectively. A minority were subtype A(H3N2) and the B/Yamagata lineage was not detected in Switzerland.

The majority of A(H1N1)pdm09 viruses isolated during the 2019/2020 influenza season were antigenically similar to the 2019/2020 vaccine strain, A/Brisbane/02/18 (6B.1A1). However, most isolates belonged to the genetic subgroup 6B.1A5. A

subpopulation of viruses carrying the N156K substitution in HA1 showed reduced recognition by antisera targeting the northern hemisphere vaccine strain 2019/2020.

More than half of the A(H3N2) viruses were antigenically similar to the 2019/2020 vaccine strain A/Kansas/14/17. At the genetic level, A(H3N2) viruses were subdivided into three distinct subgroups: the 3C.3a containing A/Kansas/14/17, and the 3C.2a1b+T131K and 3C.2a1b+T135K. The 2020/2021 vaccine strain belongs to the subgroup 3C.2a1b+T135K. All influenza B viruses detected at the National Reference Centre of Influenza belonged to the B/Victoria lineage. The majority fell into the genetic subgroup 1A(Δ162-164)B and were antigenically related to the 2020/2021 vaccine strain B/Washington/02/19.

No viruses with reduced sensitivity to neuraminidase inhibitors were detected during this season in Switzerland.

Not surprisingly, a few cases of human infections with avian and swine influenza viruses were reported to the World Health Organization during the 2019/2020 season by several countries, but without human-to-human transmission. None were identified in Switzerland.

1 Introduction

Influenza virus infections are a major clinical and economic burden worldwide.[1] In Switzerland, the Sentinel surveillance system (Sentinella) 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, epidemiological and virus characterization data gathered from samples processed and analyzed by the NRCI during the 2019/2020 influenza season.

The present report also includes demographic and genetic data on the few severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viruses detected within the influenza Sentinel network.

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] They are transmitted via airborne and contact routes.

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, but the epidemiological pattern has not been 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, specific influenza D antibodies can be found in high proportions in individuals regularly in contact with cattle.[5]

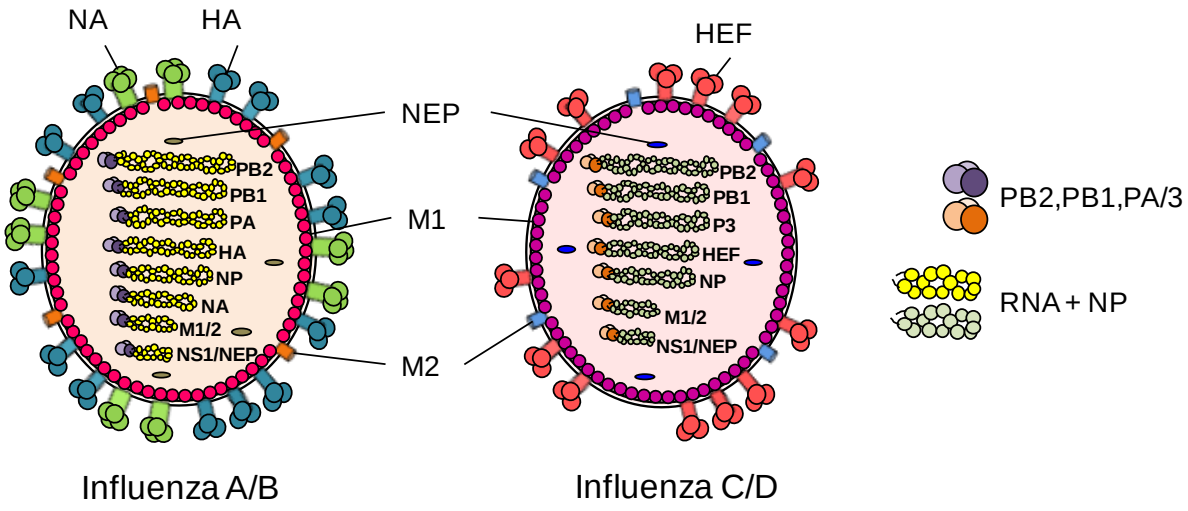

Figure 1. The structure of influenza viral particles. Basic protein 2 (PB2), 1 (PB1) and acidic protein or 3 (PA or P3) form a complex that corresponds to the RNA-dependent polymerase. The hemagglutinin (HA) and the hemagglutinin-esterase-fusion (HEF) play a role in virus attachment to sialic acids present 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 (NS) 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 viruses are known to evolve rapidly through two major mechanisms called antigenic drift and shift. The first is the consequence of the accumulation of mutations in the hemagglutinin (HA) and neuraminidase (NA) genes encoding the two major surface glycoproteins targeted by neutralizing antibodies produced against the virus. The antigenic drift drives the annual evolution of the virus and is therefore responsible for the necessity to regularly adapt the seasonal influenza vaccine strains. The antigenic shift results from the exchange (reassortment) of the influenza A HA and NA genes from different non-human species. It drives the emergence of potential pandemic strains.[6]

2.1 SARS-CoV-2 virus[7,8]

SARS-CoV-2, a beta coronavirus, is the pathogenic agent responsible for the current pandemic coronavirus disease 2019 (COVID-19) that emerged in China in December 2019.[9] The first cases of COVID-19 were linked to a wet market in Wuhan city where live wild animals were also traded. SARS-CoV-2 virus is most likely of zoonotic origin. It can be hypothesized that the virus may have been transmitted from bats to humans either directly or through a currently unidentified intermediary mammalian host.[10]

SARS-CoV-2 genome consists of a non-segmented, 5'-capped and 3'-polyadenylated, positive-sense, single-stranded RNA of around 30,000 bp coding for more than 20 proteins.[11]

Clinical manifestations of SARS-CoV-2 range from mild to severe with non-specific symptoms similar to other respiratory diseases. Asymptomatic cases have also been described.[12]

SARS-CoV-2 viruses are transmitted mainly via respiratory droplets, but can also be transmitted through fomites. Aerosol transmission is suspected, but remains controversial.[13]

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 threshold value is defined by the FOPH based on data collected over the past 10 years (excluding the pandemic season 2009/2010). For the 2019/2020 influenza season, it corresponded to 69 suspected influenza cases per 100,000 inhabitants.

3.1.1 Identification of COVID-19 cases

As COVID-19 cases shares some clinical features (fever, cough, etc.)[8] with ILI cases, it was decided to take advantage of the already existing community-based surveillance system and, as of week 12/2020, all samples sent for influenza investigation were also tested for SARS-CoV-2. Of note, this testing strategy will be renewed for 2020/2021.

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 symptom onset; pneumonia; hospitalization; travel within the previous 14 days; and influenza vaccination status.

3.3 Molecular 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.

Since the 2017/2018 season, influenza A positive samples are subtyped 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[14] (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 triple reassortant (H3N2tr),[15] 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/16/88-

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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 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).

(Yam) and B/Victoria/2/87-like (Vic)-lineages are determined using a duplex rRT-PCR.

The quality of the NRCI influenza A/B detection and subtyping is annually assessed through external quality control panels.

During the pre- and post-epidemic phases, a 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. For biosecurity and crisis management reasons, only SARS-CoV-2 negative samples were submitted to cell culture starting end of February 2020.

3.3.1 Molecular detection of SARS-CoV-2 viruses

Nasopharyngeal swabs are screened for SARS-CoV-2 genome using a one-step rRT-PCR targeting the E-gene adapted from the 2019-nCoV Charité/Berlin assay[16] version 2.[17]

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 5) 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 five 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. A random selection of samples, including some 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 be sequenced. The NA gene sequence allows to detect key mutations previously described as conferring resistance to NA inhibitors (NAI). M and NS gene sequencing allows to control the adequacy of rRT-PCR influenza A and B screening, respectively.

Whole genome sequencing protocols for influenza A and B are currently being set up by Microsynth AG (Balgach, Switzerland).

3.4.1 Genetic characterization of SARS-CoV-2 viruses

Genetic characterization of SARS-CoV-2 will be done by whole genome sequencing. Sequencing protocols are currently being set up in close collaboration with Microsynth AG (see chapter 10.4 “Work in progress” for details).

3.4.2 Cell culture

As mentioned in chapter 3.3, most of the samples received, both positive and negative, are cultured on MDCK and MDCK-SIAT1 cells in parallel during the pre- and post-epidemic phases. This allows to control to some extent that a low positivity rate observed outside the epidemic phase is not due to a rRT-PCR detection default. Of note, starting end of February 2020 and for biosafety reasons, only SARS-CoV-2 negative samples were submitted to cell culture.

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.

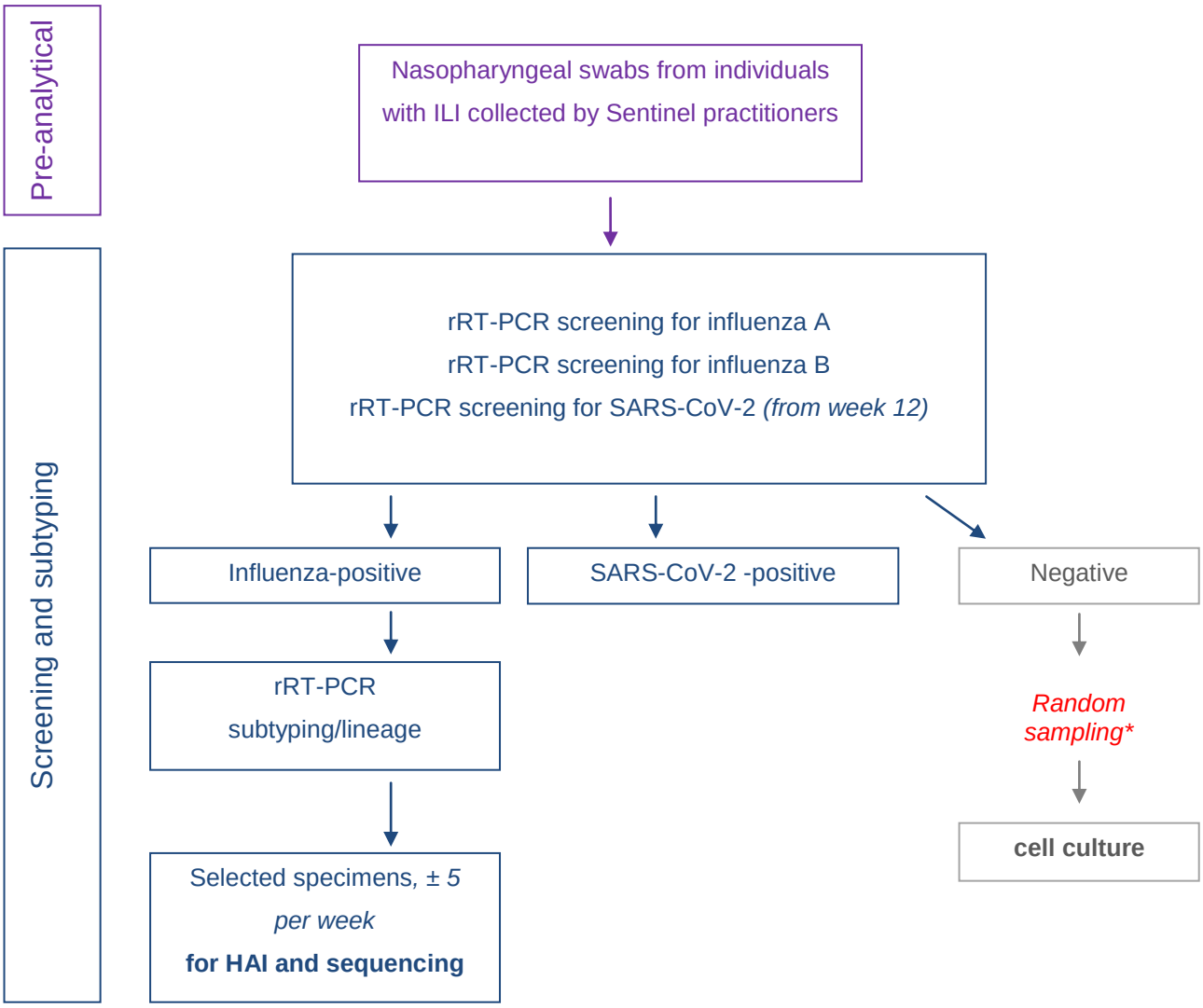


Figure 2. Flow chart of Sentinel samples collection and processing. Starting end of February 2020, only influenza positive and negative samples that were also negative for SARS-CoV-2 were submitted to cell culture.

3.4.3 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.

3.4.4 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, last updated in 26.04.2018 [accessed 21.07.2020]).

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 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). The excitation/emission wavelengths are 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 is evaluated annually through external quality panels.

a. A(H1N1)pdm09: strains/antisera	A/California/7/09	A/Michigan/45/15	A/Brisbane/02/18
A/California/7/09	**128**	128	64
A/Michigan/45/15	256	**256**	128
A/Brisbane/02/18	128	128	**128**

b. A(H3N2): Strains/antisera	A/Hong Kong/4801/14	A/Singapore/INFIM-0016-19/16	A/Switzerland/8060/17	A/Kansas/14/17
A/Hong Kong/4801/14	**128**	256	256	64
A/Singapore/INFIM-0016-19/16	32	**32**	64	64
A/Switzerland/8060/17	64	64	**64**	64
A/Kansas/14/17	64	64	64	**128**

c. IB* : Strains/antisera	B/Brisbane/60/08	B/Hong Kong/514/09	B/Johannesburg/ 3964/12	B/Hong Kong /269/17	B/Norway/240 9/17	B/Colorado/6/17
B/Brisbane/60/08	**1024**	128	128	32	128	256
B/Hong Kong/514/09	64	**64**	64	32	64	64
B/Johannesburg/3964/12	32	32	**64**	32	64	64
B/Hong Kong/269/17	64	64	64	**64**	64	64
B/Norway/2409/17	<16	<16	<16	<16	**512**	<16
B/Colorado/6/17	<16	<16	<16	<16	64	**32**

Figure 3. Antigenic tables for the 2019/2020 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: 2019/2020 influenza vaccine strains. a, b and c correspond to A(H1N1)pdm09, A(H3N2) and B influenza Victoria-lineage 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 2019/2020 influenza season

The 2019/2020 influenza season surveillance started on 30 September 2019 (week 40/2019) and continued after week 16/2020 for the first time due to the emergence of the new pandemic coronavirus SARS-CoV-2, which is now also incorporated in the influenza surveillance system.

The first positive influenza case of the season was detected during week 40 (Annex 1). The epidemic threshold of 69 ILI cases reported per 100,000 inhabitants was exceeded during week 2/2020. The number of reported ILI cases per 100,000 inhabitants then sharply decreased from weeks 7 to 10 before rising again during week 11, most probably due to the rise of COVID-19 cases in Switzerland. The influenza epidemic peak was reached during week 6/2020.

4.1 Sentinel population demographics

From weeks 40/2019 to 16/2020, the usual seasonal influenza surveillance period before the COVID-19 emergence, a total of 1118 individuals presenting with ILI in the community were sampled. Among these, 555 (49.6%) were female and 563 were male. Overall four hundred and eighty-seven participants (43.6%) were positive for influenza A or B (Table 1) or both (only one individual). Twelve individuals were sampled twice (4 females and 8 males) during the surveillance period.

Data on age were available for 1114 of 1118 individuals (median, 30 years [range, 3 months to 93 years]; 95% confidence interval [CI], 30-33). Median age of females was 32 years (range, 8 months to 93 years; 95% CI, 31-35) and 29 years (range, 3 months to 85 years; 95% CI, 29-33) for males. Individuals were stratified into different age groups defined by the FOPH, i.e. 0-4 years (101; 9%), 5-14 years (216; 19.3%), 15-29 years (220; 19.7%), 30-64 years (485; 43.4%), and ≥65 years (92, 8.2%). (Table 1).

Most individuals were sampled from 2 to 4 days (range, 1-28) after the reported onset of first symptoms. Median sampling time was 3 days (data not shown).

Among the 1118 individuals sampled, 115 (10.3%) were reported as being vaccinated against influenza (Table 1).

Table 1. Description of the subgroup of the Sentinel population whose samples were submitted to laboratory confirmation for influenza (weeks 40/2019 to 16/2020)

	Influenza A-positive	Influenza B-positive	Negative for influenza	Total
Gender				
Female	155	76	324	555
Male	153	102	308	563
Total	***308***	***178***	***632***	***1118****
**One female was influenza A- and B-positive, 2 males and 2 females were once positive, once negative; these individuals were considered positive.*				
Age group distribution (years)				
0-4	28	19	54	101
5-14	51	88	77	216
15-29	47	33	140	220
30-64	151	34	300	485
≥65	28	4	60	92
unknown	2	1	1	4
Vaccination status[£]				
Vaccinated	21	7	87	115
[£]Of note, vaccination status was reported for the majority of individuals.				

The 45 individuals sampled from weeks 17/2020 to 28/2020 were all negative for influenza and SARS-CoV-2 viruses (Figure 5). Median sampling time of 3 days remained after first symptom/s onset. Six individuals were vaccinated against influenza.

4.2 Detection of influenza in nasopharyngeal samples

From weeks 40/2019 to 16/2020, a total of 1130 samples were screened for influenza at the NRCI during the 2019/2020 season. Overall, 487(43.1%) swabs were positive for influenza by rRT-PCR (Figure 4a; Annex 1). Three hundred and nine (63.5%) were influenza A-positive; of those 205 (66.3%) were A(H1N1)pdm09 and 97 (31.4%) were A(H3N2) strains (Figure 4b). Seven (2.3%) influenza A-positive samples could not be subtyped due to a low viral load. One hundred and seventy-nine swabs (36.8%) were influenza B positive. All belonged to the B/Victoria/2/1987-lineage, except one that could not be subtyped due to a low viral load. (Figure 4b)

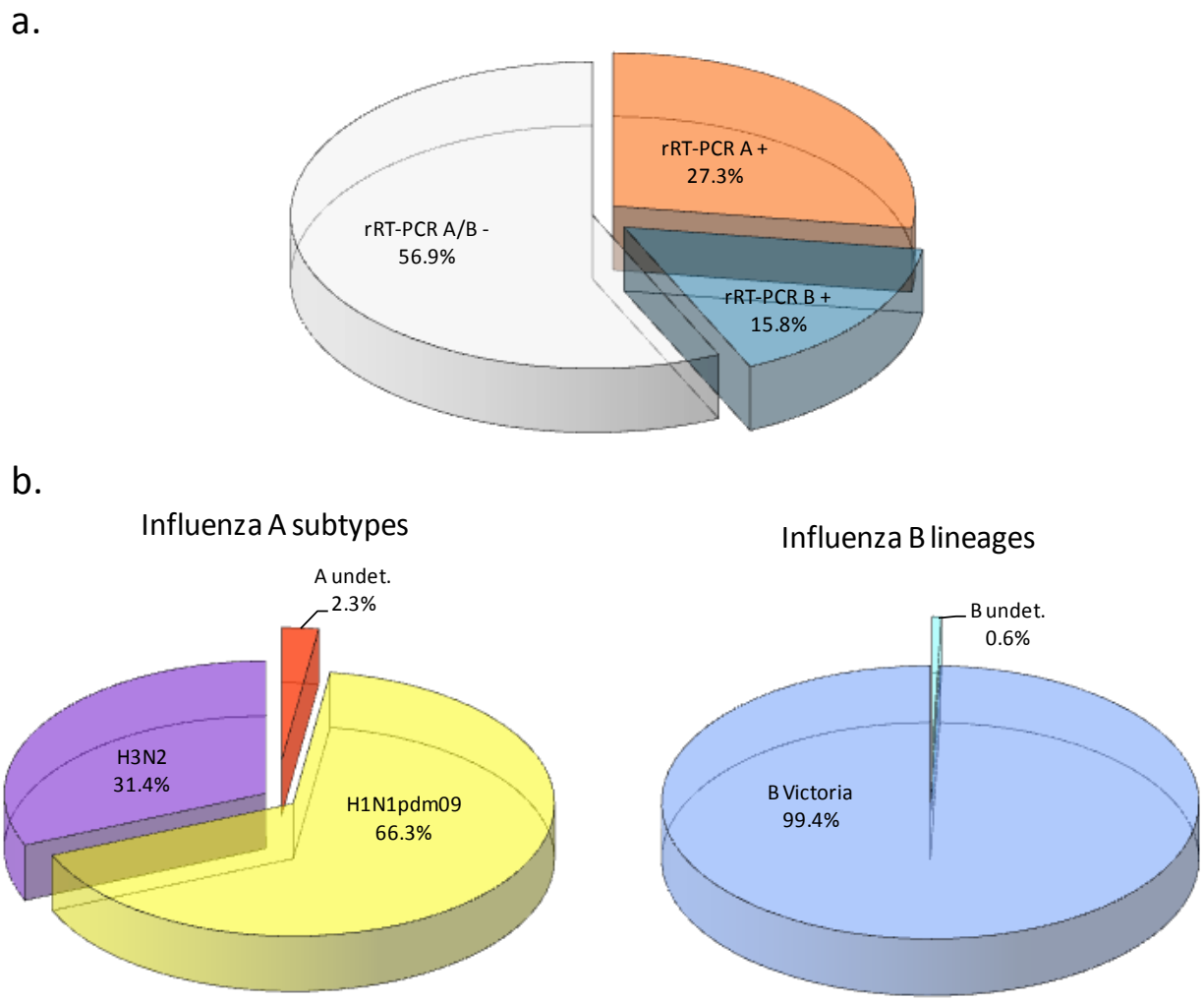


Figure 4. Distribution of influenza viruses detected in nasopharyngeal specimens collected during the 2019/2020 season. a) Percentage of rRT-PCR A- and B-positive (+) versus rRT-PCR-negative (-) specimens (n=1130). b) Distribution (%) of the different influenza A subtypes (n=309) and influenza B lineages (n=179). All positive samples were submitted to subtyping. A and B undet.: subtype not determined (negative subtyping).

A weekly positivity rate of >20% was observed from weeks 49/2019 until 12/2020. A maximum number of 109 samples (positivity rate, 63.3%) were received during week 6/2020, which also corresponded to the peak of ILI reported per 100,000 inhabitants. A positivity rate ≥50% was observed from weeks 3/2020 to 9/2020, with a peak at 79.2% during week 5/2020. During the 2019/20 season, influenza A(H1N1)pdm09 viruses were dominant. Influenza B viruses were detected in parallel to and almost as frequently as influenza A(H1N1)pdm09 viruses (Figure 5).

During the regular surveillance from weeks 12/2020 to 16/2020, 15 out of 150 samples revealed to be positive for SARS-CoV-2 viruses. These were collected from weeks 11/2020 to 13/2020, with a peak of 11 positive samples during week 12/2020 (Figure 5 and Annex 1).

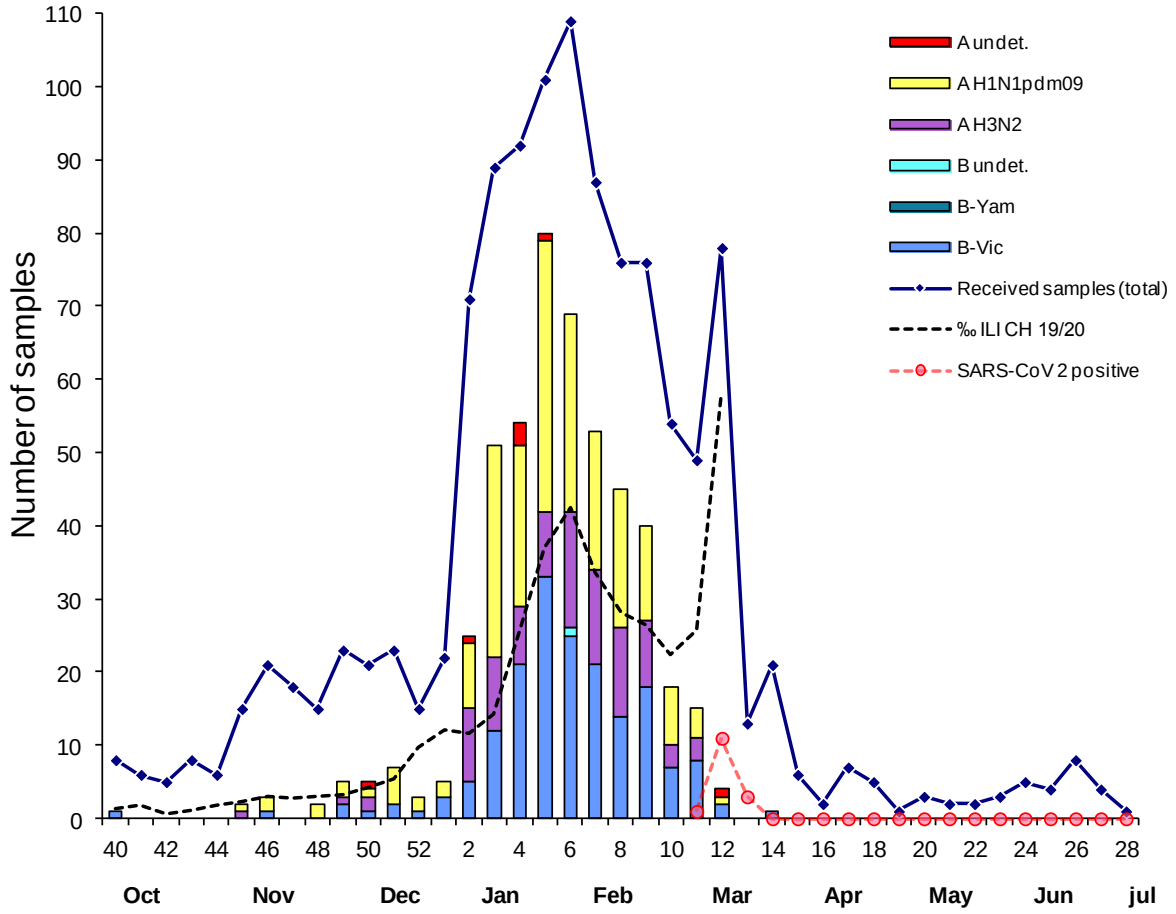


Figure 5. Schematic illustration of the 2019/2020 influenza season. A undet.: influenza A, but the type could not be determined. B undet.: influenza B, but the type could not be determined; B-Yam: influenza B of Yamagata lineage; ILI 19/20: ILI cases reported during the 2019/2020 season (‰).

As mentioned previously, all samples analyzed from week 17/2020 were negative for both influenza and SARS-CoV-2.

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. The influenza positivity rate was similar among males and females (data not shown). Apart from the 5-14 years' group, the positivity rate was <50% (Figures 6 and 7) and was similar among age groups. Information about age was lacking for four swabs.

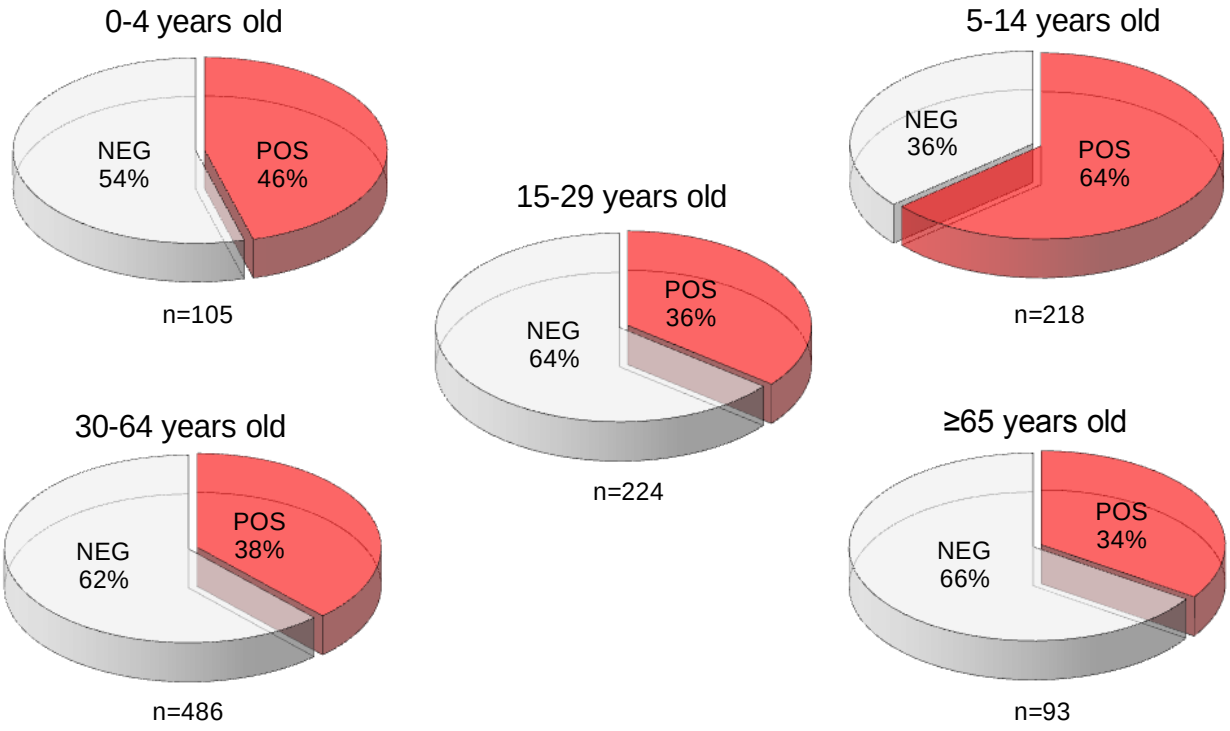


Figure 6. Influenza prevalence per age group. POS: positive; NEG: negative

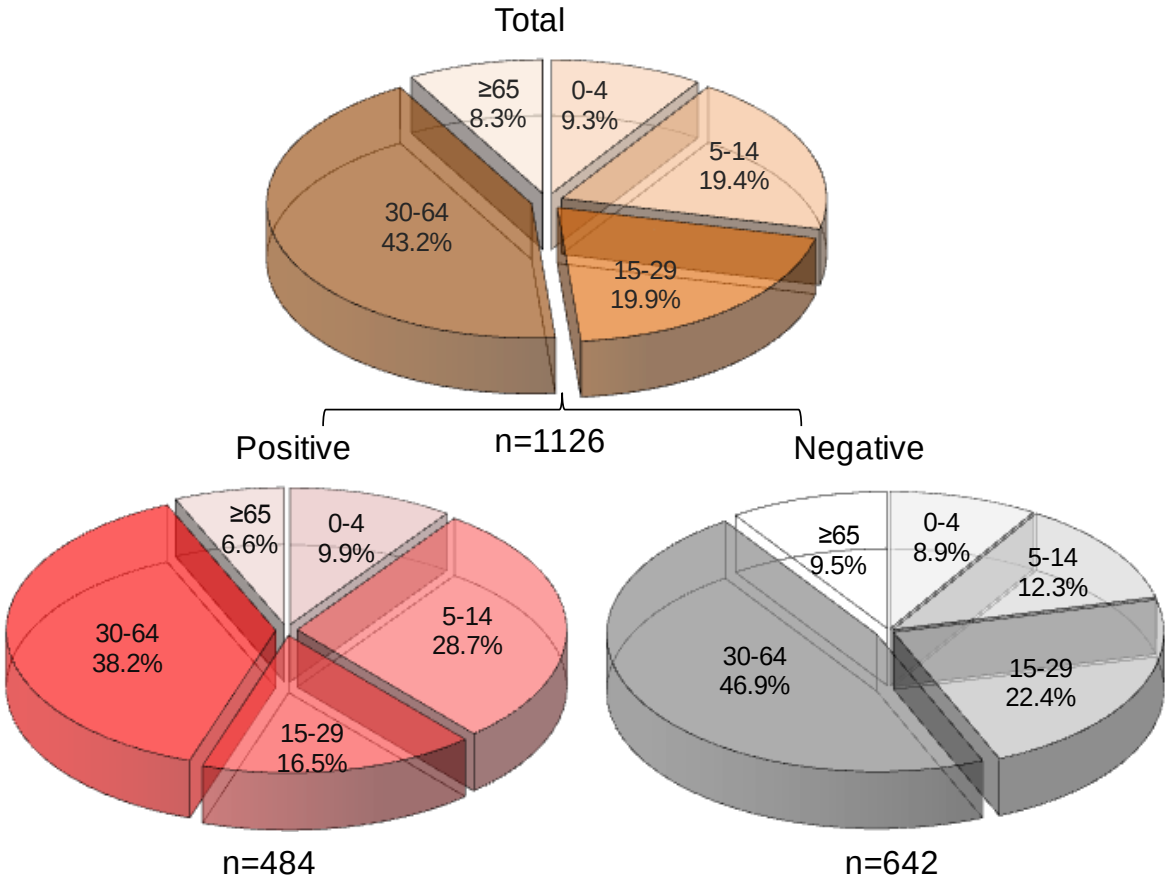


Figure 7. Distribution of the total influenza-positive and -negative samples in the different age groups.

Influenza A(H1N1)pdm09 viruses were present in all age groups at >20%, with the most prevalent strain in the 30-64 years' group (57.8%; n=107) (Figure 8). Influenza B viruses were dominant in the group 5-14 years (62.9%; n=88) and frequent in the groups 15-29 years (40%; n=32) and 0-4 years (n=19; 39.6%) Influenza A(H3N2) viruses were prevalent in the ≥65 years' group (46.9%; n=15) and present at lower percentages in the other age groups (0-4 years [14.6%; n=7]), 5-14 years [15%; n=21], 15-29 years [12.5%, n=10] and 30-64 years [22.7%, n=42]) (Figure 8).

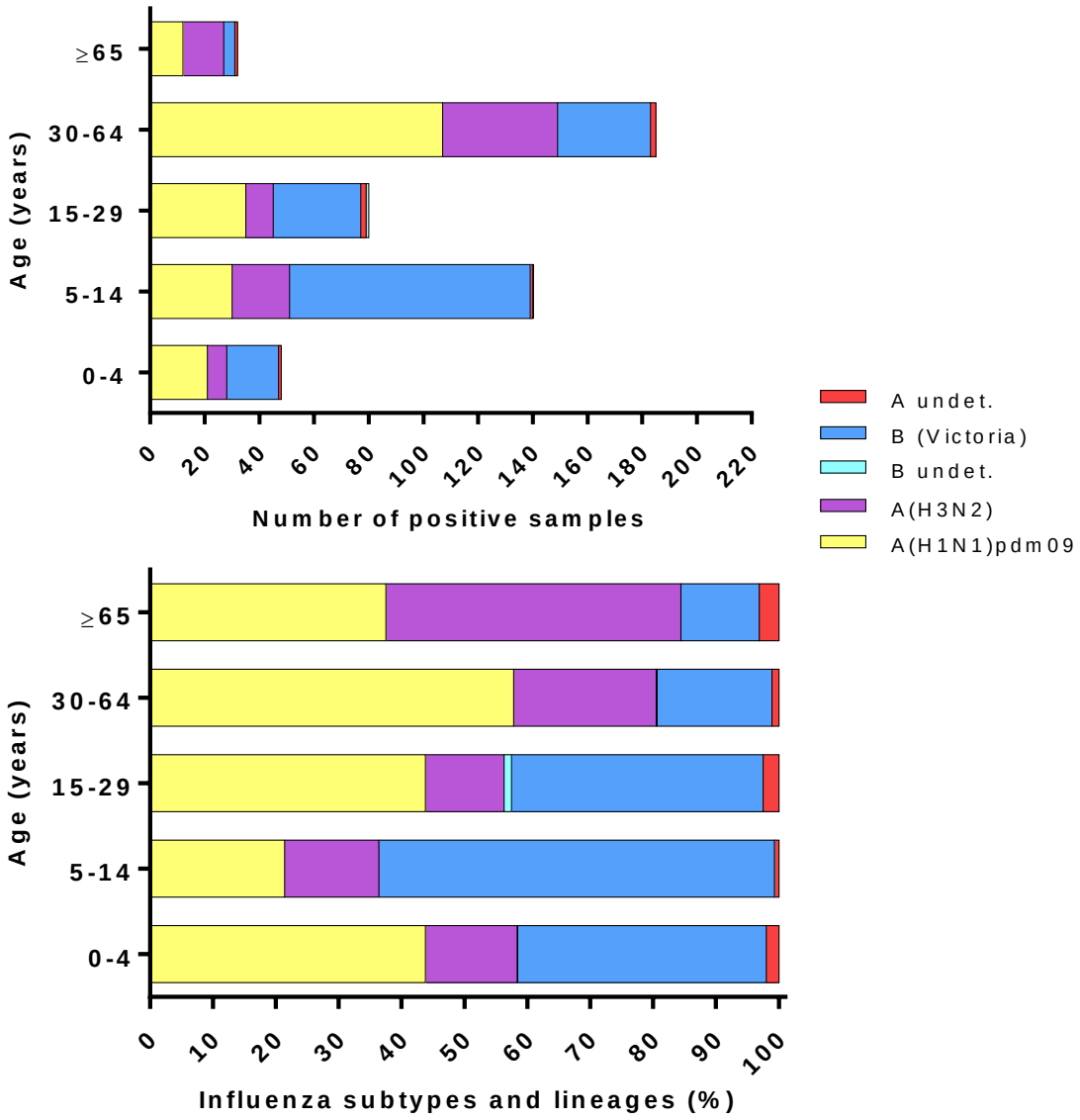


Figure 8. Distribution of influenza virus subtypes/lineages per age group. Upper panel: number of positive samples per subtype per age group. Lower panel: proportions of subtypes/lineages per age group (%); A/B undet.: not able to be subtyped.

4.4 Antigenic and genetic characterization of influenza viruses

One hundred and ninety-six samples were cultured on MDCK and MDCK-SIAT cells (104 negative and 92 influenza-positive). Among these, 79 [35 A(H1N1)pdma09, 14 A(H3N2) and 30 B/Victoria/2/1987-lineage] grew on MDCK and/or MDCK-SIAT cells and were submitted to antigenic characterization by HI (Figure 9; Annexes 2 to 4).

Of note, the culture of influenza-positive and -negative samples was delayed during the COVID-19 pandemic, notably in order to prioritize the use of the limited availability of biosecurity level 3 personal protective equipment for SARS-CoV-2

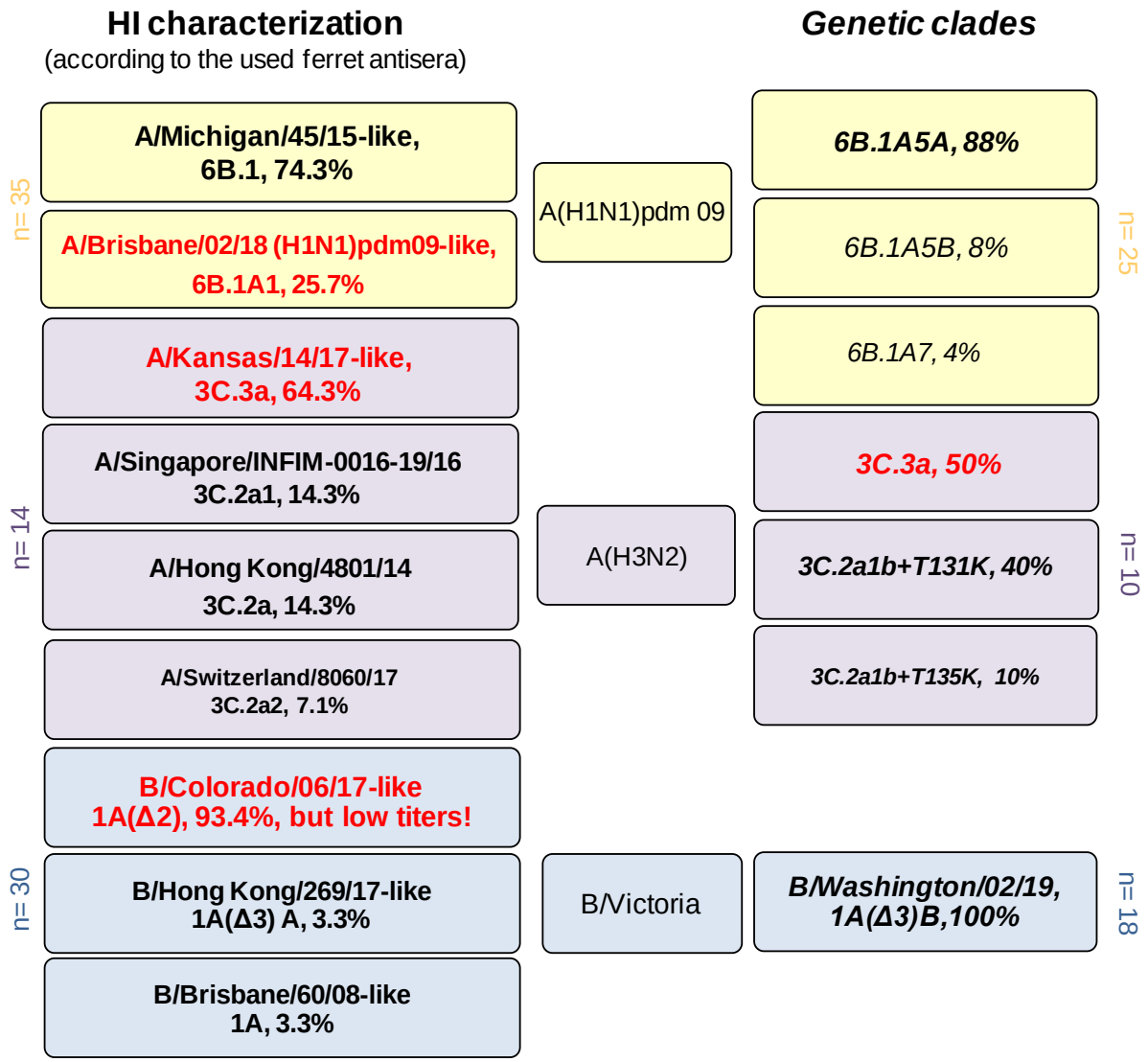
culture by the WHO reference laboratory for COVID-19. Our culture laboratory is set as a P3 laboratory and used in normal condition at the P2 level; the P3 level was implemented as soon as SARS-CoV-2 culture was implemented .

Fifty-eight HA and NA genes [25 A(H1N1)pdm09, 13 A(H3N2) and 20 B/Victoria/2/1987-lineage] from samples collected from weeks 45/2019 to 6/2020 were submitted to genetic characterization. Fifty-three/58 HA [25 A(H1N1)pdm09 and 10/13 A(H3N2) and 18/20 B/Victoria/2/1987-lineage] and 53/58 NA [25 A(H1N1)pdm09 and 9/13 A(H3N2) and 19/20 B/Victoria/2/1987-lineage] sequences were successfully recovered. One sample could not be sequenced at all due to a low viral load.

Due to constraints associated with the COVID-19 pandemic, no Sanger sequencing was performed at the NRCI from weeks 7/2020 to 16/2020. However, samples from that period will be submitted to whole genome sequencing (ongoing development in collaboration with Microsynth AG) and will be reported as a future addendum to this report.

M and NS genes will also be characterized by whole genome sequencing. Results will be reported as a future addendum to this report.

A total of 44 influenza positive samples (15 in January and 29 in April 2020) 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 (Annexes 7 to 12). The results of the second batch are still pending as of July 2020.



!All viruses tested with B/Washington/02/19 [1A(Δ3)B] antiserum, show much better reactivity, consistently with the genetic characterization. The B/Washington/02/19 antiserum was available later at the NRCI.

Figure 9. Antigenic and genetic characterization of selected influenza viruses isolated during the 2019/2020 season. *Left*. Antigenic characterization by HI (n=79 culture-positive samples). The antigenic characterization was assessed on the basis of the antigenic tables for the 2019/2020 season (Figure 3). Red: 2019/2020 vaccine strains. *Right.* Genetic characterization by HA1 sequence analysis (n=53 positive influenza samples). Reference viruses for the genetic subgroups are found in the HA1 phylogenic trees (Figures 10, 12 & 14). A(H3N2) 3C.3a (subclade of the 2019/2020 northern hemisphere vaccine strain), 3C.2a1b+T131K and 3C.2ab1+T135K are genetic subgroups of subclade 3C.2a corresponding to the 2016/2017 northern hemisphere vaccine strain. A(H1N1)pdm09 6B.1, 6B.1A1-7 are genetic subgroups of clade 6. 1A, 1A(Δ2) and 1A(Δ3) are B/Victoria genetic clades. B/Victoria subclades 1A(Δ2) and1A(Δ3) correspond to the 2019/2020 and 2020/2021 vaccine strains genetic groups, respectively.

4.4.1 Characterization of influenza A(H1N1)pdm09

All A(H1N1)pdm09 viruses (n=35) analyzed at the NRCI were well recognized by the antisera raised against the egg-propagated A/Brisbane/02/18 (F10/19) and A/Michigan/45/15 viruses (F32/16), the vaccine strains recommended for use in the northern hemisphere in 2019/2020 and 2018/2019, respectively. On the basis of the antigenic table (Figure 3), 74.3% (n=26) and 25.7% (n=9) of the test viruses were classified as A/Michigan/45/15-like and A/Brisbane/02/18-like, respectively (Figure 9).

Fifty-seven percent (n=20) were recognized at titers within 2- to 4-fold of the titer of the A/Brisbane/02/18 antiserum with the homologous virus and 43% (n=15) within 8-fold higher than the homologous titer. Four isolates had titers 2- to 4-fold reduced when tested with antisera raised against egg-propagated A/Brisbane/02/18, A/Michigan/45/15 and A/California/07/09 (F7/16) viruses (Annexes 2a & 2b).

All A(H1N1)pdm09 genotyped viruses (n=25) fell into subclade 6B.1A, which is defined by the amino acid substitutions S74R S183P and I295V in HA1, compared with the A/Michigan/45/2019 virus (clade 6B.1 reference strain) (Figure 10). Test viruses were divided into three subclades of subclade 6B.1A; 88% belonged to subclade 6B.1A5A, defined by substitutions N260D, N129D, T185I D187A and Q189E in HA1; 8% fell within subclade 6B.1A5B, characterized by substitutions E235D and N260D with K130N, K160M, T216K and H296N in HA1; and 4% belonged to subclade 6B.1A7, defined by E68D, S121N, T120A, L161I and K302T in HA1. Four isolates in the subclade 6B.1A5A formed a cluster exhibiting substitutions N156K, K130N L161I and V250A in HA1 (Figures 9 & 10). Of note, viruses with the N156K mutation were shown by the WIC to be less well recognized by the panel of ferret antisera used, which includes A/Brisbane/02/18 and A/Michigan/45/15 antisera.[18] The NA genes clustered similarly to the HA1 (Figure 11).

Data from WIC: A(H1N1)pdm09 viruses[18]

As observed at the NRCI, the six A(H1N1)pdm09 viruses sent to the WIC were generally recognized well by the antisera raised against egg-propagated A/Michigan/45/15 and A/Brisbane/02/18 viruses. Isolates A/Switzerland/9795/19 and A/Switzerland/9887/19 were better recognized by the antiserum raised against the egg-propagated A/Switzerland/2656/17 (clade 6B.1A6) than by the antiserum against A/Brisbane/02/18 (Annex 7).

As expected from the NRCI data, the six A(H1N1)pdm09 viruses sent to the WIC fell into the three 6B.1A subclades, 6B.1A5A (n=4), 6B.1A5B (n=1) and 6B.1A7 (n=1) (Annex 10). The NA genes clustered similarly (data not shown).

A(H1N1)pdm09 viruses

The majority of A(H1N1)pdm09 viruses isolated during the 2019/2020 influenza season were antigenically similar to the northern hemisphere 2019/2020 vaccine strain A/Brisbane/02/18 (clade 6B.1A1). The majority (96%) of the isolates clustered in the 6B.1A5 genetic subgroup. Sixteen percent exhibited the HA1 substitution N156K, which is associated with reduced recognition by the northern hemisphere 2019/2020 vaccine strain.

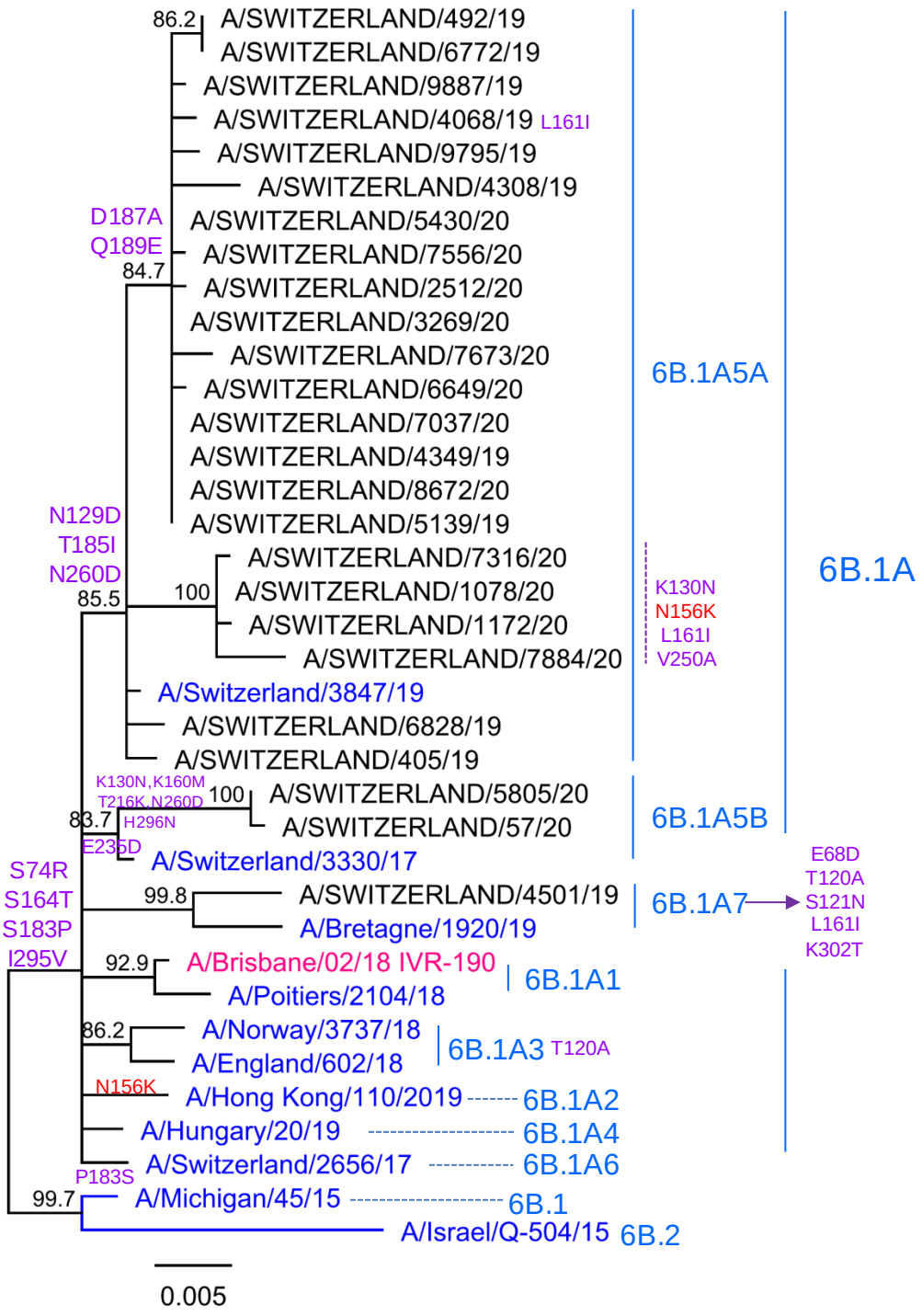


Figure 10. Phylogenetic analysis of the HA1 genes of A(H1N1)pdm09 viruses. Black: influenza virus detected in the Sentinel network during the 2019/2020 season; strain names are A/Switzerland/isolate number/year (e.g. A/Switzerland/5214/20). Pink: 2019/2020 vaccine strain. Blue: reference strains. 6B.1 (A1-7) and 6B.2: A(H1N1)pdm09 genetic clades and subclades. Red: substitution N156K. Purple: typical mutations described by the WIC and/or observed at the NRCI. Sequences were aligned using Geneious 9.1.7 MAFFT alignment (v7.017) with default settings. A consensus tree was built from 1000 original trees in ML (80% support threshold) constructed using Geneious 9.1.7 PHYML default settings.

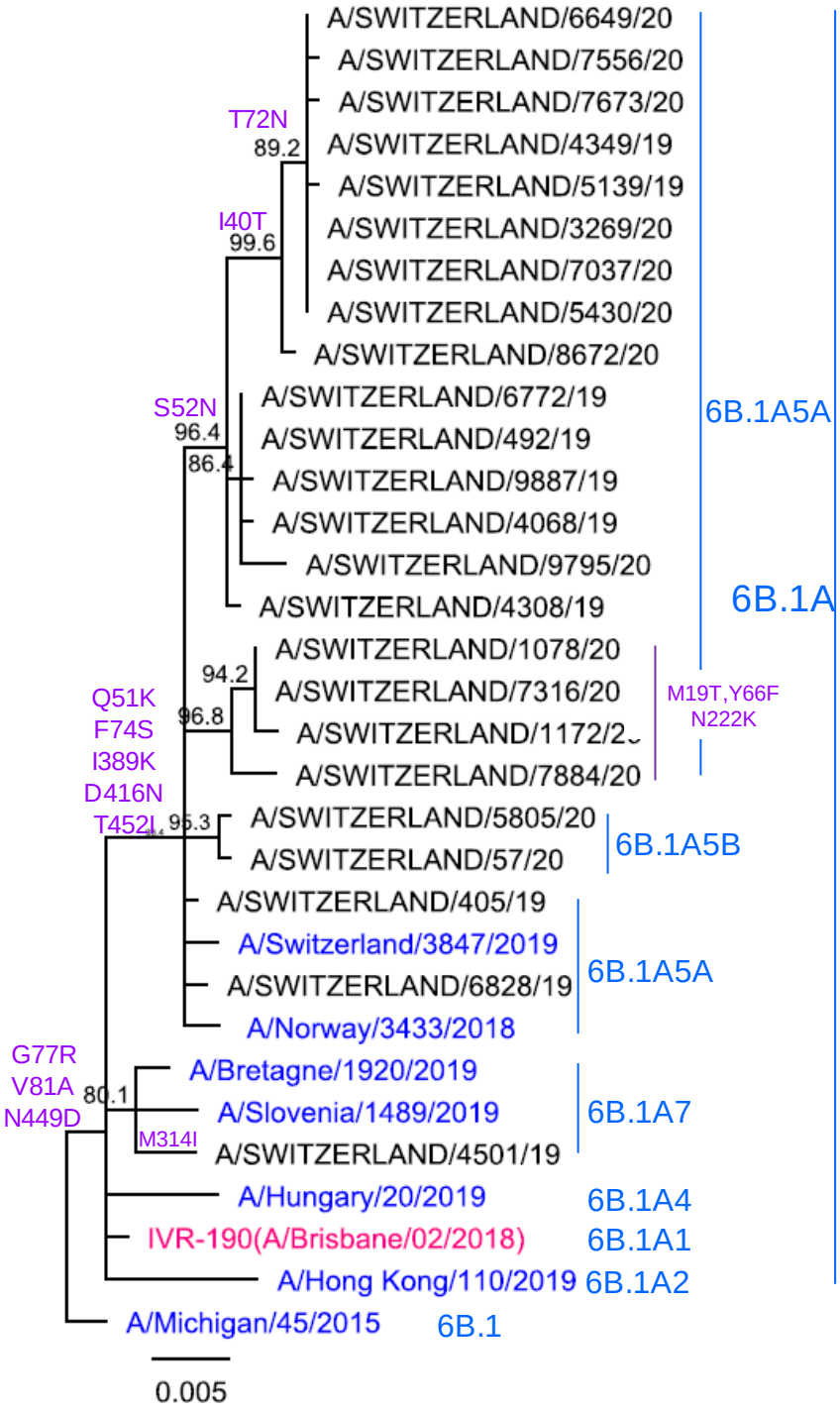


Figure 11. Phylogenetic analysis of the NA genes of A(H1N1)pdm09 viruses. Black: influenza virus detected in the Sentinel network during the 2019/2020 season; strain names are A/Switzerland/isolate number/year (e.g. A/Switzerland/5214/19). Pink: 2019/2020 vaccine strain. Blue: reference strains. 6B.1 (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 9.1.7 MAFFT alignment (v7.017) with default settings. A consensus tree was built from 1000 original trees in ML (80% support threshold) constructed using Geneious 9.1.7 PHYML default settings.

4.4.2 Characterization of influenza A(H3N2)

Antigenic characterization of Influenza A(H3N2) viruses by HA assay is increasingly challenging. Indeed, viruses from this subtype either tend to exhibit a variable agglutination pattern with fresh RBCs from guinea pigs, turkeys and humans or lose the ability to agglutinate any of these RBCs. These phenomena seem to be less present when glutaraldehyde-fixed RBCs are used, as at the NRCI.

All A(H3N2) viruses (n=14) analyzed at the NRCI were recognized within 1-, 2- or 4-fold of the homologous titers by antisera raised against A/Hong Kong/4801/14 (F41/15), A/Singapore/INFIMH-16/0019/16 (F46/17) and A/Kansas/14/17 (F12/19). Twelve were also recognized within 1- (n=5) to 2-fold (n=7) of the homologous titer by antiserum raised against A/Switzerland/8060/17 (F42/18). On the basis of the antigenic table (Figure 3), 64.3% (n=9), 14.3% (n=2), 14.3% (n=2) and 7.1% (n=1) of the test viruses were classified as A/Kansas/14/17-like, A/Singapore/INFIMH-16/0019/16-like, A/Hong Kong/4801/14-like and A/Switzerland/8060/17-like, respectively (Figure 9).

Of the 10 A(H3N2) viruses for which an HA1 sequence was successfully recovered, 50% fell within clade 3C.3a, the 2019/2020 vaccine strain clade A/Kansas/14/17, characterized by substitutions S91N, N144K, F193S, T160K and K326R in HA1 (Figure 12). The remaining 50% belonged to clade 3C.2a, defined by substitutions A128T, S138A, N144S and S159Y in HA1 (Figure 12). Four viruses clustered within subclade 3C.2a1b+T131K, the 2020 southern hemisphere vaccine strain clade A/South Australia/34/19, and one into the subclade 3C.2a1b+T135K-A of clade 3C.2a. The NA genes clustered similarly to the HA1 genes (Figure 13).

Data from the WIC: A(H3N2) viruses[18]

Of the four A(H3N2) viruses sent to the WIC, only three could be cultured and only one (A/Switzerland/7204/19) could be characterized by HI. This isolate was recognized well by antisera raised against cell culture-propagated A/La Rioja/2202/18 (clade 3C.2a1b+T135K-A), A/Norway/3275/18 (clade 3C.2a1b+T131K) and the old reference virus A/Hong Kong/5728/14 that is a A/Hong Kong/4801/14-like virus. Moreover, A/Switzerland/7204/19 was also recognized at a titer within 2-fold of the homologous titer by the antiserum raised against the egg-propagated A/Singapore/INFIMH-16/0019/16 (subclade 3C.2a1). Similar to our observations at the NRCI with antisera A/Kansas/14/17 (F12/19), the antisera raised

against the cell culture-propagated cultivar of A/Kansas/14/17 and cell culture-propagated A/England/538/18, both clade 3C.3a viruses, recognized A/Switzerland/7204/19 within 4-fold of their homologous titers (Annex 8).

As observed at the NRCI, isolates A/Switzerland/7204/19, A/Switzerland/4001/19 (Figure 12 & Annex 11) and A/Switzerland/8127/19 (Annex 11) clustered in subclade 3C.2a1b+T131K of clade 3C.2a1b. Substitutions I48M, Q197R, K207R, I214T and S219F in HA1 and V17M and a reversion E155G in HA2 were present in all three isolates. The NA genes clustered similarly (Annex 11).

A(H3N2) viruses

More than half of the A(H3N2) viruses were antigenically similar to A/Kansas/14/17, the 2019/2020 vaccine strain.

2019/2020 A(H3N2) viruses were divided into two distinct clades: the 3C.3a clade, containing A/Kansas/14/17, and subclades 3C.2a1b+T131K and 3C.2a1b+T135K-A from the 3C.2a clade.

Of note, the 2020 southern hemisphere vaccine strain, A/South Australia/34/19, is a 3C.2a1b+T131K virus and the 2020/2021 northern hemisphere vaccine strain, A/Hong Kong/2671/19, is a 3C.2a1b+T135K-B virus. A(H3N2) viruses from different (sub)clades are antigenically distinct, even if some cross-reactivity can be observed.

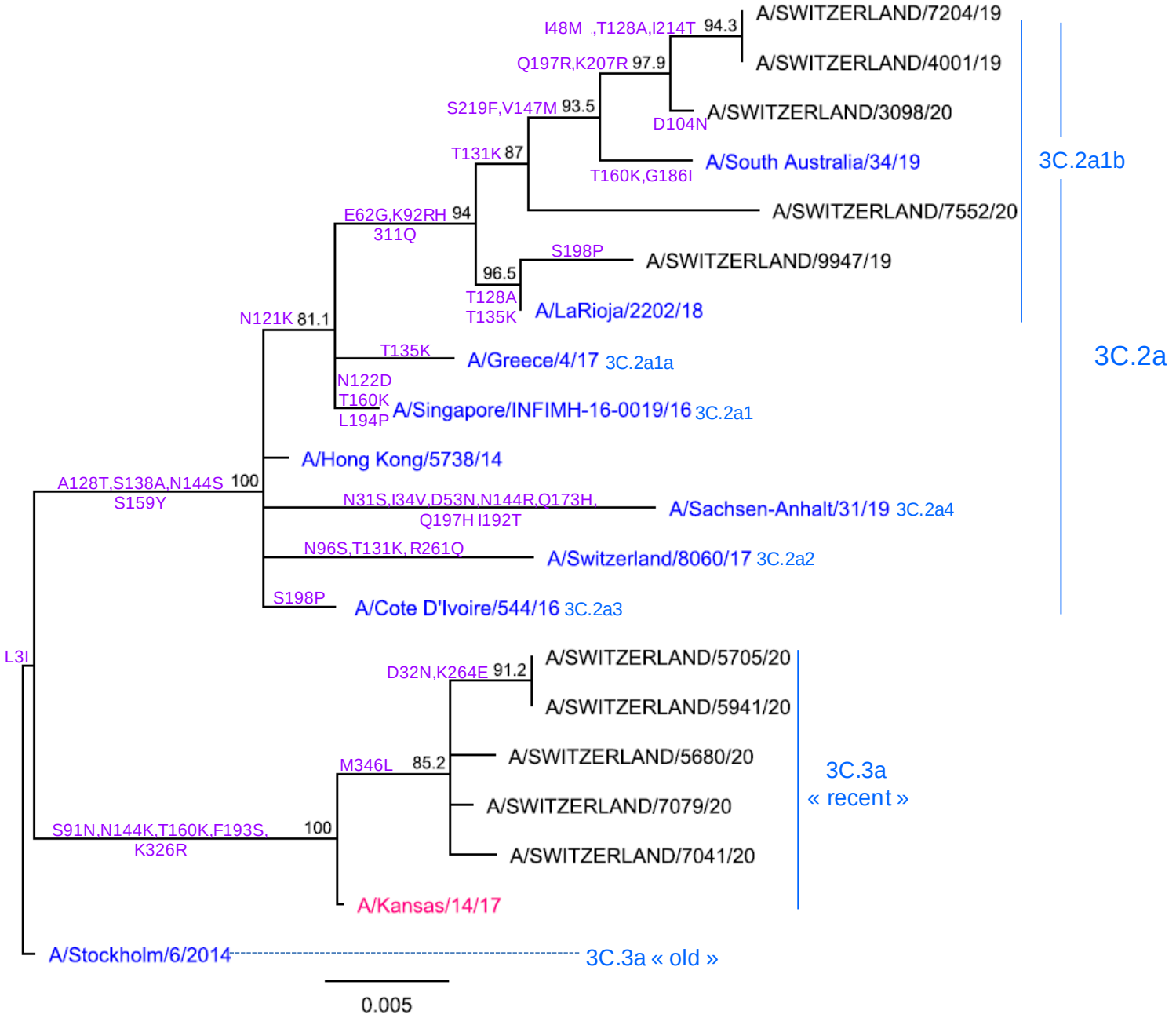


Figure 12. Phylogenetic analysis of the HA1 gene of A(H3N2) viruses. Black: influenza virus detected in the Sentinel network during the 2019/2020 season; strain names are A/Switzerland/isolate number/year (e.g. A/Switzerland/5214/19). Pink: 2019/20 vaccine strain. Blue: reference strains. Purple: typical substitutions described by the WIC and/or observed at the NRCI. 3C.2a, 3C.2a1 to 4 and 3C.3a are A(H3N2) genetic clades and subclades. Sequences were aligned using Geneious 9.1.7 MAFFT alignment (v7.017) with default settings. A consensus tree was built from 1000 original trees in ML (80% support threshold) constructed using Geneious 9.1.7 PHYML default settings.

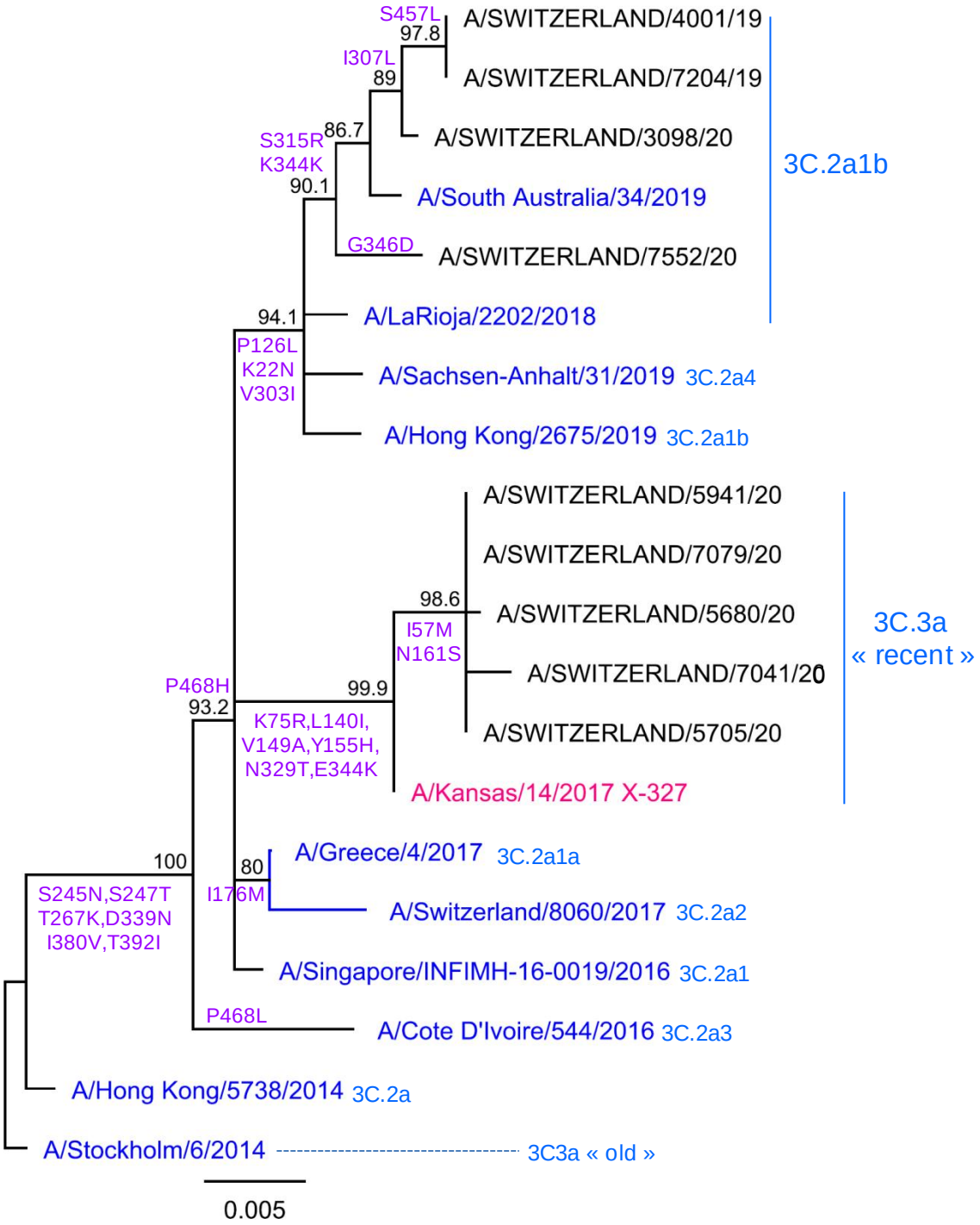


Figure 13. Phylogenetic analysis of the NA gene of A(H3N2) viruses. Black: influenza virus detected in the Sentinel network during the 2019/2020 season; strain names are A/Switzerland/isolate number/year (e.g. A/Switzerland/5214/19) Pink: 2019/20 vaccine strain. Blue: reference strains. Purple: typical 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 9.1.7 MAFFT alignment (v7.017) with default settings. A consensus tree was built from 1000 original trees in ML (80% support threshold) constructed using Geneious 9.1.7 PHYML default settings.

4.4.3 Characterization of influenza B viruses

During the 2019/2020 season, only the influenza B/Victoria/2/87-lineage was detected at the NRCI. B viruses were more abundant than A(H3N2), but less than A(H1N1)pdm09 viruses.

Most of the B/Victoria/2/1987-lineage viruses tested by HI had titers within 1- (n=9), 2- (n=17) and 4-fold (n=4) of the titer of the B/Colorado/06/17 antiserum (F10/18) with its homologous virus (the 2019/2020 vaccine strain). Of note, the homologous titer of B/Colorado/06/17 antiserum (F10/18) was low, similar to the titers of most of the tested viruses. None, except isolate B/Switzerland/7546/20, was well recognized by the antiserum against the former vaccine strain B/Brisbane/60/08 (F45/16). Indeed, most of the viruses were not reacting with the latter antiserum at all. The 25 of 30 B/Victoria/2/87-lineage viruses tested with the B/Washington/02/19 antiserum (F38/19) were recognized at titers within 1- (n=10), 2- (n=12) and 4-fold (n=3) of the homologous titer. In general, titers obtained with the latter antiserum were higher than those observed with the B/Colorado/6/17, in particular the homologous titer. Of the 15 viruses tested with antiserum B/Hong Kong/269/17 (F50/17), only four reacted within 4-fold. In contrast to B/Colorado/06/17, a so-called “double-deleted” virus (amino acid 162-163 deletion (Δ) in the HA1 gene), B/Hong Kong/269/17 and B/Washington/02/19 both bear a 162-164 deletion in the HA1 gene, but belong to distinct subclades. On the basis of the antigenic table (Figure 3), 87.5% (n=28) and 6.3% (n=1) of the test viruses were classified as B/Colorado/06/17 and B/Hong Kong/269/17, respectively. Only isolate B/Switzerland/7546/20 was characterized as B/Brisbane/60/08-like (Figure 9 and Annexes 4a to 4c).

In line with the higher titers observed with the B/Washington/02/19 antiserum compared to B/Colorado/6/17, all genotyped B/Victoria/2/87-lineage viruses fell within the subclade 1A(Δ3)B (reference: B/Washington/02/19) of clade 1A (reference: B/Brisbane/60/08) (Figure 14). In addition to the 162-164 deletion in HA1, all viruses had the substitutions G133R and K136E. Seven out of 18 also bear the substitution E128K. Isolate B/Switzerland/3805/20 clustered differently from the B/Washington/02/19 and was characterized by substitutions D129N, N233S and K272R. The NA genes clustered similarly to the HA1 genes (Figure 15).

Data from the WIC: B viruses[18]

Four of the NCRI B/Victoria/2/87-lineage viruses were recognised by the antiserum raised against egg-propagated B/Washington/02/19 at titers equal to the homologous titers of the antiserum. Isolate B/Switzerland/6569/19 was poorly recognized by this antiserum. Of note, B/Switzerland/6569/19 corresponds to isolate B/Switzerland /6570/19 at the NRCI. The antiserum raised against the cell culture-propagated cultivar of B/Washington/02/19 reacted similarly well with three isolates. Interestingly, B/Switzerland/6569/2019 was recognized at a titer 2-fold higher than the homologous titer and B/Switzerland/9761/was not recognised by this antiserum. The antisera raised against the egg-propagated and cell-propagated cultivars of B/Colorado/06/17, egg-propagated B/Brisbane/60/08 and B/Norway/2409/17 recognized the test viruses poorly (Annex 9).

As observed at the NRCI, all viruses exhibited the amino acid deletion 162-164 in the HA1, as well as the substitution K136E. Four isolates had HA genes encoding also a G133R HA1 substitution. B/Switzerland/9761/19 did not have the G133R substitution but had HA genes encoding the substitutions N150K, G184E, N197D and R279K (Annex 12). The NA genes clustered similarly (data not shown).

Influenza B viruses

All viruses detected at the NRCI belonged to the B/Victoria/2/87-lineage.

The majority of B/Victoria/2/87-lineage viruses antigenically and genetically characterized at the NRCI were 1A(Δ162-164) B viruses related to B/Washington/02/19, the 2020/2021 vaccine strain.

1A(Δ162-163) viruses, as the 2019/2020 vaccine strain, are antigenically distinct from 1A(Δ162-164) viruses.

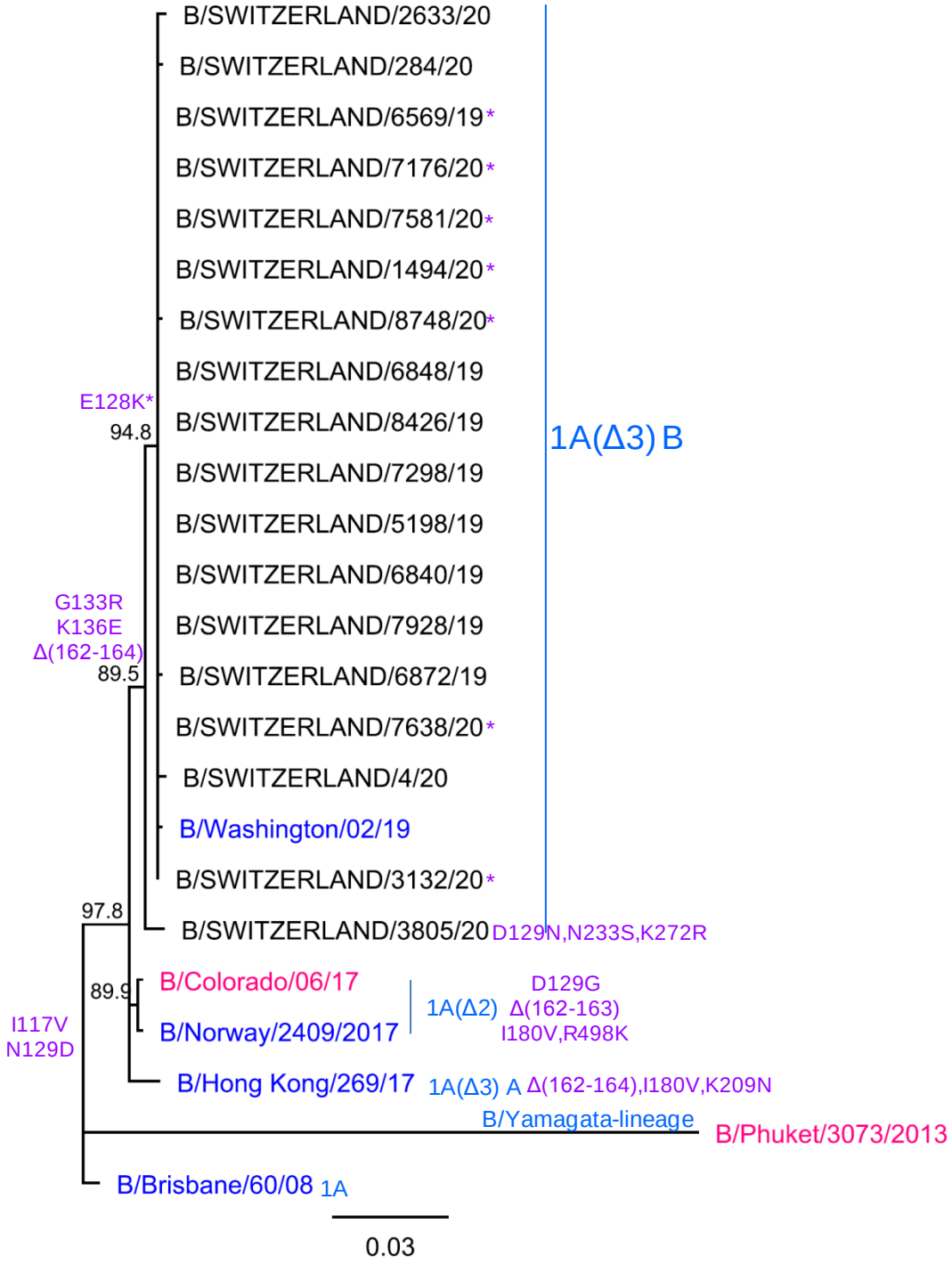


Figure 14. Phylogenetic analysis of the HA1 gene of B/Victoria-lineage viruses. Black: influenza virus detected in the Sentinel network during the 2019/2020 season; strain names are A/Switzerland/isolate number/year (e.g. B/Switzerland/5214/19). Pink: 2019/2020 vaccine strain. Blue: reference strains. Purple: typical substitutions described by the WIC and/or observed at the NRCI. 1A, 1AΔ2-3 are B/Victoria-lineage genetic subclades. Sequences were aligned using Geneious 9.1.7 MAFFT alignment (v7.017) with default settings. A consensus tree was built from 1000 original trees in ML (80% support threshold) constructed using Geneious 9.1.7 PHYML default settings.

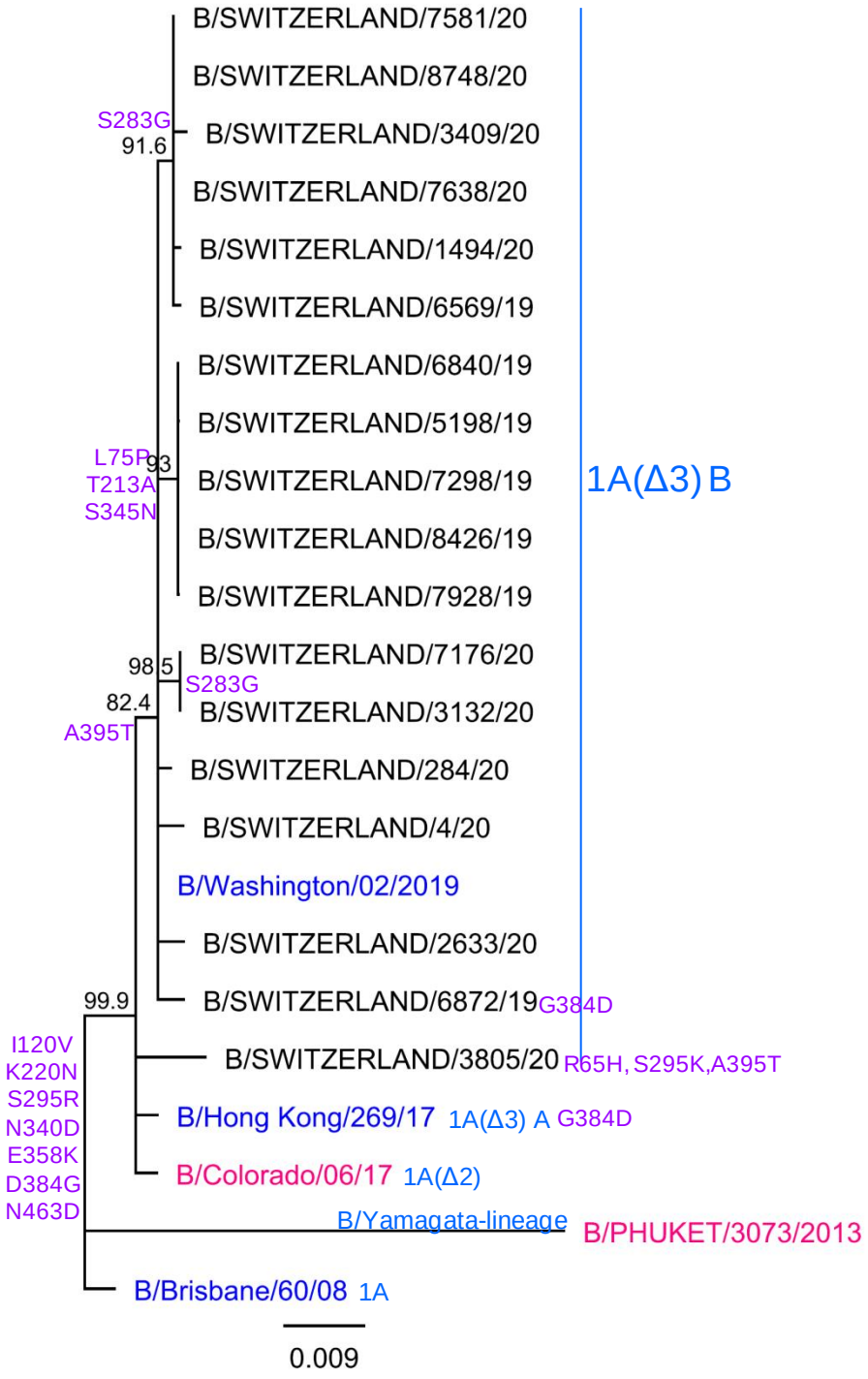


Figure 15. Phylogenetic analysis of the NA gene of B/Victoria-lineage viruses. Black: influenza virus detected in the Sentinel network during the 2019/20 season; strain names are A/Switzerland/isolate number/year (e.g. B/Switzerland/5214/19). Pink: 2019/20 vaccine strain. Blue: reference strains. Purple: typical substitutions described by the WIC and/or observed at the NRCI. 1A, 1AΔ2-3 are B/Victoria-lineage genetic subclades. Sequences were aligned using Geneious 9.1.7 MAFFT alignment (v7.017) with default settings. A consensus tree was built from 1000 original trees in ML (80% support threshold) constructed using Geneious 9.1.7 PHYML default settings.

4.5 Antiviral resistance to neuraminidase inhibitors

4.5.1 Sentinel isolates

None of the 53 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 (data not shown).

4.5.2 Non-Sentinel isolates

During the 2019/2020 influenza season, the NRCI tested two samples from two hospitalized patients for NAI susceptibility. One sample came from the “Hôpital du Valais - Institut Central” (Sion) and the other from the “Centre hospitalier universitaire vaudois” (Lausanne). Both patients were A(H1N1)pdm09 positive and both were under oseltamivir treatment, but did not show clinical improvement. None of the samples tested carried substitutions associated with oseltamivir or zanamivir treatment resistance. As we had stopped the culture of influenza-positive samples due to the COVID-19 pandemic, these samples were not submitted to the phenotypic antiviral resistance assessment.

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.

5 WHO recommendation for the composition of influenza virus vaccines for the 2020/2021 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, A(H3N2), and B/Victoria/2/1987-lineage vaccine components will be updated for the next influenza season. The vaccine strains recommended for the 2020/2021 northern hemisphere influenza vaccine by the WHO experts are[19]:

Table 2. Recommended influenza vaccine composition for the 2020/2021 influenza season.
a: for egg-based vaccines. b: for cell-based vaccines

a	Virus strains
A(H1N1)pdm09	A/Guangdong-Maonan/SWL1536/2019 (H1N1)pdm09-like
A(H3N2)	A/Hong Kong/2671/2019 (H3N2)-like
B/Yamagata/16/1988 lineage	B/Phuket/3073/2013-like
B/Victoria/2/1987 lineage	B/Washington/02/2019 (B/Victoria lineage)-like*

**Only B strain included in the trivalent vaccine*

b	Virus strains
A(H1N1)pdm09	A/Hawaii/70/2019 (H1N1)pdm09-like
A(H3N2)	A/Hong Kong/45/2019 (H3N2)-like
B/Yamagata/16/1988 lineage	B/Phuket/3073/2013-like
B/Victoria/2/1987 lineage	B/Washington/02/2019 (B/Victoria lineage)-like*

**Only B strain included in the trivalent vaccine*

6 A(H1N2) viruses

No human infection with A(H1N2), A(H1N1)pdm09 and A(H3N2) reassortant viruses was reported to WHO as of 24 July 2020.

7 Human infection with influenza viruses of zoonotic origin

Transmission of influenza viruses of zoonotic origin to humans can potentially lead to severe limited outbreaks and to pandemics in the case of efficient human-to-human transmission. Recombination events between porcine/avian and human viruses due

to concomitant circulation can drive human adaptation of zoonotic strains. In order 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

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(H1N2)v, A(H3N2)v and A(H1N1)v.

7.1.1 In Switzerland

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 2019/2020 influenza season, four samples from two different farms were sent to the NRCI for influenza testing. One swab was positive for human influenza B Victoria (A/Switzerland/[redacted]/20) and three swabs (from a different farm) were all negative for influenza (Table 3). Of note, the three swabs originated from three individuals.

Table 3. Pig breeders influenza rRT-PCR results.

Sample ID	Result	Sample date
[redacted]	Influenza B/Vic	[redacted]2020
5699	IA/IB NEG	[redacted]2020
6084	IA/IB NEG	[redacted]2020
8515	IA/IB NEG	[redacted]2020

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

7.1.2 Worldwide

Since 2010, 465 [430 A(H3N2)v, 10 A(H1N1)v and 25 A(H1N2)v] human cases of variant influenza have been reported in several states in the USA.[20]

On June 2020, Brazil reported a human infection by influenza A(H1N2)v virus. The patient did not require hospitalization and recovered. Since 2005, 26 cases of influenza A(H1N2)v have been reported to WHO. These cases were often defined as mild and there was no evidence of further human-to human transmission.

On July 2020, Germany reported a case of A(H1N1)v infection.

7.1.3 Brief summary of Eurasian avian-like A(H1N1) genotype 4 viruses

On July 2020, an article in the Proceedings of the National Academy of Sciences of the USA[21] raised concern about a group of swine influenza viruses, referred to as genotype 4 (G4) Eurasian avian-like A(H1N1) viruses, that may have a high pandemic potential as they can bind and replicate well in human airway epithelial cells. In addition, they were shown to infect and spread efficiently in the ferret model. These viruses are reassortants of Eurasian avian-like viruses (HA and NA genes), 2009 pandemic-like viruses (PB1, PB2, PA, NP and M genes), and North American triple-reassortant-like viruses (NS gene). They have been present and predominant in the Chinese porcine population since 2016.

China has implemented a very effective surveillance system for zoonotic influenza and around 400,000 samples are analyzed annually. Since 2010, only 13 human cases of Eurasian avian-like A(H1N1) virus infection have been reported (in China); among these, three were due to G4 viruses.[22] None of them resulted in secondary transmissions.

Eurasian avian-like A(H1N1) viruses (no G4) are also present in the European swine population. In 2016, two independent severe cases of Eurasian avian-like A(H1N1) virus infection were reported in humans in Italy[23] and the Netherlands.[24]

Of note, as for any zoonotic virus with pandemic potential, the G4 viruses are closely monitored by the Global Influenza Surveillance and Response System and its partners.[22]

The G4 viruses have a 2009 pandemic-like M gene that is, on the basis of a sequence alignment, detectable by the NRCI diagnostic rRT-PCR, but will most likely

be unsubtypable with our h1 combination. Such viruses will be mandatorily further characterized at the NRCI.

7.2 Avian influenza A subtypes in humans[25]

As for porcine influenza, human cases of infection with various avian influenza viruses are sporadically reported. As of July 2020 and since 2003, a total of 861 laboratory-confirmed human cases of A(H5N1), including 455 deaths, have been reported from 17 countries. The last case was reported in 2019.

Since February 2014, 25 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 2020.

Since February 2013, 1568 human cases of A(H7N9) infection, including 32 with HPAI viruses, have been reported; among these, at least 615 had a fatal outcome. All cases were of Chinese origin and most were isolated in China; no new case has been identified since April 2019.

Since 1998, 66 laboratory-confirmed human cases of A(H9N2) infections have been reported, mainly in Mainland China. Four were identified from March to May 2020, certainly due to the increased COVID-19 testing. The majority of cases A(H9N2) were mild and in children under 10 years old with known exposure to live or slaughtered poultry. Only one death from A(H9N2) infection has been reported.

Despite the constant circulation of H5N2 and H5N8 viruses within the wild and domestic avian population worldwide, no human cases have been reported so far.

8 Avian influenza A in animals[25]

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.

A(H5) virus outbreaks are frequent in wild and captive birds and in poultry worldwide. The majority of those reported in 2020 were due to HPAI A(H5N8) viruses. HPAI A(H5N1), A(H5N2) and A(H5N6) and LPAI A(H7N1) outbreaks have also been reported at lower numbers.

9 Discussion

Until week 10/2020, the 2019/2020 influenza epidemic kinetics was quite similar to the 2018/2019 influenza season, i.e., exceeding the epidemic threshold during week 2/2020, peaking during week 6/2020, and starting to decrease from weeks 7 to 10/2020. This was consistent with what was observed for most northern hemisphere countries, as was also the increase in the number of ILI cases, despite a decrease in detection of influenza viruses. This discrepancy reflected most probably the spread of the COVID-19 that can match the ILI definition; while influenza viruses were naturally declining as observed during previous seasons for the same period. Concomitant influenza-COVID-19 detection was introduced in the context of the Sentinel system during week 12/2020. Unexpectedly, a significant drop in the number of samples sent to the NRCI for testing was already observed from week 13/2020, certainly consequent to the introduction of the semi-confinement during week 12/2020 and the associated measures.

The total number of samples tested at the NRCI during the 2019/2020 season, was slightly higher than in 2018/2019. Most samples were collected from weeks 1 to 12/2020. The male and female ratio was close to one (1.01) and, as usual, there was no difference in the positivity rates between males and females.

The age group distribution and overall positivity rate (43.1%) was comparable to previous seasons. Except for the 5-14 years old group (64%), the positivity rate was lower than 46%. The majority of the positive cases in the 5-14 age group were due to influenza B/Victoria/2/1987-lineage viruses, while influenza A strains were largely predominant in the groups aged 30-64 years and ≥65 years. A higher attack rate for influenza B in older children was not unexpected[26]. A similar distribution of influenza A and B viruses types among the different age groups was observed in the USA.[27] Of note, independent of the dominant circulating strain, the positivity rate of the 5-14 age group has been systematically higher than 60% at least since 2015/2016 (2015/16 [68%], 2016/2017 [63%] and 2017/2018 [69%]).

As for previous seasons, influenza vaccine uptake was low (10%). This observation was not unexpected as there were only few changes in the network participants. Vaccine effectiveness studies from six European studies have shown variable vaccine effectiveness, which ranged from 29% to 61% in the context of primary care across all ages, and 35% to 60% in hospitalized patients ≥65 years.[28] Comparable

vaccine effectiveness estimates were observed in Canada (58%, interim data)[29] and the USA (39%, preliminary estimate).[30] Not surprisingly, the vaccine effectiveness was strain-dependent with higher rates against influenza B and A(H1N1)pdm09 than A(H3N2) viruses.[28] Variations in vaccine effectiveness rates between regions may be explained by differences in the study population, study design and circulating influenza strains. There are no vaccine effectiveness data available for Switzerland.

Influenza A(H1N1)pdm09 strains were dominant (42%) in Switzerland during the 2019/20 season, followed by influenza B/Victoria/2/87-lineage (36.5%) and A(H3N2) viruses (19.9%). The three strains co-circulated at variable proportions from weeks 2 to 12/2020. A similar pattern in strain circulation was observed for Canada.[31] In the USA,[32] the season started with a dominance of B/Victoria/2/87-lineage viruses, but switched to A(H1N1)pdm09 strain prevalence during week 4/2020.

As at the NRCI, the majority of A(H1N1)pdm09 viruses analyzed worldwide were recognized well by the antisera raised against the egg-propagated A/Brisbane/02/18 vaccine strain (genetic subclade 6B.1A1) recommended for use in the northern hemisphere 2019/2020. Nevertheless, an increasing proportion of A(H1N1)pdm09 viruses carrying amino acid substitutions in the HA1 150-loop region, such as N156K, tend to be poorly recognized by this antisera. As expected from the antigenicity data, A(H1N1)pdm09 viruses detected worldwide belonged to the genetic clade 6B.1A and clustered in various subclades, with the most prevalent being 6B.1A5A, 6B.1A5B, 6B.1A6 and 6B.1A7. 6B.1A5A viruses, characterized by the additional amino acid substitutions D187A and Q189E. Serological assays with human panel antisera showed reduced HI titers against recent subclade 6B.1A5A and 6B.1A5B viruses compared with those obtained against egg- and cell culture-propagated A/Brisbane/02/2018-like viruses, suggesting an antigenic drift. Thus, these data justified the replacement of the 2019/2020 A/Brisbane/02/18-like vaccine strain by A/Guangdong-Maonan/SWL1536/2019 (egg)-like and A/Hawaii/70/2019 (cell)-like viruses (subclade 6B.1A5A) for 2020/2021 northern hemisphere vaccines.[19]

As observed at the NRCI, A(H3N2) viruses circulating globally belonged to the clade 3C.3a and subclade 2C.2a1b, which were predominant in Europe and North America, respectively.[19,31-33] In general, viruses from the 3C.3a clade were well recognized by antisera raised against egg-propagated A/Kansas/14/17 (3C.3a), the 2019/2020 vaccine virus. While those from subclade 2C.2ab1 further clustered within three genetic subgroups, 3C.2a1b+T131K and 3C.2a1b+T135KA/B were recognized

well by the antisera raised against the 3C-2a1b+T135KB cell-cultivar reference virus A/HongKong/45/19 (recommended 2020/2021 northern hemisphere cell-vaccine strain). In general, viruses from both subgroups showed lower titers with the antiserum raised against the 3C-2a1b+T135KB egg-cultivar A/Hong Kong/2671/19 (recommended 2020/2021 northern hemisphere egg-vaccine strain).[19] A(H3N2) interclade antigenic cross-reactivity is unusual, but can nevertheless be observed.[18,29-31] Studies using human sera panels showed that A(H3N2) viruses belonging to the subclade 3C.2a1b were less well recognized than egg-propagated and cell-propagated A/Kansas/14/17-like strains. This decreased recognition was more pronounced when sera from children were used. In general, 3C.3a viruses reacted well with sera from vacinees.[19]

Influenza B strains co-circulated with influenza A viruses globally. Both lineages were detected worldwide but B/Victoria/2/87-like viruses were dominant, except in South America. Most of the B/Victoria/2/1987-lineage viruses characterized reacted poorly with antisera raised against the 2019/2020 vaccine reference B/Colorado/6/17 [subclade 1A(Δ162-163)]. A majority were well recognized by the antiserum against B/Washington/02/19 [subclade 1A(Δ162-164)B], the vaccine virus for the 2020/2021 northern hemisphere influenza season. In line with the antigenic characterization, most of the viruses isolated during 2019/2020 belonged to the subclade 1A(Δ162-164)B. However, approximately one-fourth of these viruses did not react well with antiserum raised against B/Washington/02/19-like reference strains.[18,29-31] These poor reactor viruses often had either N126K or E128K amino acid substitutions in HA1. In studies using post-vaccination human sera, 1A(Δ162-164) viruses of the B/Victoria/2/1987-lineage were less well recognized when compared to the egg- or cell-culture-propagated B/Colorado/06/2017-like strains.[19]

No B/Yamagata lineage viruses were isolated in Switzerland, but most of the circulating viruses characterized worldwide remained antigenically and genetically similar to the cell culture-propagated vaccine virus B/Phuket/3073/13. Similarly, human sera showed good reactivity to the majority of recent B/Yamagata/16/88-lineage viruses and to the cell culture-propagated B/Phuket/3073/13 vaccine viruses.[18]

Less than 0.5% of viruses (all subtypes confounded) tested globally for antiviral susceptibility showed a highly reduced or reduced inhibition to oseltamivir, zanamivir

and/or baloxavir. The highest resistance rate (0.94%) was observed with A(H1N1)pdm09 viruses and was due to the presence of the H275Y amino acid substitution in the NA gene, which is associated with a highly reduced susceptibility to oseltamivir and peramivir.[18,19]

Outbreaks of avian influenza are regularly reported in wild and captive birds, as well as in poultry, but only a few cases of transmission to humans have been reported recently, similar to porcine influenza.

Surprisingly, as of July 2020, influenza activity remains at interseasonal levels in most southern hemisphere countries, while it should be close to the seasonal peak. This phenomenon is thought to be associated with the COVID-19 pandemic and associated measures. One can hypothesise that the country-specific implementation of personal, social, and travel-related COVID-19 mitigation measures may have had a major impact on influenza viruses circulation. The influenza viruses testing capacity and policies were also greatly modified in some counties. In addition, a host competition between SARS-CoV-2 and influenza viruses cannot be excluded as co-infections do not seem to be so common compared to other respiratory viruses, such as respiratory syncytial viruses and rhinoviruses.[34]

10 Other activities of the NRCI

10.1 Validation and/or evaluation of assays

10.1.1 Update of the rRT-PCR ql for influenza A(H5) viruses detection

The H5 qualitative (ql) rRT-PCR combination currently used at the NRCI, called H5Viet, shows a decreased sensitivity for A(H5N6) and A(H5N8) strains. This observation was not unexpected as H5Viet was originally developed on the basis of A(H5N1) strains. However, since 2014, some cases of human A(H5N6) have been described and, despite the fact that no human cases of A(H5N8) have ever been reported, this subtype is prevalent in the avian population worldwide.

During the development phase, we tested several primers/probes' combinations, both published or in-house designed, and ended up with a good candidate that we chose to validate for regular use at the NRCI. The chosen candidate combination is a duplex targeting the N and C-termini of the H5 gene that originates from the publication of Heine et al[35] and is named H5 IVAD rRT-PCR (ql).

IVAD148H5	AAA CAG AGA GGA AAT AAG TGG AGT AAA ATT
IVAD149H5	AAA GAT AGA CCA GCT ACC ATG ATT GC
IVAH5A	(FAM)-TCA ACA GTG GCG AGT TCC CTA GCA-(TAMRA)
IVAD204f	ATG GCT CCT CGG RAA CCC
IVAD205r	TTY TCC ACT ATG TAA GAC CAT TCC G
IVA D215P	(FAM)-ATGTGTGACGAATTCMT-(MGB-NFQ)

In the original publication, rRT-PCR sensitivity was assessed on the basis of serial dilutions of the A/duck/Laos/XBY004/14 (H5N6, 5×10^9/ml "median embryo infective dose") strain and was equivalent to approximately five "median embryo infective doses" (dilution 10^{-7}, Ct values between 37.9 and 38.5, reaction efficacy of 92%).

In our validation, we compared the H5 IVAD sensitivity to the H5Viet combination using triplicate serial dilutions (10^{-4} to 10^{-8}) of three different A(H5) strains: A/Egypt/N03072/10 (H5N1)xPR8-IDCDC-RG29, A/Sichuan/26221/14 (H5N6)xPR8-IDCEC-RG42A and A/Gyrfalcon/Washington/41088-6/14 (H5N8)xPR8-IDCDC-RG43A. The H5 IVAD rRT-PCR sensitivity was superior to the H5Viet combination for A(H5N1) and A(H5N6) strains. It was equivalent for the A(H5N8) reference strain. The reaction properties were: A(H5N1) (R^2: 0.999, eff %: 100.4); A(H5N6) (R^2: 0.999, eff %: 102.5) and A(H5N8) (R^2: 0.991, eff %: 99].

Three materials (spiked) were also tested: nasopharyngal swabs (default sample), bronchoalveolar lavage and feces (not shown). The sensitivity was comparable for the three materials and H5 IVAD was always superior to H5Viet.

	H5Viet		IVADH5		A/B	matrix gene copies/ul
H5N1 A/Egypt 10-5	32.36	32.29	26.14	25.95	27.28	31'214
H5N1 A/Egypt 10-6	35.98	35.75	29.29	29.24	31.11	2'708
H5N1 A/Egypt 10-7	39.46	*42.96*	32.96	32.82	34.92	237
H5N1 A/Egypt 10-8	NEG	NEG	35.93	35.95	39.38	13.7
H5N6 A/Sichuan 10-5	32.21	32.32	29.17	29.02	28.16	17'796
H5N6 A/Sichuan 10-6	35.41	35.85	32.2	31.89	31.97	1'563
H5N6 A/Sichuan 10-7	*43.96*	38.44	35.23	35.61	35.27	189
H5N6 A/Sichuan 10-8	NEG	NEG	38.13	NEG	39.43	13.32
H5N8 A/Gyrfalcon 10-4	28.64	28.52	27.43	27.85	22.7	579'402
H5N8 A/Gyrfalcon 10-5	32.18	32.29	31.55	31.18	26.34	56'906
H5N8 A/Gyrfalcon 10-6	35.29	35.27	34.89	33.7	29.91	5'810
H5N8 A/Gyrfalcon 10-7	38.39	38.44	37.53	38.13	32.85	886
Initial sample input 400 ul, elution in 50 ul, PCR input 5 ul.						

Accuracy was assessed using known samples from different external quality assessment panels. All results were concordant for the H5 target. The N1 and N6 genes were determined with respective specific rRT-PCRs. The validation process has not been fully completed for the N6 rRT-PCR combination, but according to the quality control assessment panels, good sensitivity and specificity can be expected.

Specificity was assessed on 24 samples (6 A(H1N1)pdm09, 4 A(H3N2), five influenza B and 10 influenza A- and B-negative samples). All were negative for both rRT-PCRs, thus confirming an equivalent specificity. According to Heine et al,[35] the H5 IVAD combination do not detect A(H14N6) and A(H13N6) strains, and can detect A(H5N3) viruses.

ID WHO EQAP	[c/µl]*	Dilué	Isolate and strain	Clade	H5Viet	H5 IVAD	Concordance
Panel 13 V01-2014	2.83×10^1	1/2°	A/Turkey/15/2006 (H5)	2.2	36.39	31.22	C
Panel 13 V07-2014	6.9×10^1	1/2°	A/Turkey/15/2006 (H5)	2.2	34.95	29.93	C
Panel 13 V09-2014	5.61	1/2°	A/Black-headed Gull/13-01845/Hong Kong/2013 (H5)	2.3.2.1	38.87	32.79	C
Panel 13 V10-2014	3.79×10^1	1/2°	A/Black-headed Gull/13-01845/Hong Kong/2013 (H5)	2.3.2.1	33.18	29.42	C
Panel 14 V02-2015	3.28×10^2	-	A/Hong Kong/6841/2010 (H5N1)	2.3.2.1	30.43	24.16	C
Panel 14 V03-2015	3.32×10^2	-	A/Chicken/Egypt/F9514E/2014 (H5N1)	2.2.1	27.88	22.42	C
Panel 14 V05-2015	7.41×10^1	-	A/Hong Kong/6841/2010 (H5N1)	2.3.2.1	31.99	28.66	C
Panel 14 V09-2015	1.31×10^2	-	A/Chicken/Egypt/F9514E/2014 (H5N1)	2.2.1	28.87	25.92	C
Panel 16 V03-2017	1×10^1	1/2°	A/Hong Kong/6841/2010 (H5N1)	2.3.2.1	33.93	29.22	C
Panel 16 V09-2017	4.92×10^1	1/2°	A/Chicken/Hong Kong/16-02206-5/2016 (H5N6)	2.3.4.4	39.13	33.37	C

**Values provided by the WHO External Quality and Assessment Project (EQAP). Quantification by rRT-PCR targeting the M gene.*

°Dilution of the sample as only limited material available (new extraction)

10.1.2 Validation of two different E-mag extraction protocols

In December 2019, our usual extraction machine, the easyMAG (bioMérieux SA [Suisse]), was definitively replaced by an eMAG® bioMérieux. Although both machines use exactly the same reagents, the extraction process had nevertheless to be validated.

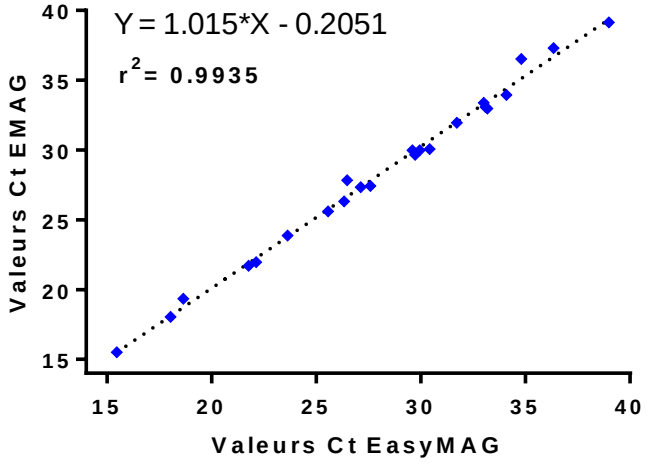
Two different protocols were evaluated: the eMAG® generic 3.0.4 (equivalent to the easyMAG® 2.0.1) and the eMAG® specific B with and without external lysis. The eMAG® generic 3.0.4 protocol is equivalent to the easyMAG® 2.0.1 previously commonly used by the NRCI. The eMAG® specific B protocol is recommended for respiratory samples, particularly sputum. Of note, the eMAG® generic 3.0.4 protocol is efficient for the extraction of non-viscous nasopharyngeal swabs. The external-lysis protocol is necessary for biosafety level 3 and 4 pathogens as the sample will be lysed for 10 minutes in a microbiological safety cabinet and not on-board.

Known influenza-positive (low and high Ct values) and -negative samples were extracted in parallel with both protocols and the influenza A/B rRT-PCR was used to assess the extraction efficiency.

Summary of the results of easyMAG® 2.0.1/eMAG® 3.0.4 extraction protocols:

	Samples	EMAG ®3.0.4	
		Positive	Not detected
easyMAG® 2.0.1	Positive	17*	0
	Not detected	0	18

When the Ct values of the extracted samples were compared, we observed a very good correlation.



Summary of the results of easyMAG 2.0.1®/eMAG® specific B with external lysis :

	Samples	e-MAG ® specific B	
		Positive	Not detected
easyMAG® 2.0.1	Positive	22	1°
	Not detected		11

°close to the rRT-PCR detection limit - one of the duplicates was negative and the second had a Ct value of 39.

We checked if the rRT-PCR sensitivity for influenza isolates was affected by the different extraction protocols, which was not the case as shown in the tables below :

	easyMag	eMAG		easyMag	eMAG
rRT-PCR	**A**/B		RT-PCR	A/**B**	
A 29257205 10-2	26.15, 26.51	26.15, 26.47	B 29237299 10-2	26.41, 26.24	26.48, 26.13
A 29257205 10-3	29.51, 29.93	29.58, 29.72	B 29237299 10-3	29.89, 29.99	30.01, 29.94
A 29257205 10-4	33.00, 33.35	32.53, 33.42	B 29237299 10-4	33.12, 33.06	33.01, 33.15
A 29257205 10-5	36.22, 36.46	38.13, 36.48	B 29237299 10-5	35.43, 34.16	37.10, 35.94
A 29257205 10-6	ND, ND	ND, ND	B 29237299 10-6	ND, 38.98	ND, 39.14
A 29257205 10-7	ND, ND	ND, ND	B 29237299 10-7	ND, ND	ND, ND

Influenza A 29257205, 18.7 Ct and influenza B 29237299, 18.64 Ct; undiluted (extraction duplicates).
(ND =not detected)

External lysis	eMAG specB
A	
A 29257205 10-2	26.67, 27
A 29257205 10-3	30.48, 30.36
A 29257205 10-4	33.19, 33.88
A 29257205 10-5	36.66, 36.85
A 29257205 10-6	ND
A 29257205 10-7	ND
B	
B 29237299 10-2	26.65, 26.97
B 29237299 10-3	29.98, 30.52
B 29237299 10-4	32.63, 33.42
B 29237299 10-5	36.84, 36.52
B 29237299 10-6	ND
B 29237299 10-7	ND

External lysis		easyMag		eMAG SpeB	
		AB			
H5N6 10-5	A	31.58	29.86	30.36	30.52
H5N6 10-6	A	35.05	34.97	34.54	34.66
H5N6 10-7	A	39.77	38.91	37.85	39.41
H5N6 10-8	A	ND	ND	ND	ND

10.1.3 SARS-CoV-2 E-gene validation (Sentinella version)

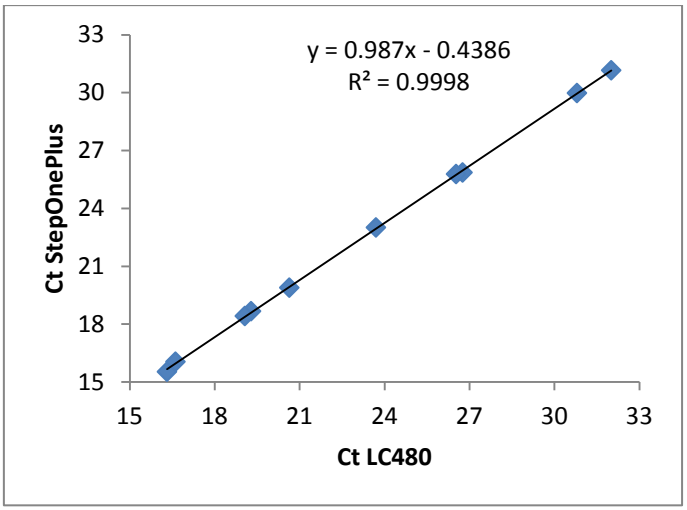
Due to the COVID-19 epidemic, we experienced sampling, extraction and rRT-PCR reagents and consumables' shortages. Even well-known companies were impacted by the global demand for key chemicals. In this context, and in order to avoid using reagents prioritized for SARS-CoV-2 routine diagnosis, we used the SARS-CoV-2 E-gene primers/probe combination[16,17] (routine diagnostic) with the same mix (SuperScript™ III Platinum™ One-Step qRT-PCR Kit w/ROX, Invitrogen, Waltham, MA, USA) as for influenza A/B surveillance. This strategy allowed us to simultaneously screen samples for influenza A/B and SARS-CoV 2, thus saving consumables and routine diagnostic reagents.

We performed a short validation of our "Sentinella" version of SARS-CoV-2 E-gene using one of the routine diagnostic E-gene rRT-PCRs as reference.

We evaluated the rRT-PCR sensitivity using 10-fold dilutions of a quantified *in vitro* transcribed E-gene and with 10 positive SARS-CoV-2 samples with known Ct values. Specificity was addressed on the basis of more than 10 SARS-CoV-2 negative samples either positive or negative for other respiratory viruses. The analytical sensitivity (table below) and specificity (not shown) of the "Sentinella" E-gene were comparable to that of the routine reference. We also observed an excellent correlation between the Ct values obtained with both mixes.

	LC480 (HUG version 1)		**« Sentinella » E-gene**	
Cible	**E-gene**	**E-gene**	**E-gene**	**E-gene**
5000 RNA*	27.91	27.94	27.96	27.86
500 RNA*	31.26	31.23	31.39	31.12
50 RNA*	34.02	34.85	35.03	34.99
5 RNA*	36.69	37.85	36.59	36.86
0.5 RNA*	**Not detected**	**Not detected**	**Not detected**	**Not detected**

**copies/5 ul in vitro transcript of E-gene*
LC480=light cycler 480; HUG: Geneva University Hospitals



We also participated in the first WHO SARS CoV-2 external quality assessment panel (2020) available (table below) and obtained equivalent results with the "Sentinella" E-gene and the routine diagnostic rRT-PCRs. The final laboratory scores have not been communicated yet.

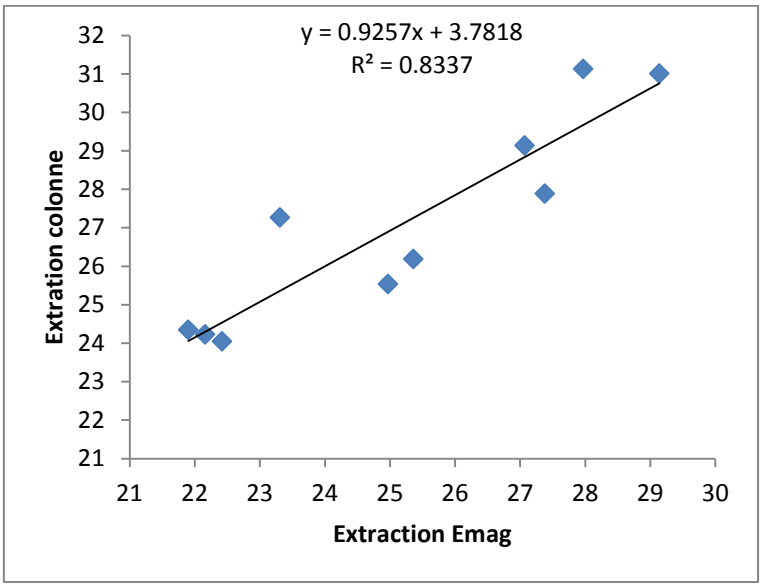
ID	Results	LC480 (HUG version 1)		Eurogentec (HUG version 2)		Invitrogen (Sentinella)
		E-gene	RdRp	E-gene	RdRp	E-Gene
2020-01	SARS-CoV-2	24.74	31.91	24.86	26.91	24.71
2020-02	SARS-CoV-2	28.50	35.83	28.61	30.62	28.34
2020-03	Negative	Not detected	Not detected	Not detected	Not detected	Not detected
2020-04	OC43	Not detected	Not detected	Not detected	Not detected	Not detected
2020-05	SARS-CoV-2	25	32.21	25.55	27.48	24.55

Of note, the RdRp, as well as a N-gene combination, were used for SARS-CoV-2 routine diagnosis at the very beginning of the COVID-19 epidemic. Later, only the E-gene was used and currently the Cobas® SARS-CoV-2 (targets: ORF-1a and E-gene) and Xpert® Xpress SARS-CoV-2 (targets: N2 and E) are the default testing methods.

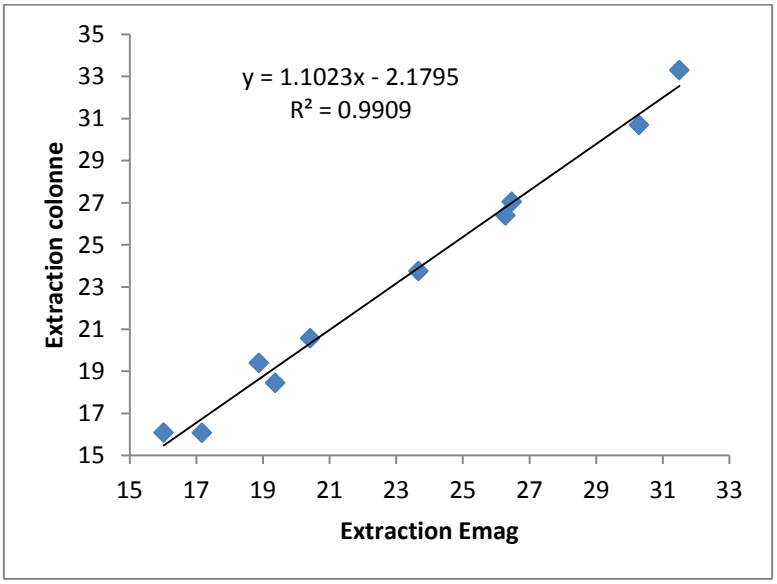
10.1.4 Column extraction pre-evaluation

With the continued aim of sparing reagents for SARS-CoV-2 routine diagnosis in the context of occurring and announced reagent shortages, we performed a pre-evaluation of the manual column extraction using QIAamp Viral RNA Mini-kits for both SARS-CoV-2 and influenza epidemiologic surveillance (Sentinel). The reference method was the e-MAG extraction. We used SARS-CoV-2 and influenza-positive and -negative samples, and Ct values from E-gene and A/B typing rRT-PCRs to assess extraction efficiency, respectively. According to our results, QIAampl column extraction could be used if needed. However, automated or semi-automated extraction methods will be preferred.

Influenza A/B e-Mag versus QIAamp extraction :



SARS-CoV-2 e-Mag versus QIAamp extraction :



Other extraction methodologies were evaluated by the routine diagnostic laboratory in the context of the COVID-19 crisis such as the IDEAL™ extraction robot coupled to the ID GENE™ Magfast extraction kit and the ampliCube coronavirus SARS-CoV-2 rRT-PCR; and the KingFisher Flex System coupled to the MagMAX™ Viral RNA Isolation kits (ThermoFisher Scientific) and the RT-PCR Taqpath Covid-19. Both platforms can extract up to 96 samples per run and are semi-automatic. We used this opportunity to run a quick pre-validation experiment in order to assess the extraction efficiency of these two extraction platforms in the context of influenza A/B positive samples. The preliminary results obtained suggest a good extraction efficiency, but further tests are needed for confirmation.

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

1. Shared material: *Influenza-positive isolates, influenza-positive clinical samples and MDCK cells.*

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

   Project: *Development of broad-spectrum antivirals, notably against influenza viruses.*

2. Shared material: *Influenza-positive isolates, influenza-positive clinical samples, RNA transcripts and MDCK cells.*

   With whom: *Professor Isabella Eckerle, Geneva Centre for Emerging Viral Diseases, Geneva University Hospitals.*

   Project: *Investigation of host-virus and virus-virus interactions and the mechanism(s) involved in the context of human epithelial tissues cultivated in an air-liquid interface system co-infected with influenza and SARS-CoV-2.*

3. Shared material: *Influenza-positive isolates and influenza reference strains.*

   With whom: *Doctor Christian Beuret, Biology Division, Spiez Laboratory, Swiss Federal Office for Civil Protection.*

   Project: *Molecular methods validation. External quality assessment was not requested for the moment.*

10.3 Collaborative projects/publications

During the COVID-19 pandemic, the NRCI supported the Laboratory of Virology and the National Reference Centre for Emerging Viral Infections (CRIVE) for SARS-CoV-2 primary and confirmatory diagnostics (mainly from mid-February to end of May 2020).

Antiviral resistance assessment and follow-up: Abed Y, Schibler M, Checkmahomed L, Carbonneau J, Venable MC, Fage C, Giannotti F, Goncalves AR, Kaiser L, Boivin G. Molecular pathway of influenza pan-neuraminidase inhibitor resistance in an immunocompromised patient. Antivir Ther. 2019; 24(8):581-587.

Support for international surveillance: Segaloff H, Melidou A, Adlhoch C, Pereyaslov D, Robesyn E, Penttinen P, Olsen SJ. WHO European Region and the European Influenza Surveillance Network. Co-circulation of influenza A(H1N1)pdm09 and influenza A(H3N2) viruses, World Health Organization (WHO) European Region, October 2018 to February 2019. Euro Surveillance. 2019; 24(9).

10.4 Work in progress

Due to the COVID-19 pandemic, most of the projects and developments expected to be conducted during 2020 were postponed to 2021 or 2022, depending on their priority.

10.4.1 SARS-CoV-2 and influenza A/B whole genome sequencing

We are currently collaborating with Microsynth AG in order to set up whole genome sequencing protocols for SARS-CoV-2 and influenza.

Two development runs have been performed for SARS-CoV-2 (Figure 1). At the present time, approximately 80% of the SARS-CoV-2 genome is covered (Ct values <32) but a new strategy is currently being tested at Microsynth. The final results will be provided as an addendum to this report. Sequencing protocols using QIAGEN columns (QIAamp® Viral RNA kit) and eMAG® extracted RNA were evaluated, both with an equivalent sequencing yield.

Briefly, the first run followed the Artic protocol as described on protocols io[36], except that the cDNA synthesis volume was reduced to 10 ul and a Maxima H Minus Reverse Transcriptase (Thermo Fisher Scientific) was used. PCR amplification was the same as in the Artic protocol, but only the first 96 forward and 96 reverse primers were combined. Two multiplex PCRs where equivoluminar pooled, purified and TruSeq Amplicon libraries were prepared. Sequencing was performed on a MiSeq with 2*250 bp.

The second testing round followed the same protocol, but the cDNA synthesis part was adapted. The total volume was still of 10 ul, but the temperature profile was modified as follows: 1) incubate at 25°C for 10 minutes; 2) incubate at 52°C for 30 minutes; and 3) terminate at 85°C for 5 minutes. In addition, a dodecamer random primer was used as advised by researchers at D-BSSE, ETH Basel. The PCR amplification step was the same as in the Artic protocol, but new primer pools inclusive of all forward and reverse primers were used. The two PCRs where equivoluminar pooled, purified and TruSeq Amplicon libraries were prepared. Sequencing was performed on MiSeq with 2*250 bp or longer reads.

Raw data were analyzed as follows: duplicate reads were removed using the cd-hit (v4.6.8) programme. Reads were then trimmed to remove low-quality and adapter sequences using Trimmomatic (v0.33). Next, reads were mapped against the reference sequence MN908947.3 using the SNAP nucleotide aligner programme. A

phylogenic tree was then built. Figure 16 shows the tree obtained after (a) the first "incomplete" protocol and (b) the one obtained after the second run. Around 80% of the genome could be covered. Microsynth is further optimizing the protocol in order to be closer to 100% coverage. The "paired" clusters often corresponded to the same sample, but either eMAG or QIAmp column extracted. Samples with Ct values >32 could not be recovered.

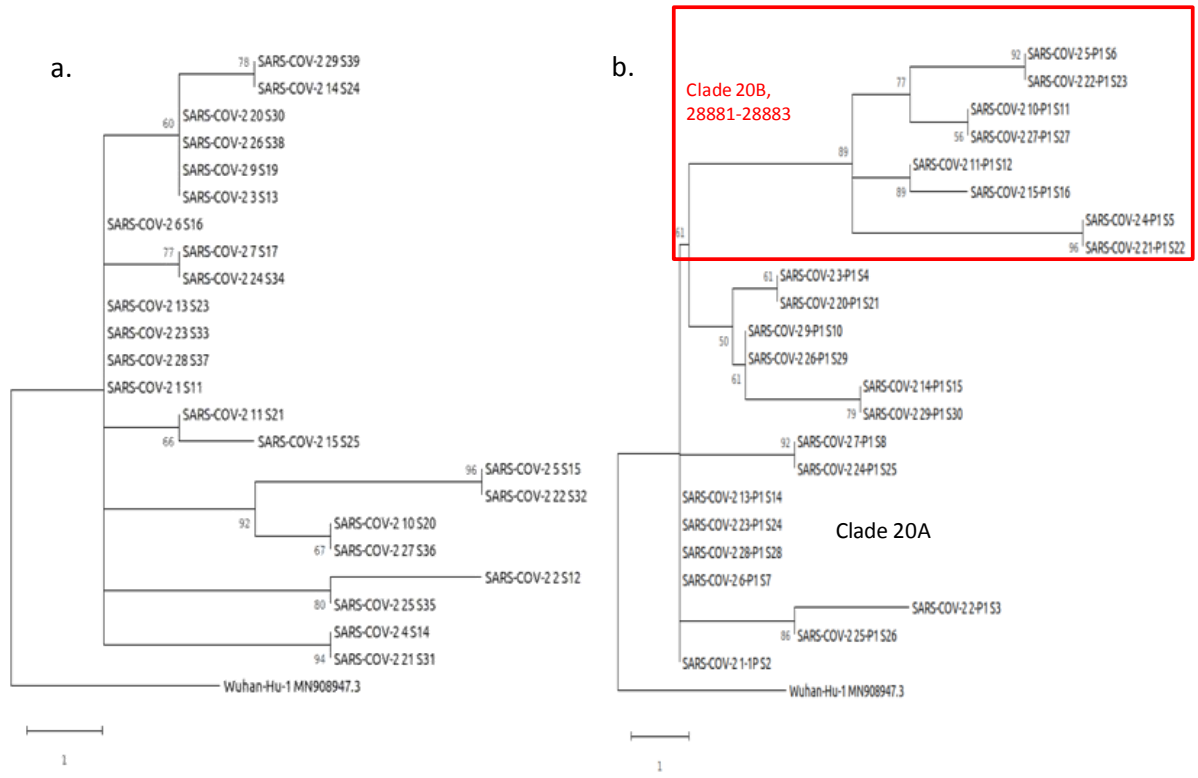


Figure 16. SARS-CoV-2 phylogenic trees. a. After the first whole genome sequencing protocol version. **b.** After the second whole genome sequencing protocol version.

The influenza A and B protocols are currently being set up. No preliminary results are available. The final results will be provided as a future addendum to this report.

10.4.2 Retrospective testing for respiratory viruses

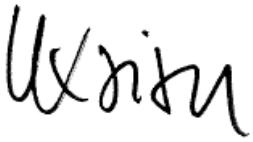
All samples collected at the NRCI are being retrospectively screened for SARS-CoV-2 virus and other common respiratory viruses, such as respiratory syncytial virus, human metapneumovirus, parainfluenza 1 to 4, bocavirus, picornavirus, adenovirus and human coronaviruses (NL63, OC43, 229E, HKU1). The results will be provided as a future addendum to this report.

Geneva, 14 August 2020

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

Sentinella Surveillance, Season 2019-20																	
Weeks	Dates		‰ ILI	Samples received	Influenza A				Influenza B				Total virus (n)	% influenza pos	Total tested for SARS-CoV 2 £	SARS-CoV 2 POS £
					Undet.	A (H1N1) pdm09	A (H3N2) seasonal	Total	Undet.	Bvic	Byam	Total				
40	29-Sep-19	5-Oct-19	1.4	8	0	0	0	0	0	1	0	1	1	12.50	ND	ND
41	6-Oct-19	12-Oct-19	1.8	6	0	0	0	0	0	0	0	0	0	0.00	ND	ND
42	13-Oct-19	19-Oct-19	0.7	5	0	0	0	0	0	0	0	0	0	0.00	ND	ND
43	20-Oct-19	26-Oct-19	1	8	0	0	0	0	0	0	0	0	0	0.00	ND	ND
44	27-Oct-19	2-Nov-19	1.9	6	0	0	0	0	0	0	0	0	0	0.00	ND	ND
45	3-Nov-19	9-Nov-19	2.4	15	0	1	1	2	0	0	0	0	2	13.33	ND	ND
46	10-Nov-19	16-Nov-19	2.9	21	0	2	0	2	0	1	0	1	3	14.29	ND	ND
47	17-Nov-19	23-Nov-19	2.8	18	0	0	0	0	0	0	0	0	0	0.00	ND	ND
48	24-Nov-19	30-Nov-19	3	15	0	2	0	2	0	0	0	0	2	13.33	ND	ND
49	1-Dec-19	7-Dec-19	3.2	23	0	2	1	3	0	2	0	2	5	21.74	ND	ND
50	8-Dec-19	14-Dec-19	4.1	21	1	1	2	4	0	1	0	1	5	23.81	ND	ND
51	15-Dec-19	21-Dec-19	5.4	23	0	5	0	5	0	2	0	2	7	30.43	ND	ND
52	22-Dec-19	28-Dec-19	9.8	15	0	2	0	2	0	1	0	1	3	20.00	ND	ND
1	29-Dec-19	4-Jan-20	12.2	22	0	2	0	2	0	3	0	3	5	22.73	ND	ND
2	5-Jan-20	11-Jan-20	11.6	71	1	9	10	20	0	5	0	5	25	35.21	ND	ND
3	12-Jan-20	18-Jan-20	14.2	89	0	29	10	39	0	12	0	12	51	57.30	ND	ND
4	19-Jan-20	25-Jan-20	25.8	92	3	22	8	33	0	21	0	21	54	58.70	ND	ND
5	26-Jan-20	1-Feb-20	37	101	1	37	9	47	0	33	0	33	80	79.21	ND	ND
6	2-Feb-20	8-Feb-20	42.5	109	0	27	16	43	1	25	0	26	69	63.30	ND	ND
7	9-Feb-20	15-Feb-20	33.6	87	0	19	13	32	0	21	0	21	53	60.92	ND	ND
8	16-Feb-20	22-Feb-20	28.1	76	0	19	12	31	0	14	0	14	45	59.21	ND	ND
9	23-Feb-20	29-Feb-20	26.4	76	0	13	9	22	0	18	0	18	40	52.63	ND	ND
10	1-Mar-20	7-Mar-20	22.4	54	0	8	3	11	0	7	0	7	18	33.33	ND	ND
11	8-Mar-20	14-Mar-20	25.8	49	0	4	3	7	0	8	0	8	15	30.61	30	1
12*	15-Mar-20	21-Mar-20	57.7	78	1	1	0	2	0	2	0	2	4	5.13	78	11
13	22-Mar-20	28-Mar-20	ND	13	0	0	0	0	0	0	0	0	0	0.00	13	3
14	29-Mar-20	4-Apr-20	ND	21	0	0	0	0	0	1	0	1	1	4.76	21	0
15	5-Apr-20	11-Apr-20	ND	6	0	0	0	0	0	0	0	0	0	0.00	6	0
16	12-Apr-20	18-Apr-20	ND	2	0	0	0	0	0	0	0	0	0	0.00	2	0
				1130	**7**	**205**	**97**		**1**	**178**	**0**		**488**		**150**	**15**
					309				**179**							

‰ 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*
ND: *Not done or not available*
**:* *Official start of SARS-CoV 2 surveillance*
£: *Preliminary results, retrospective testing ongoing*

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

			Antisera		
		Reference viral isolates	A/California/07/09	A/Michigan/45/15	A/Brisbane/02/18
		A/California/07/09	**128**	128	64
		A/Michigan/45/15*	256	**256**	128
		A/Brisbane/02/18	128	128	**128**
Isolates	HA titre	Typisation			
9888	64	A/Michigan-Like	1024	1024	1024
9796	128	A/Michigan-Like	1024	512	512
3566	32	A/Michigan-Like	256	256	256
4502	16	A/Michigan-Like	256	256	512
5140	32	A/Michigan-Like	256	256	256
4069 (8)	32	A/Michigan-Like	256	256	256
6773	64	A/Michigan-Like	512	512	512
8943	64	A/Michigan-Like	1024	1024	1024
6829	64	A/Michigan-Like	1024	1024	1024
4350	64	A/Michigan-Like	512	512	1024
4309	64	A/Michigan-Like	1024	512	1024
406	128	A/Michigan-Like	1024	512	1024
493	128	A/Michigan-Like	512	1024	1024
8021	64	A/Michigan-Like	2048	1024	1024
8060	128	A/Michigan-Like	1024	1024	1024
9275	64	A/Michigan-Like	1024	1024	1024
3761	64	A/Michigan-Like	1024	1024	1024
7448	128	A/Michigan-Like	2048	1024	1024

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

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

			Antisera		
		Reference viral isolates	A/California/07/09	A/Michigan/45/15	A/Brisbane/02/18
		A/California/07/09	**128**	128	64
		A/Michigan/45/15*	256	**256**	128
		A/Brisbane/02/18	128	128	**128**
Isolates	HA titre	Typisation			
434	32	A/Michigan-Like	512	512	512
1348	16	A/Michigan-Like	512	512	1024
873	64	A/Michigan-Like	512	512	512
913	32	A/Brisbane-Like	256	128	128
4018	64	A/Michigan-Like	1024	1024	1024
3784	64	A/Michigan-Like	512	512	512
7425	16	A/Brisbane-Like	16	64	32
7908	64	A/Brisbane-Like	32	64	64
6495	32	A/Michigan-Like	256	512	1024
6508	128	A/Brisbane-Like	64	64	128
7672	128	A/Brisbane-Like	16	64	32
4460	32	A/Brisbane-Like	128	128	128
4859	16	A/Michigan-Like	256	256	256
4581	32	A/Brisbane-Like	128	128	128
5817	32	A/Brisbane-Like	128	128	256
7604	16	A/Brisbane-Like	<16	64	64
8756	16/32	A/Michigan-Like	256	256	512

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

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

			Antisera			
		Reference viral isolates	A/Hong Kong/4801/14	A/Singapore/INFIM-0016-19/16	A/Switzerland/8060/17	A/Kansas/14/17
		A/Hong Kong/4801/14	**128**	256	256	64
		A/Singapore/INFIM-0016-19/16	32	**32**	64	64
		A/Switzerland/8060/17	64	64	**64**	64
		A/Kansas/14/17	64	64	64	**128**
Isolates	HA titre	Typisation				
9948	16	A/Kansas/14/17-Like	256	128	128	128
7593	64	A/Kansas/14/17-Like	64	64	64	256
7205	32	A/Kansas/14/17-Like	64	64	128	256
4002	32	A/Switzerland/8060/17-Like	32	64	64	64
4916	16	A/Singapore/0016-19/16-Like	32	32	32	32
4993	32	A/Kansas/14/17-Like	64	64	32	64
7485	32	A/Hong Kong/4801/14-Like	512	256	128	512
3985	16	A/Kansas/14/17-Like	128	256	64	512
7401	64	A/Kansas/14/17-Like	128	256	ND	512
7462	64	A/Kansas/14/17-Like	128	128	ND	64
5992	32	A/Kansas/14/17-Like	32	64	64	128
7722	16	A/Singapore/0016-19/16-Like	32	32	32	64
6207	16	A/Hong Kong/4801/14-Like	128	128	128	64
8483	64/64	A/Kansas/14/17-Like	64	128	64	64

HA titers were established in MDCK-SIAT (S/1) cells or MDCK (MD/1). **HI titers should be multiplied by 8**. Bold: also sent to the WIC. Vaccine strain: green. ND: not done.

Annex 4a: Hemagglutination inhibition data of influenza B/Victoria/2/87-lineageviruses

			Antisera				
		Reference viral isolates	B/Brisbane/60/08	B/Hong Kong /269/17 1A(Δ3)A	B/Norway/2409/171 A(Δ2)	B/Colorado/02/17 1A(Δ2)	B/Washington/02/19 1A(3Δ)B
		B/Brisbane/60/08	**1024**	32	128	256	ND
		B/Hong Kong/269/17	64	**64**	64	64	ND
		B/Norway/2409/17	<16	<16	**512**	<16	ND
		B/Colorado/02/17	<16	<16	64	**32**	ND
		B/Washington/02/19	32	<16	64	64	**256**
Isolates	**HA titre**	**Typisation**					
6570	32	B/Colorado/06/17-Like	<16	<16	64	16	ND
9762	128	B/Colorado/06/17-Like	<16	<16	64	16	ND
5198	32	B/Colorado/06/17-Like	<16	<16	64	8	ND
7299	32	B/Colorado/06/17-Like	<16	<16	32	8	ND
7929	32	B/Colorado/06/17-Like	<16	<16	64	8	ND
6873	64	B/Colorado/06/17-Like	<16	<16	128	16	256
8427	32	B/Colorado/06/17-Like	<16	<16	64	16	128
6841	64	B/Colorado/06/17-Like	<16	<16	128	16	128
3806	128	B/Colorado/06/17-Like	<16	ND	128	32	128
3839	32	B/Colorado/06/17-Like	<16	ND	128	16	512
4043	16	B/Colorado/06/17-Like	<16	ND	128	32	512
7456	64	B/Colorado/06/17-Like	<16	ND	256	32	256
412	64	B/Colorado/06/17-Like	<16	ND	256	64	256
8329	32	B/Colorado/06/17-Like	<16	ND	128	32	512

HA titers were established in MDCK-SIAT (S/1) cells or MDCK (MD/1). **HI titers should be multiplied by 8**. Bold: also sent to the WIC. Vaccine strain: green. ND: not done.

Annex 4b: Hemagglutination inhibition data of influenza B/Victoria/2/87-lineageviruses

			Antisera				
		Reference viral isolates	B/Brisbane/60/08	B/Hong Kong /269/17 1A(Δ3)A	B/Norway/2409/171 A(Δ2)	B/Colorado/02/17 1A(Δ2)	B/Washington/0 2/19 1A(3Δ)B
		B/Brisbane/60/08	**1024**	32	128	256	ND
		B/Hong Kong/269/17	64	**64**	64	64	ND
		B/Norway/2409/17	<16	<16	**512**	<16	ND
		B/Colorado/02/17	<16	<16	64	**32**	ND
		B/Washington/02/19	32	<16	64	64	**256**
Isolates	HA titre	Typisation					
4942	16	B/Colorado/06/17-Like	<16	ND	256	64	1024
296	64	B/Colorado/06/17-Like	32	ND	256	64	512
7764	128	B/Colorado/06/17-Like	<16	ND	128	32	128
9112	64	B/Colorado/06/17-Like	<16	ND	64	16	64
9209	64	B/Colorado/06/17-Like	<16	ND	32	8	64
9392	64	B/Colorado/06/17-Like	<16	ND	64	16	128
7546	16	B/Brisbane/60/08-Like	256	128	ND	64	256
7626	32	B/Hong Kong/269/17-Like	64	64	ND	64	256
6412	32	B/Colorado/06/17-Like	32	16	256	32	256
6330	64	B/Colorado/06/17-Like	<8	<8	64	16	256
6338	32	B/Colorado/06/17-Like	32	16	512	32	128
5774	32	B/Colorado/06/17-Like	<16	<16	64	32	256
4396	32	B/Colorado/06/17-Like	<16	<16	32	64	256
84550	32	B/Colorado/06/17-Like	<16	ND	64	64	512

HA titers were established in MDCK-SIAT (S/1) cells or MDCK (MD/1). **HI titers should be multiplied by 8**. Vaccine strain: green. ND: not done.

Annex 4c: Hemagglutination inhibition data of influenza B/Victoria/2/87-lineageviruses

			Antisera				
		Reference viral isolates	B/Brisbane/60/08	B/Hong Kong /269/17 1A(Δ3)A	B/Norway/2409/171 A(Δ2)	B/Colorado/02/17 1A(Δ2)	B/Washington/02/19 1A(3Δ)B
		B/Brisbane/60/08	**1024**	32	128	256	ND
		B/Hong Kong/269/17	64	**64**	64	64	ND
		B/Norway/2409/17	<16	<16	**512**	<16	ND
		B/Colorado/02/17	<16	<16	64	**32**	ND
		B/Washington/02/19	32	<16	64	64	**256**
Isolates	**HA titre**	**Typisation**					
5735	64	B/Colorado/06/17-Like	<16	ND	64	32	256
4493	32	B/Colorado/06/17-Like	<16	ND	128	64	512

HA titers were established in MDCK-SIAT (S/1) cells or MDCK (MD/1). **HI titers should be multiplied by 8**. Vaccine strain: green. ND: not done.

Annex 5: List of reference antisera provided by the WIC for the 2019/2020 season

Reference antisera	Ferret	
A Hong Kong/4801/14	F41/15	Egg
A/Singapore/INFIMH-16-0019/16	F46/17	Egg
A/Switzerland/8060/17	F42/18	Egg
A/Kansas/14/17	F12/19	Egg
A/California/07/09	F07/16	Egg
A/Michigan/45/15	F32/16	Egg
A/Brisbane/02/18	F10/19	Egg
B/Hong Kong/514/09	F9/13	Egg
B/Johannesburg/3964/12	F4/16	Egg
B/Norway/2409/17	F20/17	T/C
B/Wisconsin/1/10	F10/13	Egg
B/Hong Kong/269/17	F50/17	Egg
B/Novosibirsk/1/12	F31/12	T/C
B/Phuket/3073/13	F51/16	Egg
B/Brisbane/60/08	F45/16	Egg
B/Colorado/6/17	F10/18	Egg
B/Washington/02/19	F38/19	Egg

Annex 6: Sequencing primers

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, HPA,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	

MRC-NIMR: Medical Research Council-National Institute for Medical Research (London, UK).

Annex 7: Antigenic analyses of influenza A(H1N1)pdm09 viruses, WIC (2020-01-21)

	Viruses	Other information	Passage history	Collection date	Passage history	Haemagglutination inhibition titre: Post-infection ferret antisera									
						A/Mich 45/15	A/Bayern 69/09	A/Lviv N6/09	A/Slov 2903/2015	A/Paris 1447/17	A/Swit 2656/17	A/Swit 3330/17	A/Norway 3433/18	A/Ire 84630/18	A/Bris 02/18
			Passage history			Egg	MDCK	MDCK	Egg	MDCK	Egg	Egg	MDCK	MDCK	Egg
			Ferret number			F31/16[*1]	F09/15[*1]	F13/18[*1]	NIB F48/16[*1]	F03/18[*2]	F20/18[*1]	F23/18[*1]	F04/19[*1]	F08/19[*1]	F09/19[*1]
			Genetic group			6B.1			6B.1	6B.1A	6B.1A	6B.1A5	6B.1A5	6B.1A6	6B.1A1
	REFERENCE VIRUSES														
	A/Michigan/45/2015		6B.1	2015-09-07	E3/E3	640	160	160	640	640	1280	320	1280	640	640
	A/Bayern/69/2009	G155E		2009-07-01	MDCK5/MDCK1	80	320	320	40	160	160	80	320	40	80
	A/Lviv/N6/2009	G155E, D222G		2009-10-27	MDCK4/SIAT1/MDCK3	320	640	640	160	320	320	320	640	160	160
	A/Slovenia/2903/2015	clone 37	6B.1	2015-10-26	E4/E2	1280	320	160	1280	1280	1280	640	1280	640	640
	A/Paris/1447/2017		6B.1A	2017-10-20	MDCK1/MDCK3	640	320	80	1280	1280	1280	640	1280	1280	640
	A/Switzerland/2656/2017		6B.1A	2017-12-21	E5/E3	2560	640	640	1280	1280	2560	1280	2560	1280	1280
	A/Switzerland/3330/2017	clone 35	6B.1A5B	2017-12-20	E6/E2	640	160	80	640	640	640	640	1280	640	320
	A/Norway/3433/2018		6B.1A5A	2018-10-30	MDCK3	640	160	80	640	640	640	640	1280	640	320
	A/Ireland/84630/2018		6B.1A6	2018-11-28	MDCK1/MDCK3	1280	320	160	1280	1280	1280	640	2560	1280	640
	A/Brisbane/02/2018		6B.1A1	2018-01-04	E3/E1	1280	320	320	1280	2560	2560	1280	2560	1280	1280
	TEST VIRUSES														
	A/Switzerland/4068/2019		6B.1A5A	2019-12-11	SIAT1/MDCK1	640	320	160	1280	1280	1280	1280	2560	1280	640
	A/Switzerland/5139/2019		6B.1A5A	2019-12-02	SIAT1/MDCK1	640	320	160	1280	1280	1280	1280	2560	1280	640
	A/Switzerland/4501/2019		6B.1A7	2019-11-26	SIAT1/MDCK1	1280	320	80	1280	1280	1280	640	1280	1280	640
	A/Switzerland/3565/2019		6B.1A5B	2019-11-26	SIAT1/MDCK1	1280	320	80	1280	1280	1280	640	1280	1280	640
	A/Switzerland/9795/2019		6B.1A5A	2019-11-14	SIAT1/MDCK1	640	320	160	640	640	640	320	1280	640	320
	A/Switzerland/9887/2019		6B.1A5A	2019-11-06	SIAT1/MDCK1	320	160	80	640	640	640	320	640	320	320
Assay	HI (Turkey RBC)					Vaccine									Vaccine
RBC	Turkey					NH 2018-19									NH 2019-20
Virus	Influenza A(H1N1)pdm09					SH 2019									SH 2020
Date															

* Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used)

[1] < = <40

ND Not Done

Sequences in phylogenetic trees

Annex 8: Antigenic analyses of influenza A(H3N2) viruses, WIC (Guinea Pig RBC with 20nM Oseltamivir)2020-01-17

Viruses	Other information		Collection date	Passage history	Haemagglutination inhibition titre									
					Post-infection ferret antisera									
					A/HK 5738/14	A/Bretagne 1413/17	A/Singapore 0019/16	A/La Rioja 2202/18	A/Eng 538/18	A/Norway 3275/18	NYMC X-327 A/Kansas/14	A/Kansas 14/17	A/Sth Aus 34/19	A/HK 2671/19
		Passage history			MDCK	SIAT	Egg 10^{-4}	SIAT	SIAT	SIAT	Egg	SIAT	Egg	Egg
		Ferret number			St Judes F60/17[*1]	F01/18[*1]	F13/19[*1]	F26/18[*1]	F31/18[*1]	F03/19[*1]	F16/19[*1]	F17/19[*1]	F45/19[*1]	F44/19
		Genetic group			3C.2a	3C.2a2	3C.2a1	3C.2a1b	3C.3a	3C.2a1b	3C.3a	3C.3a	3C.2a1b	
REFERENCE VIRUSES														
A/Hong Kong/5738/2014		3C.2a	2014-04-30	MDCK1/MDCK3/SIAT2	160	160	320	80	160	160	160	80	160	<
A/Bretagne/1413/2017		3C.2a2	2017-10-09	MDCK1/SIAT4	160	640	320	80	160	160	80	80	320	<
A/Singapore/INFIMH-16-0019/2016		3C.2a1	2016-04-14	E5/E2	160	80	320	160	80	40	40	<	80	40
A/England/538/2018		3C.3a	2018-02-26	MDCK1/SIAT4	40	40	40	<	640	<	160	320	40	<
NYMC X-327 (A/Kansas/14/17)		3C.3a	2017-12-14	Ex/E1	40	40	80	<	320	40	1280	320	80	160
A/Kansas/14/2017		3C.3a	2017-12-14	SIAT3/SIAT2	40	40	80	<	320	40	160	320	40	<
A/South Australia/34/2019		3C.2a1b+T131K		E6/E1	160	640	320	80	80	1280	40	40	1280	80
A/Hong Kong/2671/2019		3C.2a1b+T135K-B	2019-06-17	E8	40	<	80	40	160	<	320	80	80	320
TEST VIRUSES														
A/Switzerland/7204/2019		3C.2a1b+T131K	2019-12-06	SIAT1/SIAT1	160	160	160	160	160	640	80	80	160	<
							Vaccine NH 2018-19				Vaccine NH 2019-20		Vaccine SH 2019-2020	

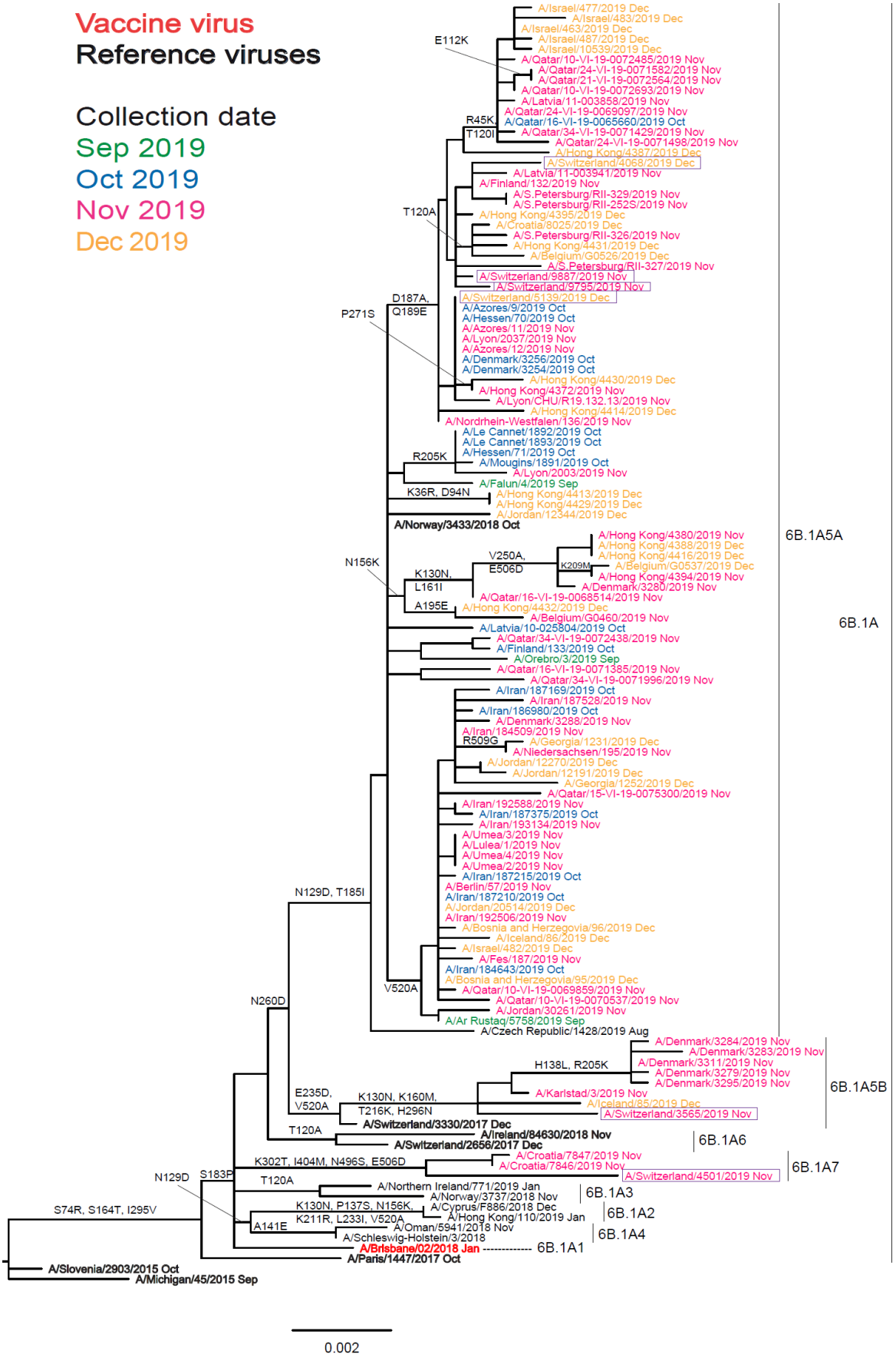
Assay HI (Guinea Pig RBC with 20nM Oseltamivir)
RBC Guinea Pig
Virus Influenza A(H3N2)
Date
* Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used)
[1] < = <40
ND Not Done
Sequences in phylogenetic trees

Annex 9: Antigenic analyses of influenza B viruses, WIC (Victoria lineage) 2020-01-22

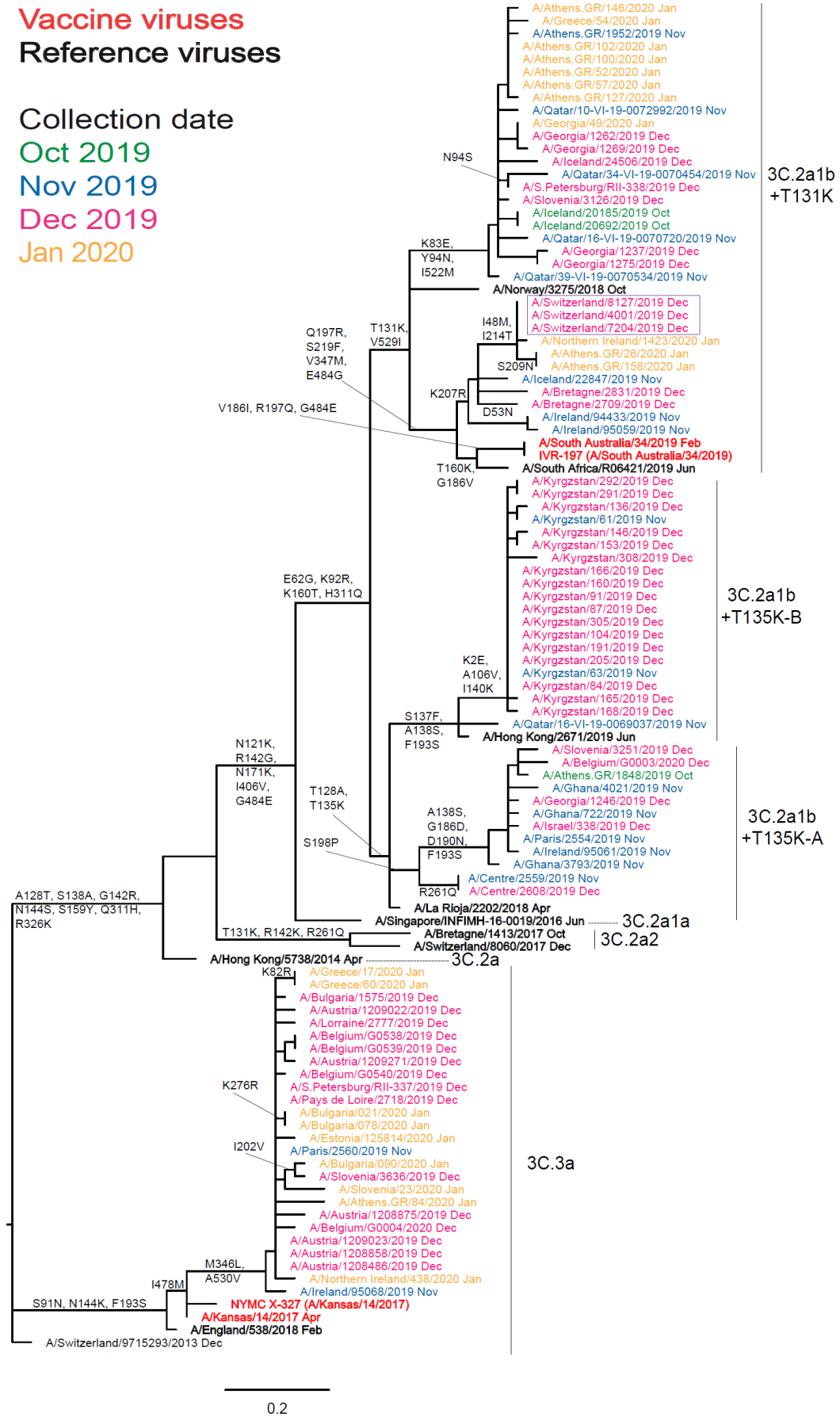
Viruses	Other information	Passage history	Collection date	Passage history	Haemagglutination inhibition titre: Post-infection ferret antisera									
					B/Bris 60/08	B/Bris 60/08	B/Sth Aus 81/12	B/Ireland 3154/16	B/Nord-West 1/16	B/Norway 2409/17	B/Colorado 06/17	B/Colorado 06/17	B/Wash'ton 02/19	B/Wash'ton 02/19
		Passage history			Egg	Egg	Egg	MDCK	MDCK	MDCK	MDCK	Egg	MDCK	Egg
		Ferret number			Sh 539, 540, 543, 544, 570, 571, 574[*1,3]	F44/17[*2]	F25/16[*2]	F15/16[*2]	F38/17[*2]	F40/17[*2]	F09/18[*4]	F11/18[*4]	F37/19[*4]	F38/19[*4]
		Genetic group			1A	1A	1A	1A	1A	1A(Δ2)	1A(Δ2)	1A(Δ2)	1A(Δ3)	1A(Δ3)
REFERENCE VIRUSES														
B/Brisbane/60/2008		1A	2008-08-04	E4/E4	1280	640	640	40	160	<	20	40	<	160
B/South Australia/81/2012		1A	2012-11-28	E4/E2	2560	640	640	40	160	<	20	40	<	160
B/Ireland/3154/2016		1A	2016-01-14	MDCK1/MDCK4	1280	80	40	80	320	<	<	<	<	<
B/Nordrhein-Westfalen/1/2016		1A	2016-01-04	C2/MDCK2	1280	80	40	40	160	<	<	<	<	<
B/Norway/2409/2017		1A(Δ2)	2017-04-27	MDCK1/MDCK3	80	10	10	<	<	80	20	40	<	<
B/Colorado/06/2017		1A(Δ2)	2017-02-05	MDCK1/MDCK2	160	40	20	<	<	80	40	80	<	<
B/Colorado/06/2017		1A(Δ2)	2017-02-05	E5/E2	640	160	80	<	<	20	40	160	<	80
B/Washington/02/2019		1A(Δ3)B	2019-01-19	C2/MDCK2	320	80	40	<	<	<	<	40	40	320
B/Washington/02/2019		1A(Δ3)B	2019-01-19	E3/E2	640	80	80	<	<	<	<	80	20	320
TEST VIRUSES														
B/Switzerland/7929/2019		1A(Δ3)B	2019-12-09	SIAT1/MDCK1	160	20	20	<	<	<	<	<	40	320
B/Switzerland/7299/2019		1A(Δ3)B	2019-12-06	SIAT1/MDCK1	160	40	40	<	<	<	<	<	40	320
B/Switzerland/5198/2019		1A(Δ3)B	2019-12-02	SIAT1/MDCK2	160	20	20	<	<	<	<	<	40	320
B/Switzerland/9761/2019		1A(Δ3)B	2019-11-15	MDCK1/MDCK1	640	80	20	<	<	<	<	<	<	320
B/Switzerland/6569/2019		1A(Δ3)B	2019-10-03	SIAT1/MDCK1	160	40	40	<	<	<	<	<	80	40
												Vaccine NH 2018-19 SH 2019 NH 2019-20		Vaccine SH 2020

Assay HI (Turkey RBC)
RBC Turkey
Virus Influenza B/Victoria-lineage
Date
* Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used)
1-4 [1] < = <40; [2] < = <10; [3] hyperimmune sheep serum; [4] < = <20
ND Not Done
Sequences in phylogenetic trees

Annex 10: Phylogeny of A(H1N1)pdm09 viruses (HA), WIC



Annex 11: Phylogeny of A(H3N2) viruses (HA), WIC



Annex 12: Phylogeny of B(Victoria) viruses (HA), WIC