Influenza virus surveillance in Switzerland
Season 2017–2018

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Abbreviations and Acronyms

CDC:	Centers for Disease Control and Prevention
CPE:	cytopathic effect
Ct:	cycle threshold
EEA:	European Economic Area
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
HUG:	Geneva University Hospitals
ILI:	influenza-like illness
M:	matrix
MC-ILI:	medical consultations for influenza-like illness
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
OIE:	World Organization for Animal Health
PA:	acidic protein
PB:	basic protein
RBC:	red blood cells
RFU:	relative fluorescent units
RNA:	ribonucleic acids
RNP:	ribonucleoprotein
rRT-PCR:	real-time reverse-transcription polymerase chain reaction
USA:	United States of America
Vic:	Victoria
WHO:	World Health Organization
WIC:	Worldwide Influenza Centre
Yam:	Yamagata

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Résumé – Zusammenfassung – Summary

Résumé

L'épidémie de grippe de 2017/18 a débuté plus tôt que ces dernières années, excepté 2016/17. Celle-ci s’est prolongée de 4 semaines par rapport à la saison 2016/17. Deux pics de consultations médicales pour des syndromes grippaux ont été observés au cours des semaines 2/2018 et 4/2018.

Sur les 1296 échantillons dépistés pour l’influenza durant la saison 2017/18, 57,6% étaient positifs. L'épidémie a été marquée par une prédominance de virus de l’influenza de type B par rapport aux virus grippaux de type A. La plupart des virus influenza B identifiés appartenaient à la lignée B/Yamagata/16/1988. Ceux qui ont été caractérisés génétiquement appartenaient au clade 3 et étaient soit bien, soit faiblement reconnus par des antisérums (produits chez le furet) dirigés contre la souche vaccinale B/Phuket/3073/2013 (clade 3) notamment en fonction du lot d'antisérum utilisé. Le second sous-type de virus le plus répandu cette saison, en Suisse, était l’influenza A(H1N1)pdm09. Dans l’ensemble les virus A(H1N1)pdm09 étaient antigéniquement semblables à la souche vaccinale A/Michigan/45/2015 (clade 6B.1). Un nombre limité de souches A(H3N2) et de virus apparentés à la lignée B B/Victoria/2/1987 ont circulé pendant la saison 2017/18 en Suisse. Tous les virus A(H3N2) génétiquement caractérisés se répartissaient dans différentes sous-clades du sous-groupe génétique 3C.2a. En général, ces derniers étaient bien reconnus par les antisérums dirigés contre les souches vaccinales 2017/18 (A/Hong Kong/4801/14) et 2018/19 (A/Singapore/INFIMH-16-0019/2016). La majorité (3 sur les 4 détectés) des virus B/Victoria/2/1987 présentaient une délétion des acides aminés 162 et 163 dans le gène HA et n'étaient, en général, pas reconnus par les antisérums dirigés contre les souches similaires à la référence vaccinale B/Brisbane/60/2008.

Comme les années précédentes, tous les virus influenza A testés, pendant la saison 2017/18 en Suisse, présentaient la mutation S31N dans le gène de la matrice associée à une résistance aux adamantanes. Un seul virus A(H3N2), selon l’analyse effectuée par le « Worldwide Influenza Centre », présentait une sensibilité à l'oseltamivir réduite au niveau phénotypique. Parallèlement à la surveillance annuelle régulière de la grippe effectuée dans la population générale, deux isolats porteurs de

la substitution H275Y, associée à une sensibilité à l'oseltamivir fortement réduite, ont été identifiés chez deux patients immunodéprimés hospitalisés.

Un cas A (H1N1)v positif (origine porcine) a également été identifié chez un employé agricole cette saison.

Zusammenfassung

Die Grippeepidemie hat in der Schweiz dieses Jahr früher begonnen als sonst üblich, abgesehen von derjenigen von 2016/17. Im Vergleich zur Saison 2016/2017 hat sie vier Wochen länger gedauert. Die ärztlichen Konsultationen für grippeartige Erkrankungen erreichten zwei Maxima im Verlaufe der Wochen 2/2018 und 4/2018.

Von den 1296 Proben welche während der Saison 2017/2018 auf Influenza Viren untersucht wurden, waren 57,6% positiv. In der diesjährigen Epidemie waren die Influenza B Viren gegenüber Influenza A vorherrschend. Die meisten der Influenza B Viren gehörten zur Linie von Influenza B/Yamagata/16/1988. Diejenigen welche genetisch charakterisiert wurden, gehörten zur Klade 3 und wurden, abhängig vom verwendeten Lot, entweder gut oder schlecht erkannt vom Frettchen Antiserum gegen den im Impfstoff enthaltenen Influenza B/Phuket/3073/2013 (Klade 3). Der zweite, diese Saison in der Schweiz häufig zirkulierende Typ, war Influenza A(H1N1)pdm09. Im Gesamten waren die Influenza A(H1N1)pdm09 Viren vergleichbar mit dem im Impfstoff enthaltenen Stamm A/Michigan/45/2015 (Klade 6B.1). Eine kleine Anzahl von Influenza A(H3N2) und Verwandte von der Linie Influenza B Viktoria/2/1987 zirkulierten in der Saison 2017/18 in der Schweiz. Alle der der genetisch charakterisierten Influenza A (H3N2) Viren verteilt sich auf verschiedene Sub-Kladen der genetischen Klade 3C.2a. Im Allgemeinen wurden letztere durch die Antiseren gegen die im Impfstoff enthaltenen Stämme 2017/2018 (A/Hong Kong/4801/14) und 2018/19 (A/Singapore/INFIMH-16-0019/2016) gut erkannt. Die Mehrheit (3/4 nachgewiesenen) der Influenza B/Viktoria/2/1987 wiesen eine Deletion der Aminosäuren 162 und 163 im Hämagglutinin Gen auf und wurden im Allgemeinen vom Serum gegen den Impfstoff Stamm B/Brisbane/60/2008 nicht erkannt (oder nicht gut).

Wie in den vorangegangenen Jahren, wiesen alle während der Saison 2017/2018 geprüften Influenza A Viren die Resistenz Mutation S31N im Matrix Gen auf welche für eine Resistenz gegen Amantadine verantwortlich ist. Ein einziges Influenza A

(H3N2) welches durch das « Worldwide Influenza Centre » geprüft wurde, wies eine reduzierte phänotypische Empfindlichkeit gegen Oseltamivir auf. Parallel zur jährlichen Grippeüberwachung in der allgemeinen Bevölkerung wiesen zwei Isolate welche von zwei hospitalisierten, immunsupprimierten Patienten stammten, die Substitution H275Y auf, welche mit einer stark reduzierten Empfindlichkeit gegenüber Oseltamivir verbunden ist.

Bei einem Angestellten aus der Landwirtschaft wurde in dieser Saison ein Fall von einem Influenza A (H1N1)v (Herkunft aus dem Schwein) nachgewiesen.

Summary

2017/18 influenza outbreak started earlier than in recent years in Switzerland, and lasted 4 weeks more than in 2016/17. Two peaks of medical consultations for influenza like-illnesses were observed at weeks 2/2018 and 4/2018.

Of the 1296 samples screened for influenza during the 2017/18 season, 57.6% were positive. The outbreak was marked by a dominance of influenza B over influenza A viruses. Most of the identified influenza B viruses were B/Yamagata/16/1988. All B Yamagata viruses characterized belonged to the genetic clade 3 and were either well or poorly recognized or by ferret antisera raised against the B/Phuket/3073/2013 vaccine strain (clade 3) depending on the antisera lot. The second most prevalent virus subtype was influenza A(H1N1)pdm09. In general, A(H1N1)pdm09 viruses were antigenically similar to the A/Michigan/45/2015 vaccine strain (clade 6B.1). Only a limited number of A(H3N2) strains and influenza B B/Victoria/2/1987-like viruses circulated in Switzerland during this season. All A(H3N2) viruses belonged to several subclades of the genetic clade 3C.2a. In general, they were in well recognized by the antisera raised against the 2017/18 and 2018/19 vaccine strains, i.e., A/Hong Kong/4801/14 and A/Singapore/INFIMH-16-0019/2016, respectively. Most (3) of the B/Victoria/2/1987-like viruses exhibited a deletion of amino acids 162 and 163 in the Hemagglutinin gene and were generally not (or not well) recognized by antiserum raised against B/Brisbane/60/2008-like reference viruses.

As observed during previous seasons, all influenza A viruses tested for adamantanes resistance in Switzerland exhibited the S31N resistance mutation. Only one A(H3N2) virus was shown by the Worldwide Influenza Centre to have a phenotypic reduced inhibition by oseltamivir. In parallel to the regular annual influenza surveillance

performed in the general population, two isolates bearing the H275Y substitution, which is associated with highly-reduced inhibition by oseltamivir, were identified in two immunocompromised hospitalized patients.

One A(H1N1)v positive case (swine origin) was identified in a farm employee during the 2017/18 season.

1. Introduction

Influenza virus infections are a major clinical and economic burden worldwide.[1,2] In Switzerland, the Sentinella surveillance system is a community based network of primary care medical practitioners who report medical consultations for influenza-like illnesses (MC-ILI) to the Federal Office of Public Health (FOPH). A subgroup of Sentinella practitioners randomly collects respiratory samples from patients diagnosed with ILI and sends these to the National Reference Centre of Influenza (NRCI) in Geneva for further characterization. This report summarizes the demographic, epidemiologic and virus characterization data gathered from samples processed and analyzed by the NRCI during the 2017/18 influenza season.

2. The influenza virus

Influenza viruses are orthomyxoviruses, a family of enveloped negative single-stranded ribonucleic acid (RNA) viruses (Figure 1) known to be causative agents of respiratory tract infections referred to as influenza disease or “flu”. Influenza viruses are divided into four genera, A, B, C and D.[3,4] Influenza A viruses have a wide host tropism, while influenza B viruses are found in humans[5] and in harbour seals.[6] The two former influenza types are responsible for the annual influenza epidemics. Influenza C viruses can be isolated from swine and humans in whom they can cause minor symptoms, while influenza D viruses are mainly found in swine and cattle.[4] Even if the pathogenic potential of influenza D virus in humans remains unknown, a recent study estimated that specific influenza D antibodies could be found in approximately 1.3% of the general human population.[7]

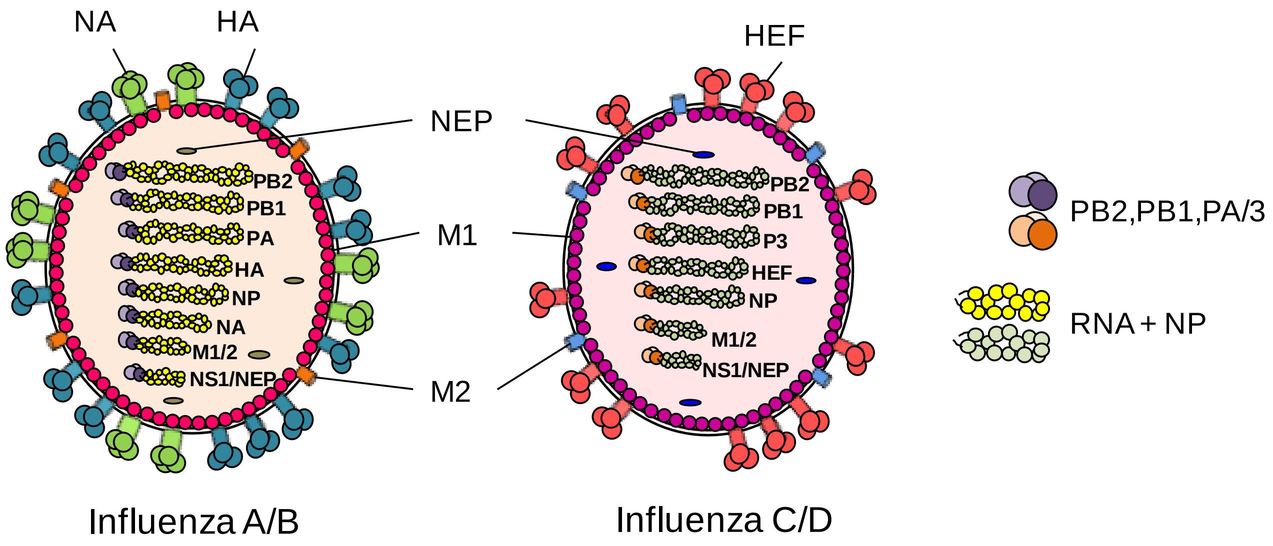


Figure 1. The structure of influenza viral particles. Basic protein 2 (PB2), 1 (PB1) and acidic protein or 3 (PA or P3) form a complex that corresponds to the RNA-dependent polymerase. The hemagglutinin (HA) and the hemagglutinin-esterase-fusion (HEF) play a role in virus attachment to sialic acids present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

3. Methodology

3.1. Clinical identification of influenza cases

During the annual influenza season, starting at week 40 and lasting until week 16 the following year, 150 to 200 primary care practitioners participate in the national influenza surveillance network. They are requested to notify MC-ILI on a weekly basis. ILI is defined by fever >38°C with or without a feeling of sickness, myalgia, or an alteration of general status, together with at least one acute respiratory symptom, such as cough and/or sore throat.[8] A subgroup of Sentinella practitioners, often around half of those participating, collect nasopharyngeal swabs from patients with ILI for subsequent viral detection and characterization. The sampling procedure of specimens is performed according to the following protocol:

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

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

The threshold value is defined by the FOPH based on data collected over the past 10 years (excluding the pandemic season 2009/10). For the 2017/18 influenza season, It corresponded to 68 suspected influenza cases per 100,000 inhabitants.

3.2. Sampled population data

Sentinella practitioners who send samples to the NRCI are asked to complete a case report form to collect the following data: sample type; age; sex; symptoms at the time of swab collection (abrupt disease onset, fever <38°C, temperature 37-38°C, myalgia, headache, cough, pneumonia and others); presence of chronic disease(s): pregnancy, time of onset of symptoms; influenza vaccination status; and antiviral treatment.

3.3. Virological detection of influenza viruses

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 United States (US) Centers for Disease Prevention and Control (CDC) protocol is used to detect the presence of influenza A and/or B viral genomes in the clinical samples. The rRT-PCR targets are the matrix protein (M) and the non-structural protein (NS) genes for influenza A and B viruses, respectively. Influenza A and B positive samples are then subtyped using rRT-PCRs targeting the hemagglutinin (HA) and neuraminidase (NA) genes in order to discriminate between influenza A H1N1 and H3N2 subtypes and B Yamagata (Yam) and Victoria (Vic) lineages, respectively.

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

3.4. Antigenic and genetic characterization of influenza viruses

A selection of influenza viruses are submitted to phenotypic and genotypic analysis (Figure 2). In brief, during the pre- and post-epidemic phases all positive samples with sufficient Hemagglutinin (HA) titers are phenotypically characterized using the hemagglutination inhibition (HI) assay, which evaluates the antigenic similarity between reference and circulating influenza strains. During the epidemic phase, approximately 5 positive samples per week with a cycle threshold (Ct) value ≤30 and sufficient HA titers, are analyzed. When judged relevant, samples with Ct values ≥30 will also be selected for characterization. Similarly, a microneutralization (MN) test can be used for samples that do not (or only poorly) hemagglutinate red blood cells (RBC). Reference antisera (Annex 19) and corresponding viral reference strains used for the HI and MN were kindly provided by the WHO Collaborating Centre Reference Laboratory at The Francis Crick Worldwide Influenza Centre (WIC), London, United Kingdom (UK)). Reference viruses stocks for the “current influenza season” have been produced on cells (Madin-Darby canine kidney [MDCK] and MDCK-sialic acid-enriched [MDCK-SIAT]). HIs are performed with glutaraldehyde-fixed guinea pig (Charles River, Lyon, France).

To assess the phylogeny of the circulating strains and to determine how genetically close they are to vaccine strains, the HA genes, in particular the HA1 part, of the samples previously chosen for phenotypic characterization with a Ct ≤30 are submitted to Sanger sequencing (approximately 5 per week). The corresponding NA genes are also sequenced. To a lesser extent, influenza A M and influenza B NS genes. The NA gene sequence allows to detect key mutations previously described as conferring resistance to NA inhibitors (NAI). M and NS genes sequencing allows to control the adequacy of rRT-PCR influenza A and B screening, respectively.

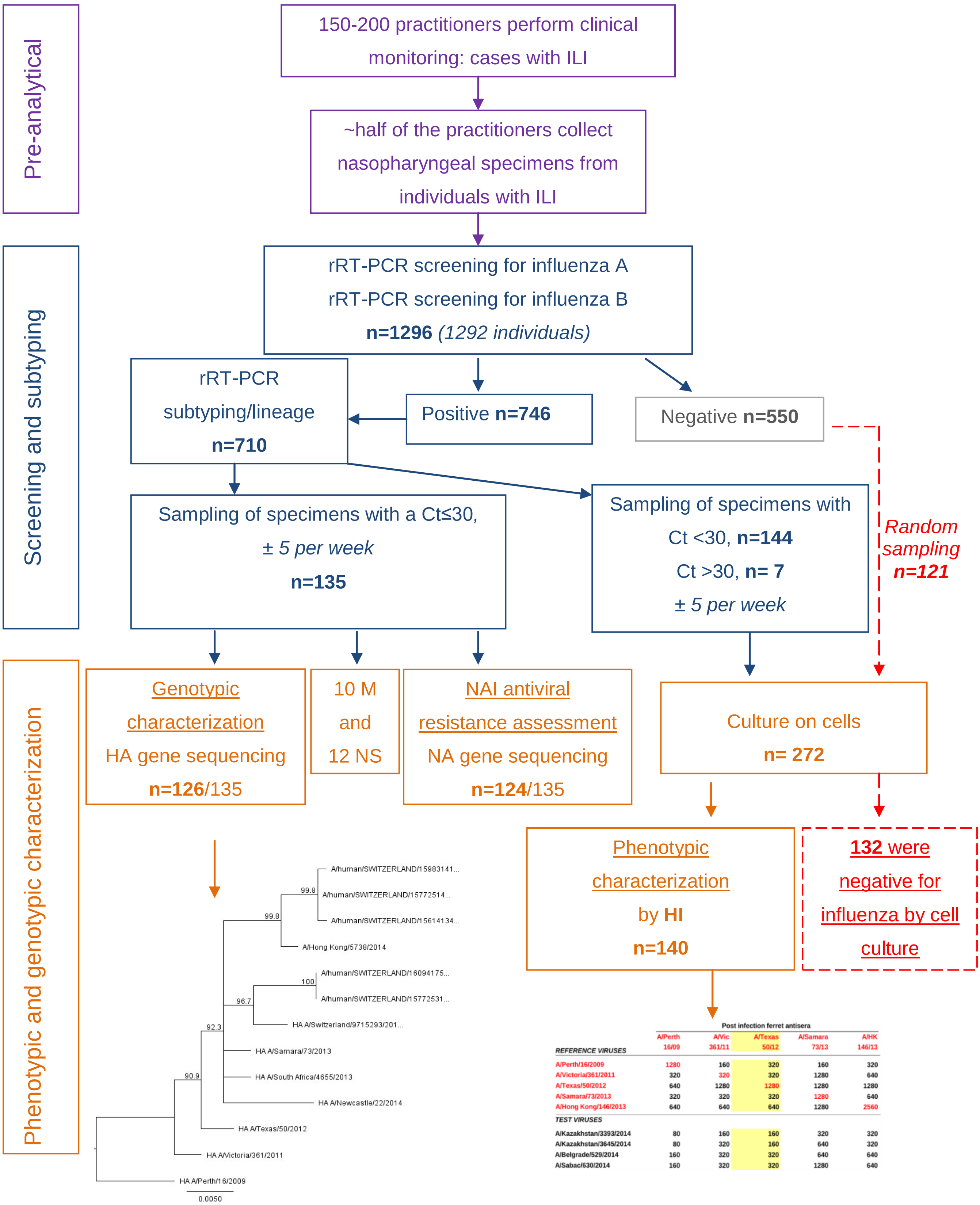


Figure 2. Flow chart of Sentinella sample collection and processing. Numbers (n) represent the number of samples submitted to the described step during the 2017/18 season.

3.4.1. Cell culture

As HI analysis requires a high concentration of influenza virus, a viral amplification step is performed by inoculating the clinical samples on MDCK and MDCK-SIAT1 cells in parallel. According to our predefined selection criteria, a subgroup of five specimens per week detected positive by rRT-PCR and with a Ct value <30 are inoculated on cells. In brief, 0.4 ml of transport medium containing nasopharyngeal swab are incubated for 7 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 FITC-conjugate (Merck-Millipore, Chemicon®, Schaffhausen Switzerland). Positive samples are submitted to a hemagglutination test in order to determine the virus titer. The HA and HI assays are dependent on the ability of the viral HA to bind to sialic acids present at the surface of RBCs.

3.4.2. Hemagglutination inhibition assay

A two-fold serial dilution is performed using 50 µl of viral suspension buffer in SALK solution (5%) and 25 µl of glutaraldehyde-fixed guinea pig RBC (1.5%) are added for a 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 solution (5%) in the second well. 25 µl are then collected from the same well and the procedure repeated to the end of each line. 25 µl of viral suspension containing 4 HA units are added to the ferret antisera dilution and incubated for 1 h at room temperature. 25 µl of guinea pig RBC are then 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 5 should be multiplied by 8 to obtain the final titers.

a. H1N1pdm09 / antisera	A/California/7/09	A/Michigan/45/15	A/St Petersburg/27/11	A/Hong Kong /3934/11
A/California/7/09	**64**	128	128	128
A/Michigan/45/15	64	**128**	128	64
A/St Petersburg/27/11	64	64	**64**	64
A/Hong Kong/3934/11	64	128	64	**64**

b. H3N2 / antisera	A/Switzerland/9715293/13	A/Hong Kong/4801/14	A/Slovenia/3188/15	A/Singapore/INFIM-16-0019/16
A/Switzerland/ 9715293/13	**64**	64	64	64
A/Hong-Kong/4801/14	64	**128**	64	64
A/Slovenia/3188/15	32	32	**32**	32
A/Singapore/INFIM-16-0019/16	32	32	32	**32**

<table>
<tr><th>c. B / antisera</th><th>B/Wisconsin/ 1/10</th><th>B/Novosibirsk/ 1/12</th><th>B/Phuket/3073/ 13</th><th>B/Brisbane /60/08</th><th>B/Hong Kong/ 514/11</th><th>B/Johannesburg/ 3964/12</th><th>B/Norway/240 9/17</th></tr>
<tr><td>B/Wisconsin/1/10</td><td>256</td><td>128</td><td>512</td><td colspan="4" rowspan="3"><16</td></tr>
<tr><td>B/Novosibirsk/1/12</td><td>32</td><td>128</td><td>64</td></tr>
<tr><td>B/Phuket/3073/13</td><td>256</td><td>64</td><td>256</td></tr>
<tr><td>B/Brisbane/60/08</td><td colspan="3" rowspan="4"><16</td><td>128</td><td>64</td><td>64</td><td>64</td></tr>
<tr><td>B/Hong Kong/514/11</td><td>32</td><td>32</td><td>32</td><td>64</td></tr>
<tr><td>B/Johannesburg/3964/12</td><td>256</td><td>64</td><td>128</td><td>32</td></tr>
<tr><td>B/Norway/2409/17</td><td><16</td><td><16</td><td><16</td><td>128</td></tr>
</table>

Figure 3. Antigenic tables for the 2017/18 influenza season. These tables correspond to the HI titers of reference influenza strains incubated with 2017/18 ferret reference antisera. 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: 2017/18 influenza vaccine strains. a, b and c correspond to A(H1N1pdm09), A(H3N2) and B influenza virus antigenic tables, respectively. The first line and column of each influenza type/subtype table correspond to the ferret antiserum and virus strain tested, respectively.

Antigenic similarity

A tested 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.

3.4.3. Influenza gene sequencing

A subset of the influenza samples isolated at the NRCI are genetically characterized by sequencing the HA1 part of their HA genes. As HA genes tend to evolve rapidly, comparing HA sequences of the circulating strains with reference sequences, including those from the vaccine strains, allows to evaluate viral diversity.

Viral genomes of samples selected for sequencing are processed as follows. 400 μl of the initial respiratory specimens are extracted using the NucliSens easyMAG magnetic bead system (BioMérieux, Geneva, Switzerland) according to the manufacturer's instructions and viral RNA is recovered in a 50 μl elution volume. After sample screening and subtyping by rRT-PCR, viral genomes of samples with a Ct value <30 are used for the synthesis of cDNA using the SuperScript® II Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) with influenza A/B-specific primers. Strain-specific HA1 cDNAs are further amplified using either a nested PCR for influenza B/HA1 or a first-round PCR with strain-specific primers, followed by two independent hemi-nested PCRs for influenza A(H1N1)pdm09 and A(H3N2) HA1, respectively. The amplified products are then sequenced with strain-specific primers using conventional Sanger sequencing performed with the ABI 3500xL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). A list of primers used for sequencing analysis is presented in Annex 20. Primer sequences and PCR conditions are described in the standard operating procedures of the WHO Collaborating Centre at the National Institute for Medical Research (London, UK). Similar sequencing procedures are applied for NA, M and NS gene sequencing but with gene-specific primers.

HA1, NA, M and NS sequences are edited and stored in the Smartgene ISDN database (SmartGene, Switzerland; www.smartgene.com) and analyzed with the software platform Geneious 6.1.6.[9] The MAFFT v7.017[10] programme is used for sequence alignments and maximum-likelihood trees (Figures 12-15) are estimated using the PhyML programme.[11] Reference sequences used in the phylogenic trees were imported from the Global Initiative on Sharing Avian Influenza Data platform (http://platform.gisaid.org, restricted access).

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.[12]

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

4. 2017/18 Influenza season

The 2017/18 influenza surveillance started on 2 October 2017 (week 40/2017) and ended on 22 April 2018 (week 16/2018). Data published by the FOPH show that the epidemic threshold of 68 ILI identified clinically per 100,000 inhabitants was exceeded from weeks 51/2017 to 13/2018, with a first peak of MC-ILI during week 2/2018 and a second peak on week 4/2018. A “double-peak” influenza epidemic has not been observed since the 2003/04 season. The epidemic phase of 2017/18 influenza season lasted for 15 weeks. While the beginning was comparable to 2016/17 (week 50/2016), the end was four weeks later compared to last year (week 8/2017).

4.1. Sentinella population description

A total of 1292 individuals identified in the community were sampled during the 2017/18 influenza season. Among these four were tested twice throughout the season. Information on sex was available for all individuals and age was provided for 1284 out of 1292. Of the sampled individuals, 683 (52.9%) were female (385 influenza-positive and 298 negative) and 609 (47.1%) were male (358 influenza-positive and 251 negative) (Table 1). Median age of sampled individuals was 38 years (range: 2 months to 94 years, 95% CI [37-41]). Data were missing for eight individuals. Females had a median age of 41 years (range: 2 months to 94 years, 95% CI [34-40]) and males of 37 years (range: 6 months to 94 years old, 95% CI [38-43]). Sampled individuals were further stratified into age groups as defined by the FOPH i.e. 0-4 years; 5-14 years; 15-29 years; 30-64 years; and ≥ 65 years. Six hundred and fifty-two (50.8%) individuals belonged to the 30-64 years’ group, 222 (17.3 %) to the 15-29 years, 193 (15%) to the 5-14 years, 136 (10.6%) to the ≥65 years and 81 (6.3%) to the 0-4 years’ group (Table 1).

Concerning the reporting of symptoms, cough was present in 82.3% of patients, followed by abrupt disease onset (72.8%), fever <38°C (69.8%), headache (69.2%), myalgia (65.4%), temperature 37-38°C (24.8%) and pneumonia (2.7%). Other symptoms included rhinitis, diarrhoea, nausea with(out) vomiting and conjunctivitis. (Table 1).

Most individuals were sampled 3 days (median) after disease onset (range: 1-28; 2.96 geometric mean 95% [2.87,3.06] days) (Table 1).

Information on vaccination status was provided for 1261 (97.6%) of the 1292 sampled individuals (Table1). Among these, 133 (10.5%) Sentinella patients received the 201/18 influenza vaccine and 1128 (89.5) were not vaccinated (Table 1).No vaccination data were available for 31 (2.4%) patients. In Switzerland, vaccination is recommended for some specific populations, such as elderly patients (≥65 years), pregnant women and individuals suffering from several chronic diseases. During the 2017/18 season, the NRCI received samples from 136 elderly patients. Of these 49 (36%) were vaccinated. None of the sampled females was pregnant. Twenty-four patients were identified as chronically ill (e.g. diabetes, asthma, human immunodeficiency virus). Eight were vaccinated against influenza

Data on patient treatment with an influenza-specific antiviral drug were not consistently reported. However according to the data provided, none of the Sentinella individuals was treated with antiviral drugs, such as adamantanes and neuraminidase inhibitors.

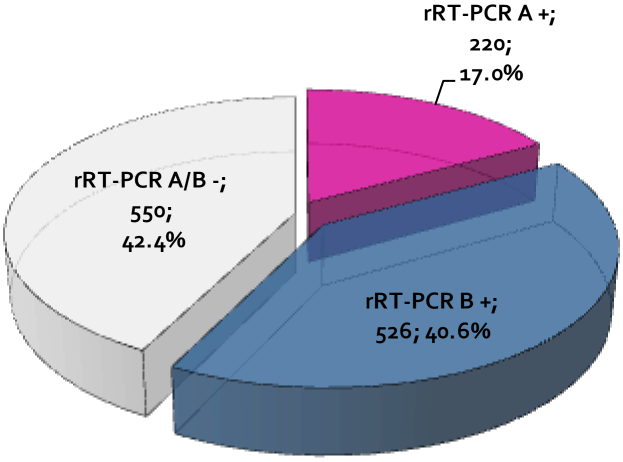
Table 1. Description of the subgroup of the Sentinella population whose samples were submitted to laboratory confirmation for influenza

Laboratory-tested influenza population *(samples)*				
	Influenza A-positive	**Influenza B-positive**	**Negative for influenza**	**Total**
Sex				
Female	112 *(113)*	273 *(275)*	298 *(298)*	683 *(686)*
Male	107 *(107)*	251 *(251)*	251 *(252)*	609 *(610)*
Total	219 *(220)*	524 *(526)*	549 *(550)*	1292 *(1296)*
Proportion	*17%*	*40.5%*	*42.5%*	
Age group distribution (y)*				
0-4	28	12	41	81
5-14	37	97	59 (60)	193 (194)
15-29	32	71	119	222
30-64	108 (109)	280 (282)	264	652 (655)
≥65	14	58	64	136
**Age was missing for 8 individuals.*				
Reported symptoms per sampling event	Proportion of n=220 (%)	Proportion of n=526 (%)	Proportion of n=550 (%)	Proportion of n=1296 (%)
Abrupt onset	165 (75)	391 (74.3)	387 (70.4)	943 (72.8)
Fever >38°C	181 (82.3)	388 (73.8)	335 (60.9)	904 (69.8)
37-38°C	31 (14.1)	110 (20.9)	180 (32.7%	321 (24.8)
Myalgia	144 (65.5)	350 (66.5)	353 (64.2)	847 (65.4)
Headache	150 (68.2)	368 (70)	379 (68.9)	897 (69.2)
Cough	190 (86.4)	459 (87.3)	417 (75.8)	1066 (82.3)
Pneumonia	6 (2.7)	9 (1.7)	20 (3.6)	35 (2.7)
Time to disease onset geometric mean, range (days)	2.68 (1-28)	3.07 (1-14)	2.98 (1-18)	2.96 (1-28)
Vaccination status n=1292				
Vaccinated	16	44	73	133
Non-vaccinated	200	469	459	1128
Vaccination status unknown	4	13	14	31

4.2. Detection of influenza in nasopharyngeal samples

A total of 1296 samples were screened for influenza at the NRCI during the 2017/18 season. Overall, 746 (57.6%) were positive for influenza by rRT-PCR (Figure 4a, Annex 1). Among these, 526 were of type B (70.5%) and 220 (29.5%) of type A. Four hundred and ninety (93.2%) of 526 influenza B belonged to the B/Yamagata/16/88 lineage, 7 (1.3%) were B/Victoria/2/87-like viruses, and 29 (5.5%) could not be attributed to a specific lineage. Concerning the influenza A viruses, 174 of 220 (79.1%) were A(H1N1)pdm09 viruses, 39/220 (17.7%) were A(H3N2), and 7/220 (3.2%) could not be further characterized due to a low viral load (Figure 4b).

a.



b.

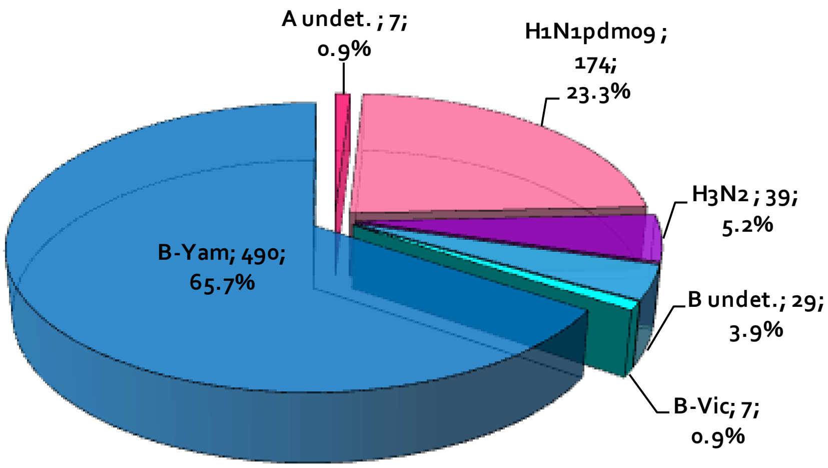


Figure 4. Distribution of influenza viruses detected in nasopharyngeal specimens collected during the 2017/18 season. A) Percentage of rRT-PCR A and B-positive (+) versus rRT-PCR-negative (-) specimens (n=1296). B) Distribution of the different subtypes (influenza A) and lineages (influenza B) of the viruses in % (n=746). All positive samples are submitted to subtyping. A undet.: subtype not determined (negative subtyping). B undet.: lineage not determined (negative subtyping). B-Yam: B/Yamagata/16/88-like. B-Vic: B/Victoria/2/87-like.

The number of influenza-positive samples processed started to increase at week 50/2017 and peaked at week 2/2017 (n=141; 63.8% positivity). The positivity rate remained above 50% from weeks 51/2017 to 12/2018 (Figure 5). B Yamagata viruses were predominant until week 8/2018 and then co-circulated with influenza A strains, mainly A(H1N1)pdm09. Some A(H3N2) viruses and a few sporadic cases of B Victoria were also observed (Figure 5).

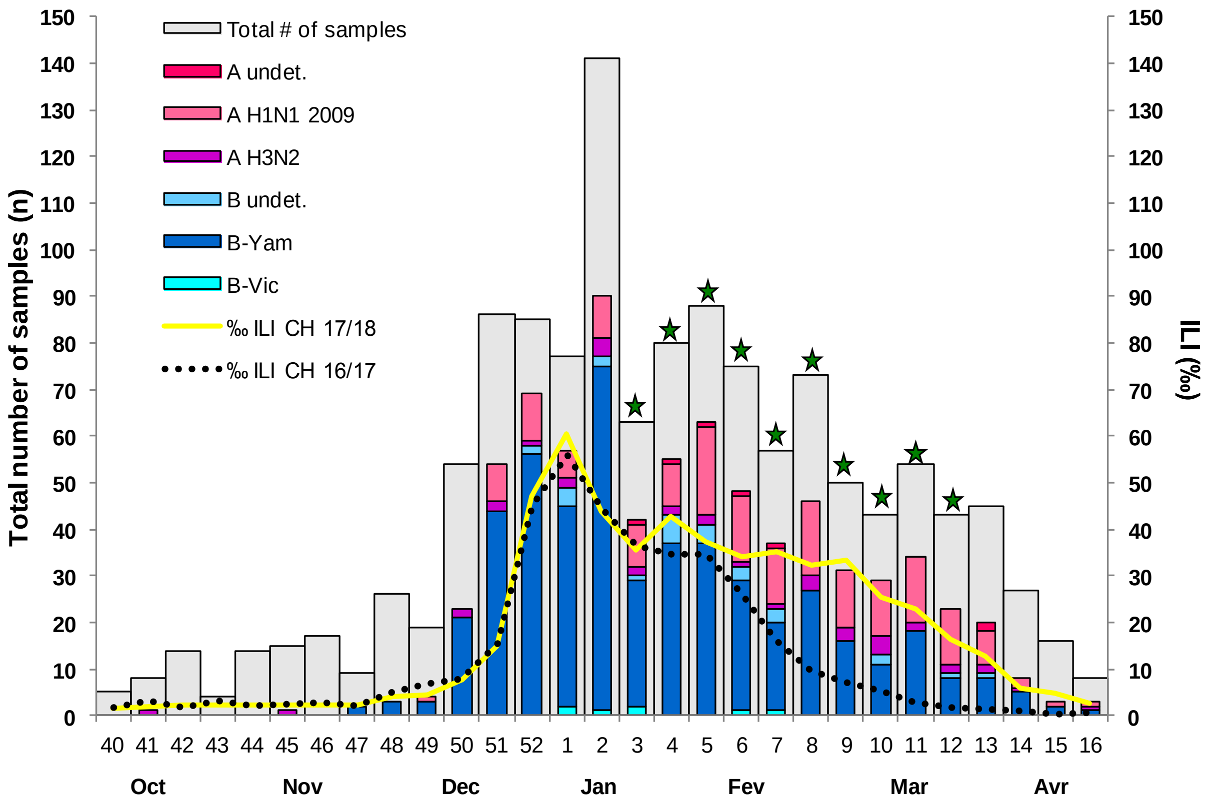


Figure 5. Schematic illustration of the 2017/18 influenza season. A undet.: influenza A, but the type could not be determined; A H1N1 2009: influenza A(H1N1)pdm09; A H3N2 seasonal: influenza A(H3N2) viruses; B undet.: influenza B, but the type could not be determined; B-Yam: influenza B of Yamagata lineage; B-Vic: influenza B of Victoria lineage; ILI 17/18 and 16/17: ILI suspected cases registered during the 2017/18 and 2016/17 season (‰);green stars (sampling period): indicate the weeks when Sentinella practitioners sent 1 of 5 samples for influenza screening (weeks 3 to 12/2018).

Influenza outbreak summary

Duration of the epidemic phase	: **15 of 29 weeks**
Total number of samples	: **1296** *(1292 individuals)*
Percentage of positive samples	: **57.6%** (n=746)
	: **70.5% influenza B** (93.2%, B Yamagata)
	: **29.5% influenza A** (79.1%, A(H1N1)pdm09)

4.3. Epidemiology of influenza viruses detected by the Sentinella network

4.3.1. Stratification by sex and age

Influenza-positive and -negative samples were first analyzed according to the sex and age of the "source" individuals". The highest prevalence of positive samples (69%) was observed in the age group 5-14 years, followed by the 30-64 years (60%) (Figure 6). The groups ≥65, 0-4 and 15-29 years groups exhibited similar positivity rates (53%, 49% and 46%, respectively). Eight samples (6 positive and 2 negative) could not be attributed to an age group.

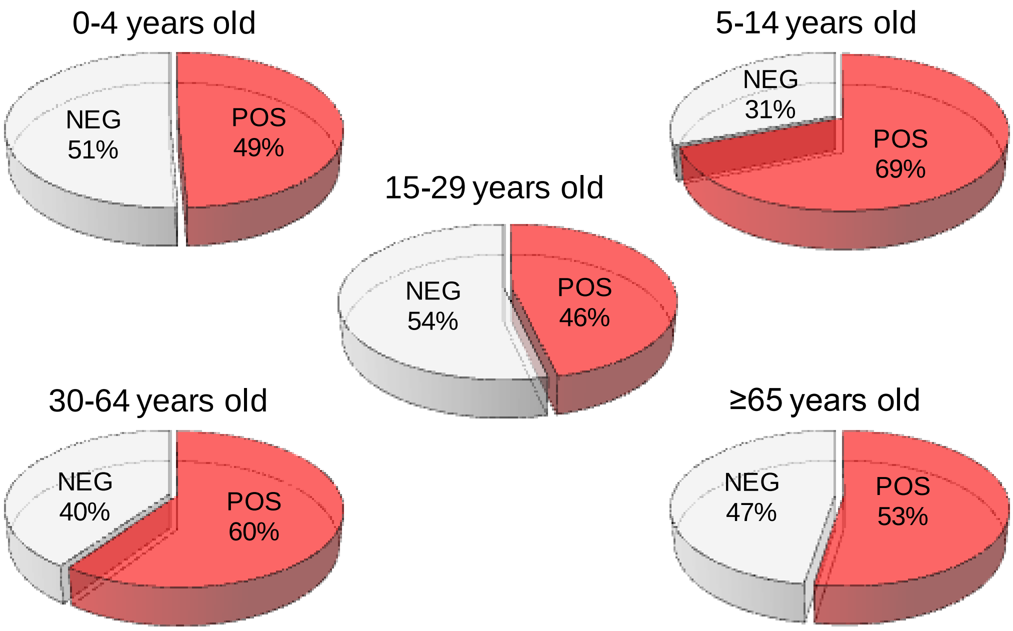


Figure 6. Influenza prevalence per age group. 0-4 years: n=81, 5-14 years: n=194, 15-29 years: n=222, 30-64 years: n=655 and ≥65 years: n =136.

Figure 7 illustrates the proportions of the total influenza-positive and –negative samples according to each age group.

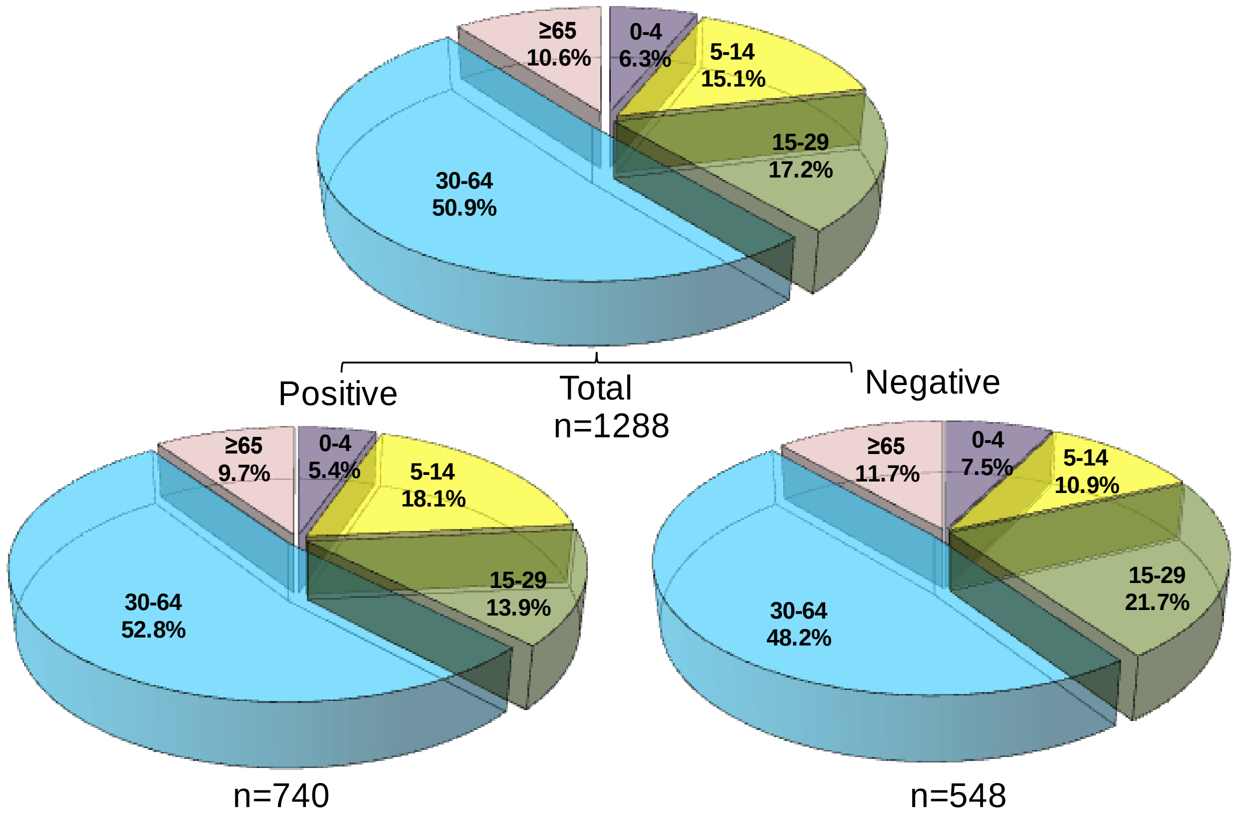


Figure 7. Proportions of the total influenza-positive and -negative samples according to each age group.

B Yamagata viruses were dominant across almost all age groups: 22.5% for 0-4 years (n=40); 70.1% for 5-14 years (n=134); 60.2% for 15-29 years (n=103); 67.3% for 30-64 years (n=391); and 77.8% for ≥65 years old group (n=72). Influenza A(H1N1)pdm09 strains were detected to a lower extent in all age groups (≥65: 15.3%; 15-29: 17.5%; 5-14: 22.4% and 30-64: 22.5%), except in the 0-4 years group where they represented the majority of the detected viruses (67.5%). Some A(H3N2) strains also were also indentified across all age groups (0-4: 2.5%; ≥65: 4.2%; 30-64: 4.3%; 5-14: 4.5% and 15-29: 11.5%). B Victoria viruses (n=7) were only sporadically detected and not in all age groups (Figure 8).

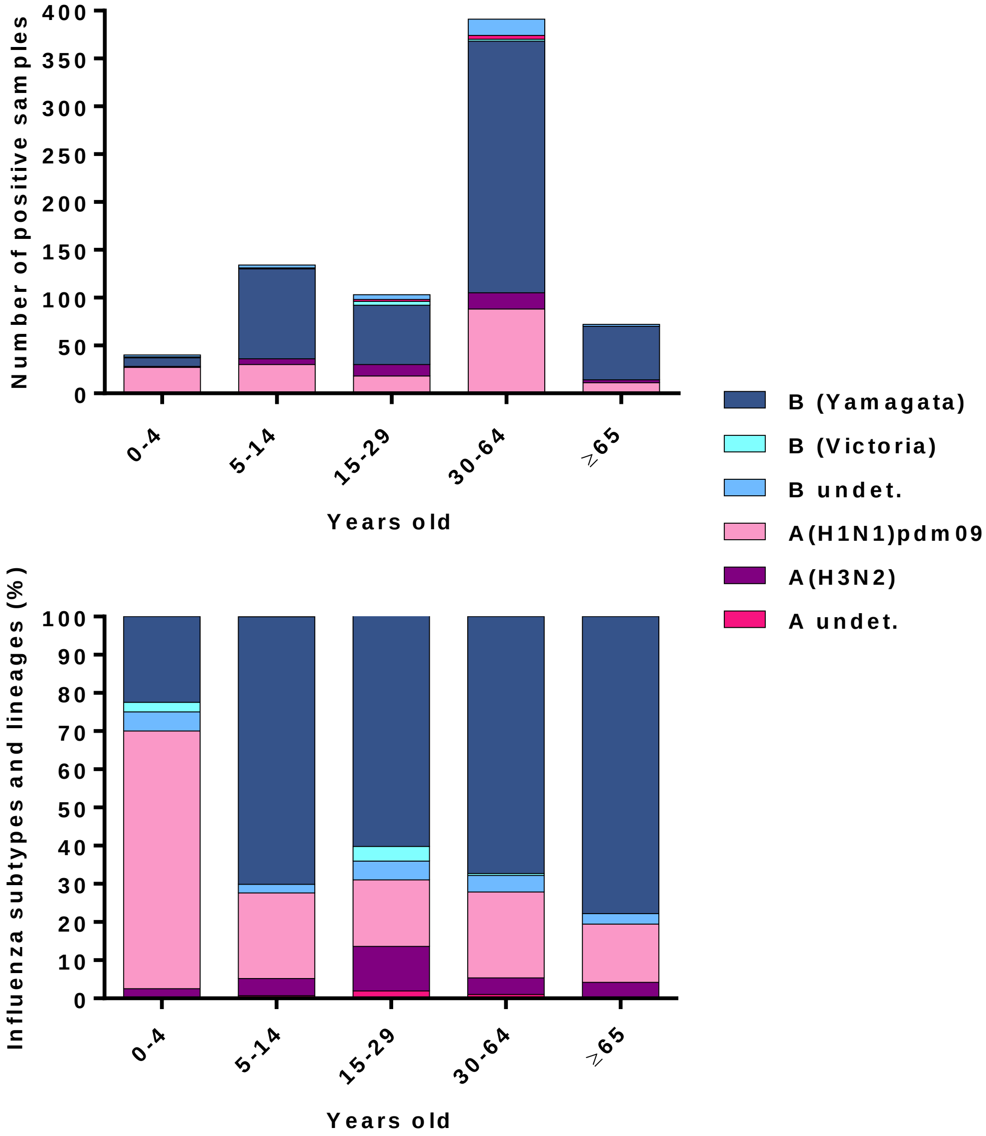


Figure 8. Distribution of influenza virus subtypes/lineages per age group. Upper panel: number of positive samples per subtype per age group. Lower panel: subtypes/lineages proportions per age group (%). B Vic = B Victoria; B Yam = B Yamagata. Undet.= not able to be subtyped.

4.3.2. Stratification by influenza vaccination status

As mentioned previously, vaccination status was available for 1261 individuals; 133 samples originated from vaccinated individuals. Sixty (45.1%) of 133 were positive and 73 (54.9%) were negative for influenza. Among the 60 positive samples, 44 that were infected with an influenza B (73.3%; 42 Yamagata; 2 not subtyped) and 16 with an A strain (26.7%; 10 A(H1N1)pdm09, 5 A(H3N2); and 1 not subtyped). Of note, 19 (37.7%; influenza B, 17; influenza A, 2) belonged to the ≥65 years' group. Five of eight samples collected from chronically-ill vaccinated patients were positive for influenza B Yamagata.

4.4. Antigenic and genetic characterization of influenza viruses

One hundred and fifty-one influenza-positive samples were cultured on MDCK and MDCK-SIAT cells. Among these, 141 grew on MDCK and/or MDCK-SIAT cells and were submitted to antigenic characterization by HI. Apart from one with insufficient HA activity, all isolates were successfully subtyped (Figure 9; Annexes 2 to 5).

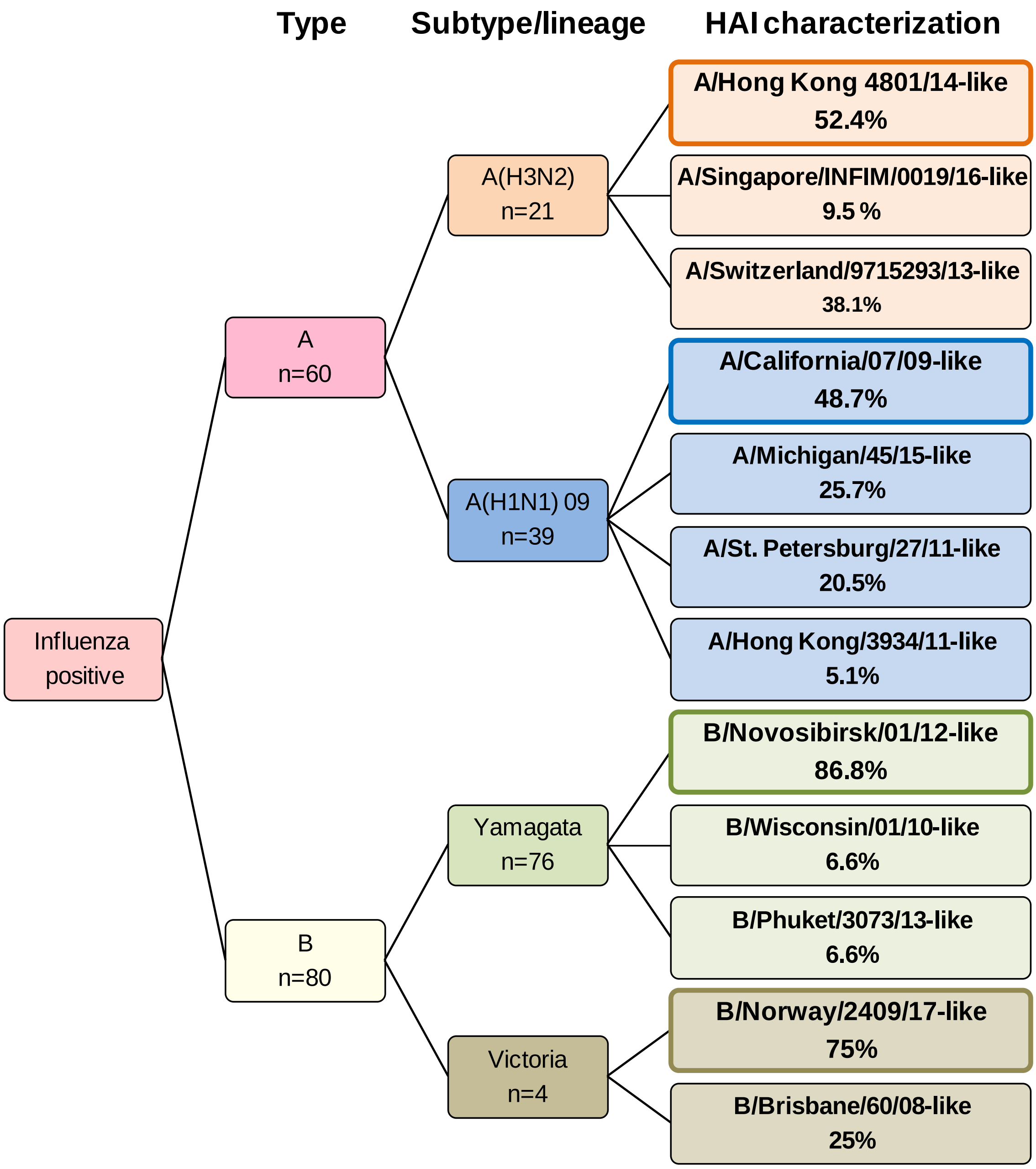


Figure 9. Antigenic characterization by HI of selected influenza viruses isolated through the 2017/18 season (n=140 culture positive samples).

One hundred and thirty-five samples were submitted for genetic characterization by HA and NA gene sequencing. Ten M and 12NS genes were also sequenced. One hundred and twenty-six HA sequences were successfully recovered. Among these, 73 were B Yamagata, four B Victoria, 33 A(H1N1)pdm09 and 16 A(H3N2) subtypes/lineages (Figure 10).

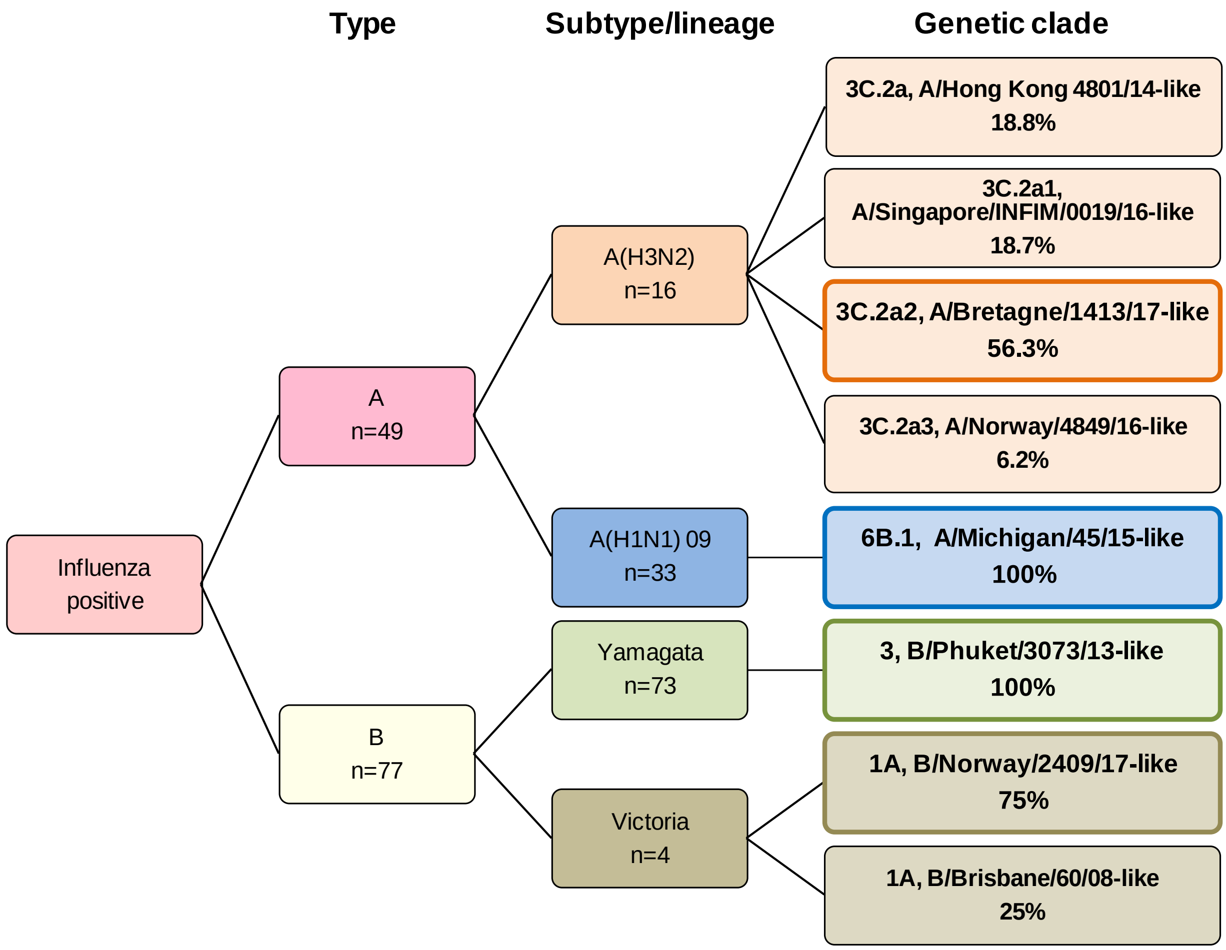


Figure 10. Genetic characterization of selected influenza viruses isolated through the 2017/18 season by the sequencing the HA1 (n=126 positive influenza samples).

One hundred and twenty-four NA were successfully sequenced: B Yamagata, 69; B Victoria, 3; A(H1N1)pdm09, 36; and A(H3N2), 16. All of the 10M sequences (5 A(H3N2); 5 A(H1N1)pdm09) and 12 B NS (B Yamagata, 9; B Victoria, 3) sequences were recovered successfully. Of note, no significant changes were observed in the sequenced portions of the M and NS genes. The few sequence-primers/probe mismatches observed are unlikely to have a significant impact on the rRT-PCR screening sensitivity to circulating strains.

Ninety-four samples (B, 54; A(H1N1)pdm09, 28; A(H3N2), 12 were shared with the WIC for additional characterization (four independent dispatches: November 2017, January 3 and 22, and May 5 2018). The results of the phenotypic characterization performed in London are available in Annexes 6 to 9 for HI/MN; in Annexes 10 to 17 for phylogenic analysis; and in Annexes 18a and 18b for antiviral resistance. Of note, the results for the last dispatch are not yet available.

4.4.1. Characterization of influenza A(H1N1)pdm09

Of 60 influenza A isolates, 39 A(H1N1)pdm09 strains were successfully characterized by HI (Figure 9; Annexes 2a to 2c). Nineteen isolates were identified as A/California/7/2009-like, 10 as A/Michigan/45/2015-like, eight as A/Saint-Petersburg/27/11-like, and two as A/Hong-Kong/3934/11-like (Figure 9; Annexes 2a to 2c). All viruses were recognized by the antiserum directed against the currently used vaccine strain A/Michigan/45/2015 at two-, equal to or four-fold the homologous titer. This was also the case with the antiserum raised against the former vaccine strain A/California/7/2009; apart from five viruses that exhibited titers that were eight-fold higher than the homologous titer (Annexes 2a to 2c). Our results matched with the 14 samples analysed by the WIC for (Annexes 6a to 6c).

A sequence analysis was successfully performed for 33 of 39 HA1 genes of randomly selected A(H1N1)pdm09 viruses. It showed that all isolates belonged to clade 6B.1 with additional mutations S74R, S164T and I295V when compared to A/Michigan/45/2015 (Figure 11). TheS164T mutation was notified as affecting the glycosylation motif at residues 162-164 in the HA1. Other mutations were observed in more than six isolates including: T120A, S183P and N260D (Figure 11).

The 36 NA sequences that were successfully recovered clustered similarly to the HA1 genes. All belonged to the clade 6B.1 with the additional mutations G77R, V81A and N449D. Some isolates had also other specific change, such as V62I, T72I and N222D. The results we obtained were concordant with the 13 samples analyzed by the WIC (Annexes 10 & 11).

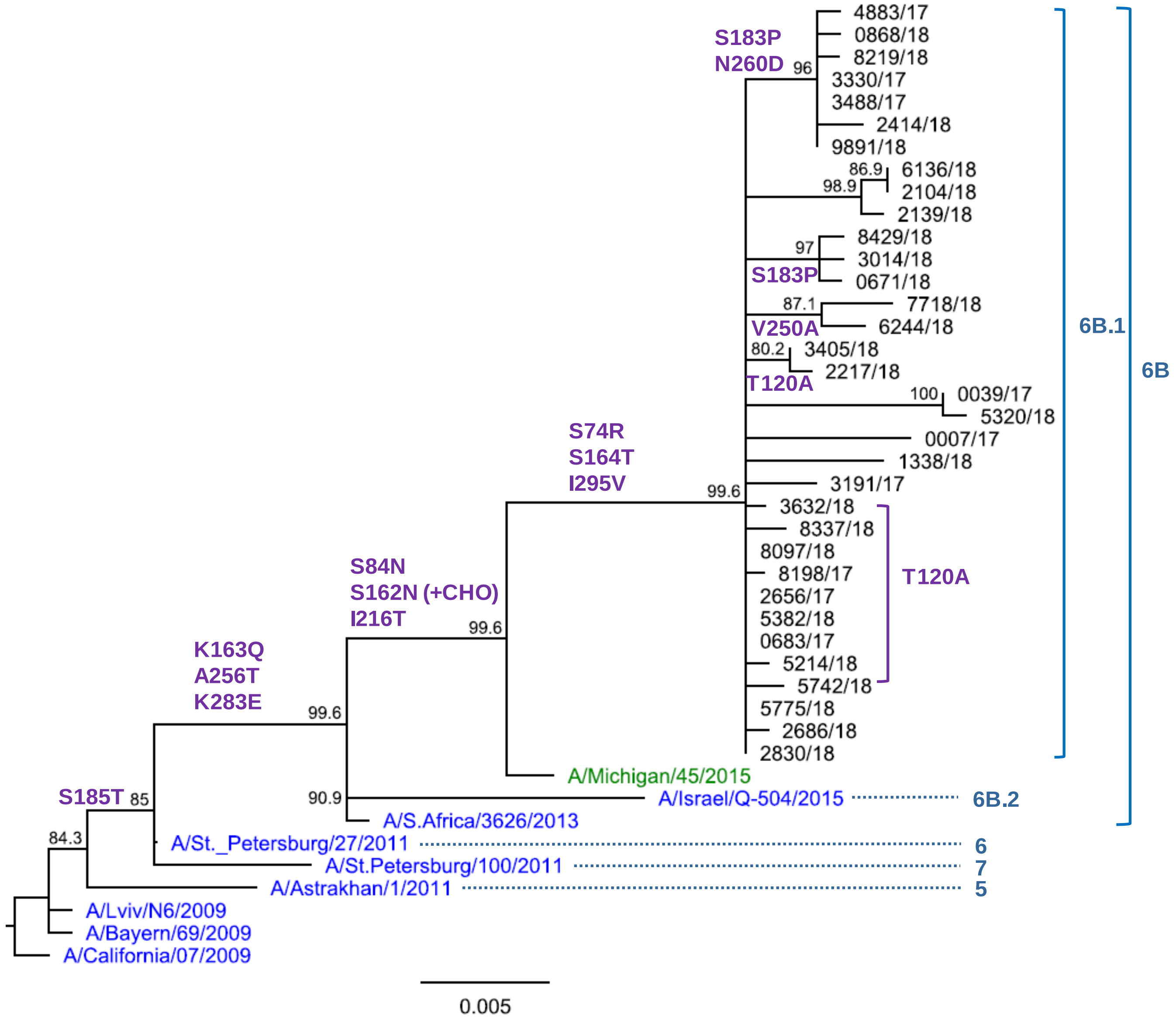


Figure 11. Phylogenetic analysis of the HA1 gene of A(H1N1)pdm09 viruses. Black: influenza virus detected in the Sentinella network during the 2017/18 season; strain names are A/Switzerland/in tree number (subtype) (e.g. A/Switzerland/5214/2018(H1N1)pdm09). Green: 2017/18 vaccine strain. Blue: reference strains. 6B, 6B.1 and 6B.2: A(H1N1)pdm09 genetic clades and subclades. Purple: typical mutations described by the WIC and/or observed at the NRCI. Sequences were aligned using Geneious 6.1.8 MAFFT alignment (v7.017) with default settings. A consensus tree was built from 1000 original trees in ML (80% support threshold) constructed using Geneious 6.1.8 PHYML default settings.

Comments from the WIC

“The main characteristics of viruses in the 6B.1 group are the carriage of the amino acid substitutions S84N, S162N (introducing a new potential glycosylation site) and I216T in HA1, e.g., A/Slovenia/2903/2015. A(H1N1)pdm09 viruses, notably in group 6B.1, have been causing problems in many parts of the world during the 2015/2016 Northern hemisphere influenza season. Within this subgroup, a new cluster, defined by the HA1 amino acid substitutions S74R, S164T has emerged, which alters the glycosylation motif at residues 162 to 164) and I295V.”

A(H1N1)pdm09 viruses

All A(H1N1)pdm09 viruses isolated during the 2017/18 influenza season were antigenically and genetically similar to the vaccine strain A/Michigan/45/2015.

4.4.2. Characterization of influenza A(H3N2)

Similar to the previous influenza seasons, WIC reported that antigenic characterization of A(H3N2) viruses by HI was difficult due to a variable agglutination of RBC from guinea pig, turkey and humans and the NA-mediated agglutination of RBC. This phenomenon was particularly observed for viruses belonging to the 3C.2a clade and related subclades. Nevertheless, in contrast to last season, we did not have any problems to characterize our A(H3N2) isolates by HI. Thus we did not use MN (or plaque reduction neutralization) assays during the 2017/18 season.

Twenty one viruses were submitted to the HI assay and all could be characterized (Figure 9). Eleven were identified as A/Hong Kong/4801/2014-like, the strain included in the 2017/18 vaccine, eight as A/Switzerland/9715293/2013-like and two as A/Singapore/INFIMH-16-0019/2016-like strains. In the WHO recommendation for the 2018 Southern and 2018/19 Northern influenza seasons, A/Hong Kong/4801/2014 is replaced by A/Singapore/INFIMH-16-0019/2016 as a vaccine component. All viruses were recognized by the antisera targeting A/Hong Kong/4801/2014. Nine had titers two-fold lower than the homologous titer and A/Switzerland/4325/2017 showed a titer that was four-fold lower than the homologous titer. The A/Switzerland/3052/2018, A/Switzerland/1515/2018 and A/Switzerland/8327/2018 isolates were recognized by the antiserum raised against A/Hong Kong/4801/2014 at two and four-fold higher the homologous titer. Almost all isolates were recognized at one, two- or four-fold the homologous titer by A /Singapore/INFIMH-16-0019/2016 antiserum. Interestingly, A/Switzerland/8327/2018 exhibited a titer that was eight-fold higher than the A/Singapore/INFIMH-16-0019/2016 homologous titer. Of note, as some antisera, including that targeting A/Singapore/INFIMH-16-0019/2016, were not available at the NRCI at the beginning of the 2017/18 season, the A/Switzerland/0882/2017 (A/Switzerland/882/2017 at the WIC) and A/Switzerland/2159/2017 isolates were not tested with the antiserum raised against A/Singapore/INFIMH-16-0019/2016.

Of the nine A(H3N2) viruses sent to the WIC, eight viruses could be recovered but only two were analyzed by HI as the others failed to agglutinate RBCs. A/Switzerland/882/2017 (A/Switzerland/0882/2017) was poorly recognized by the antisera raised against either egg-propagated A/Hong Kong/4801/2014 or A/Singapore/INFIMH/16-0019/2016. This virus was better recognized by antisera raised against reference viruses propagated in cell culture (Annex 3a). The

A/Switzerland/143/2017 isolate was poorly recognized by the antiserum raised against egg-propagated A/Hong Kong/4801/2014 and it was recognized at a titer eight-fold lower than the homologous titer by an antiserum raised against egg-propagated A/Singapore/INFIMH-16-0019/2016. Similar to A/Switzerland/0882/2017, A/Switzerland/143/2017 was generally well recognized by antisera raised against cell culture-propagated viruses, such as A/Hong Kong/5738/2014 and A/Bretagne/1413/2017 (Annex 3b).

At the genetic level, 16 A(H3N2) HA1 and NA sequences were successfully recovered. All viruses clustered in subclades 3C.2a1, in particular 3C.2a1b, 3C.2a2 and 3C.2a3 of the 3C.2a clade defined by substitutions L3I, N128T (resulting in the gain of a potential glycosylation site [+CHO]), N144S (resulting in the loss of a potential glycosylation site [-CHO]), N145S, F159Y, K160T ([+CHO] at residue 158), P198S, F219S, N225D and Q311H in HA1. Most A(H3N2) isolates belonged to the subclade 3C.2a2 characterized by the additional substitutions T131K, R142K and R261Q in HA1. Three viruses clustered in subclade 3C.2a1b defined by the additional N171K (3C.2a1) and K92R mutations in HA, but bear Q311 instead of K311 residue. Two of 3 viruses, A/Switzerland/7099/2018 and A/Switzerland/1511/2018 had the substitution T135K (-CHO) in the HA1, which is typical of viruses of subclade 3C.2a1a. The A/Switzerland/1515/2018 isolate was the only one to fall in subclade 3C.2a3 characterized by substitutions of clade 3C.2a plus N121K and S144K in HA1. We could also observe the T135K [-CHO] and R150K substitutions in this virus (Figure 12). The NA (data not shown) and HA1 genes clustered similarly.

Almost all samples sent to the WIC fell into the genetic subclade 3C2a2, except for A/Switzerland/431/2017 that fell into clade 3C2a4 (Figure 11). The HA gene of A/Switzerland/882/2017 has polymorphism at residue 158 of HA1 (N158N/H), affecting the glycosylation associated with reduced agglutination of RBCs, and this probably accounts for its ability to agglutinate the RBCs. The NA genes clustered similarly (Annex 13).

Overall, the genetic characterization and subsequent cluster/sub-cluster attribution for the different Swiss A(H3N2) isolates analyzed at both sites was concordant in-between the WIC and NRCI (Figure 12; Annexes 12 and 13)

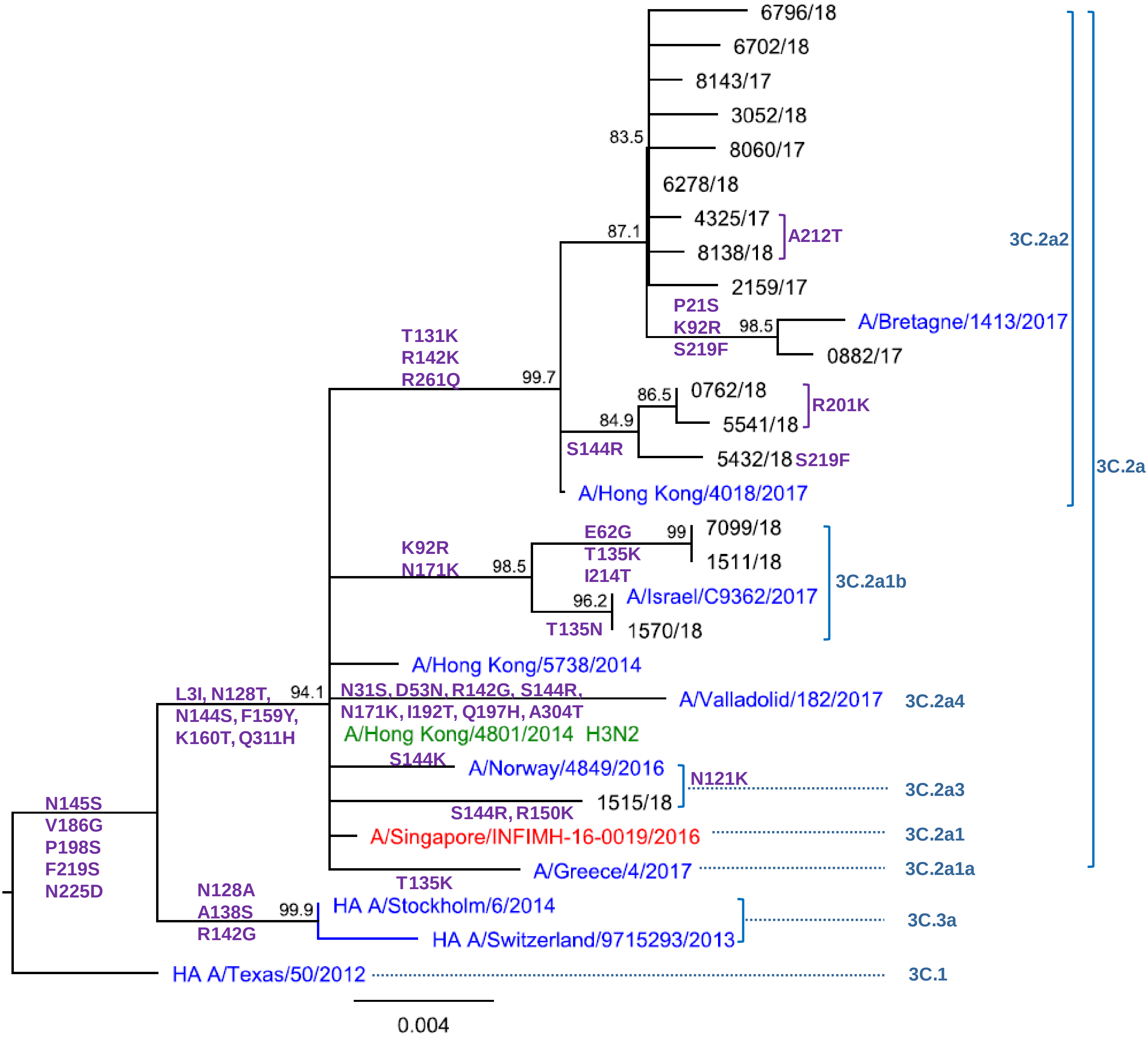


Figure 12. Phylogenetic analysis of the HA1 gene of A(H3N2) viruses. Black: influenza virus detected in the Sentinella network during the 2017/18 season; strain names are A/Switzerland/in tree number (subtype) (e.g. A/Switzerland/1515/2018(H3N2)). Green: 2017/18 vaccine strain. Red: 2018/19 vaccine strain. Blue: reference strains. 3C.1, 3C.2, 3C.2a, 3C.2a1, 3C.2a1a and b, 3C.2a2, 3C.2a3, 3C.2a4 and 3C.3a correspond to A(H3N2) viruses genetic clades and subclades. Purple: some typical mutations described by the WIC and/or observed at the NRCI. Sequences were aligned using Geneious 6.1.8 MAFFT alignment (v7.017) with default settings. A consensus tree was built from 1000 original trees in ML (80% support threshold) constructed using Geneious 6.1.8 PHYML default settings.

Comments from the WIC

*“*The HA genes of the circulating A(H3N2) viruses have fallen in recent years within subclades 3C.3a, 3C.2a and 3C.2a1 but the genetic diversity of the HA genes has increased and new subclades and genetic groups (3C.2a1a, 3C.2a1b, 3C.2a2, 3C.2a3 and 3C.2a4) have been defined. All share a N225D substitution in HA1. Of note, a difference in the NA gene for the 3C.2a2 viruses has been seen recently with many viruses with a 3C.2a4 HA having a 3C.2a1 NA.”

A(H3N2) viruses

All of the A(H3N2) viruses isolated in Switzerland were antigenically related to the A/Hong-Kong/4801/2014 (genetic clade 3C.2a) and A/Singapore/INFIMH-16-0019/2016 (genetic subclade 3C.2a1 of clade 3C.2a) strains.

At the genetic level, most Sentinella A(H3N2) isolates belonged to the subclade 3C.2a2 of clade 3C.2a.

For the 2018 Southern and 2018/19 Northern hemisphere influenza seasons, A/Hong Kong/4801/2014 is replaced by A/Singapore/INFIMH-16-0019/2016 as a vaccine component.

4.4.3. Characterization of influenza B viruses

4.4.3.1. B/Yamagata/16/1988-like viruses:

As already mentioned, B Yamagata viruses were dominant this season (65.7% of positive samples). Seventy-six isolates were characterized by HI. Sixty-six were identified as B/Novosibirsk701/2012-like, 5 as B/Wisconsin/01/2010-like and 5 as B/Phuket/3073/2013-like, the current vaccine strain (Figure 9). Surprisingly, we observed a poor (eight-fold) and variable recognition of 59% (45 out of 76) of our B Yamagata isolates by the antisera raised against B/Phuket/3073/2013 and B/Wisconsin/01/2010 strains received specifically for the 2017/18 season (Annexes 4a to 4e). Interestingly, when we tested the same isolates with the B/Phuket/3073/2013 antiserum used during the 2016/17 season the titers were higher and at two and four-fold the homologous titer (Annexes 4a to 4e, grey values). As we had a limited amount of 2016/17 B/Phuket/3073/2013 antiserum, we only tested a random selection of isolates that reacted poorly with the 2017/18 equivalent antiserum. Nevertheless, 31 viruses were well recognized by 2017/18 B/Phuket/3073/2013 antiserum at equal (n=2), two- (n=6) and four- (n=23)-fold the homologous titer. Apart from A/Switzerland/0873/2018 (Annex 4c) and A/Switzerland/8278/2018 isolates (Annex 4e), all viruses were recognized at equal, two and four-fold the homologous titer by B/Novosibirsk/1/2012 antiserum.

Among the B Yamagata samples sent to the WIC, 40 were recovered and analyzed. Similar to the NRCI, the WIC generally observed a low and variable reactivity of our isolates with the chosen reference antisera. Indeed, their report mentioned that in the HI analysis done on 06.03.18, the viruses were generally well recognized by the panel of antisera raised against egg and/or cell culture-propagated reference viruses in clade 3 (e.g. B/Wisconsin/1/2010 and B/Phuket/3073/2013) or clade 2 (e.g., B/Massachusetts/2/2012), but somewhat less well on the assay done on 20.02.18 (Annexes 8a to 8d).

Seventy-six HA1 and 69 NA sequences were successfully recovered among the 76 B Yamagata isolates tested. According to both the HA1 (Figure 13) and NA (data not shown) genes, all our B Yamagata viruses belonged to clade 3, the B/Wisconsin/1/2010 and B/Phuket/3073/2013 clade. The HA gene was similar to other viruses by having the substitutions L172Q and M251V that differentiate it from the vaccine virus B/Phuket/3073/2013. Two distinct clusters of viruses could be observed one characterized by mutations P31Q and K253S, and a second that had a mixed distribution of mutations (e.g., D232N [8633/18, 5105/18, 8252/18, 6985/18 and 9428/18], V15I [3422/18 and 5675/18], T75I and I150V [1430/18], and G255R [7032/17], Figure 13). B/Switzerland/5203/2018 exhibited three mutations (V73M, Q122K and T181A) that had all been observed in other parts of the world (Annex 14). Interestingly, the NA sequences of recent viruses also differed at several amino acids positions compared with B/Phuket/3073/2013 (e.g., I49M, R65H, L74P, I717M, D342K, A395S, S402P). Substitution D342K in the NA was present in all viruses isolated at the NRCI, as well as substitutions I49M and I171M in the vast majority. Results of the genetic analysis performed by the WIC (Annexes 14 and 15) were fully concordant with those obtained at the NRCI for both HA1 (Figure 13) and NA (data not shown) genes.

B/Yamagata/16/1988 viruses

Depending on the chosen antisera lot, the isolated B Yamagata lineage viruses could either be antigenically close or distant from the 2017/18 vaccine strain, B/Phuket/3073/13.

The exact origin of the high variability of the measured HI titers, observed by the NRCI and the WIC is not yet fully understood.

All viruses clearly belonged to the genetic clade 3, corresponding to the vaccine strain B/Phuket/3073/2013.

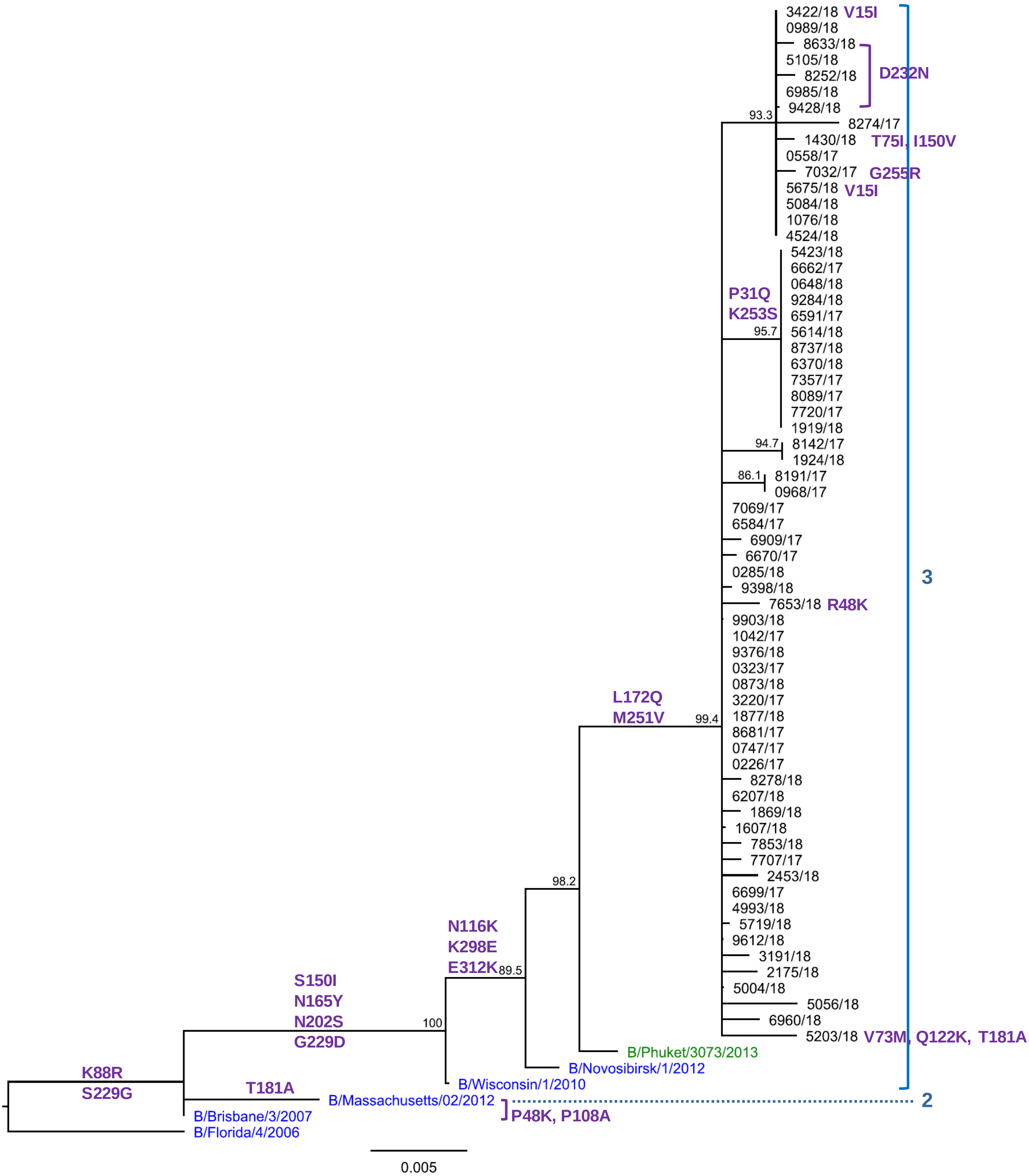


Figure 13. Phylogenetic analysis of the HA1 gene of B Yamagata viruses. Black: influenza virus detected in the Sentinella network during the 2017/18 season. Green: 2017/18 vaccine strain. Blue: selected reference sequences. Two and 3 correspond to the genetic clades of B/Yamagata/16/1988 viruses. Purple: some typical mutations described by the WIC and/or observed at the NRCI. Sequences were aligned using Geneious 6.1.8 MAFFT alignment (v7.017) with default settings. A consensus tree was built from 1000 original trees in ML (80% support threshold) constructed using Geneious 6.1.8 PHYML default settings.

4.4.3.2. *B/Victoria/2/1987-like viruses:*

Only four B Victoria viruses were identified at the NRCI during the 2017/18 season. Three were recognized at two- and four-fold lower than the homologous titer of the B/Brisbane/60/2008, the current vaccine strain. The B/Switzerland/9952/2018 isolate did not react at all with B/Brisbane/60/2008, B/Johannesburg/3964/2012 and B/Hong Kong 514/2009 antisera but it was recognized by an antiserum raised against B/Norway/2409/2017, a newly identified strain known to have a deletion in the HA gene at positions 162 to 163. The four viruses isolated at the NRCI were recognized at two to four-fold the homologous titer by B/Norway/2409/2017 antiserum (Annexe 5). In HI analysis performed at the WIC, the B/Switzerland/952/2017 (corresponds to B/Switzerland/9952/2017) virus was also not recognized by the standard antisera raised against the ”usual” reference viruses, either egg- or cell-cultured, but it was well recognized by an antiserum raised against B/Norway/2409/2017 propagated in cell culture (Annex 9). Interestingly the B/Norway/2409/2017 antiserum lot we had cross-reacted with our cell-propagated B/Brisbane/60/2008, B/Johannesburg/3964/2012 and B/Hong Kong 514/2009 reference viruses, which was not the case for the B/Norway/24092017 antiserum raised against the cell-propagated B/Norway/24092017 virus at the WIC (Annex 9).

Four HA and three NA sequences of B Victoria viruses were successfully recovered. All isolates belonged to the clade 1A as the vaccine strain B/Brisbane/60/2008 (Figure 14). However, consistent with the HI results, B/Switzerland/9952/2018, B/Switzerland/4139/2018 and B/Switzerland/4727/2018 viruses clustered with B/Norway/2409/2017 and B/Colorado/6/2017, two reference strains bearing the 162 to 163 deletions in the HA1 gene. Residues 162 and 163 were present in the B/Switzerland/5355/2018 virus. We did not observe any triple-deleted (162-164) strain represented by the B/Hubei-Jiangan/1974/2017 reference strain (Figure 114). HA1 substitutions I117V and N129D/G, and V146I alone were observed in all four NRCI viruses when compared to B/Brisbane/60/2008; and I180V was present in two of the double-deleted viruses, as well as in the corresponding reference viruses B/Norway/2409/2017 and B/Colorado/6/2017 (Figure 14). The NA genes of these viruses clustered similarly (data not shown).

The genetic analysis of the NRCI and WIC were fully concordant for both HA1 (Figure 14 and Annex 16, respectively) and NA (data not shown and Annex 17, respectively) genes.

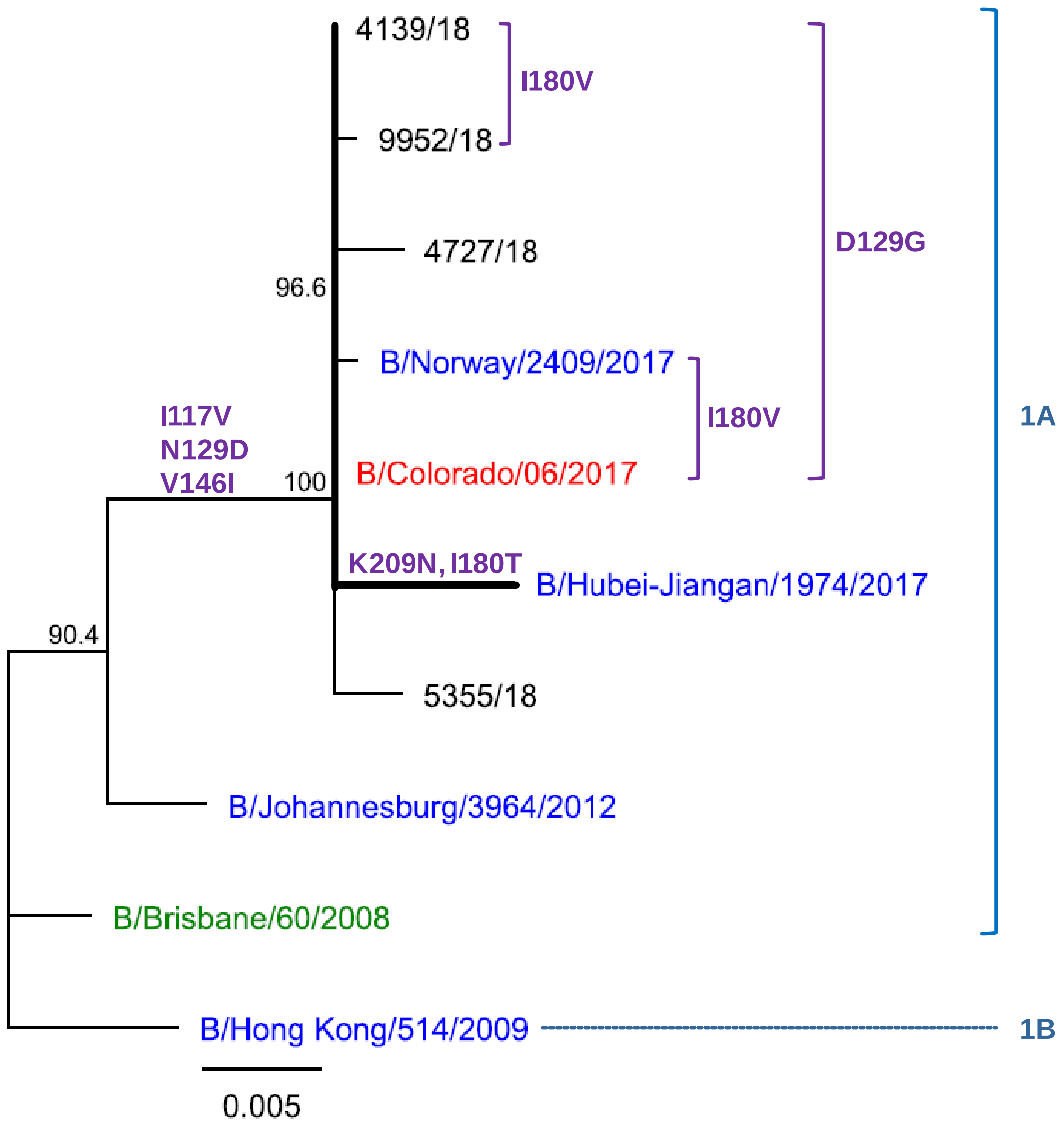


Figure 14. Phylogenetic analysis of the HA1 gene of B Victoria-like viruses. Black: influenza virus detected in the Sentinella network during the 2017/18 season. Green: 2017/18 vaccine strain. Red: 2018/19 vaccine strain. Blue: selected reference sequences. Purple: some typical mutations described by the WIC and/or observed at the NRCI. 1A and 1B: genetic groups (clades) of B/Victoria/2/1987 lineage. Sequences were aligned using Geneious 6.1.8 MAFFT alignment (v7.017) with default settings. A consensus tree was built from 1000 original trees in ML (80% support threshold) constructed using Geneious 6.1.8 PHYML default settings.

B/Victoria/2/87 viruses

During this season, we observed the emergence of a recent B/Victoria/2/1987 subgroup within clade A1-containing viruses (deletion 162-163) that are or can be antigenically distinct from B/Brisbane/60/2008 depending on the antisera lot used.

4.5. Antiviral resistance

4.5.1. Sentinella isolates

One hundred and thirty-five influenza viruses were submitted to NA gene sequencing analysis to assess the antiviral resistance of circulating strains. Among the 69 of 76 B/Yamagata, 36 of 39 A(H1N1)pdm09, 16 of 16 A(H3N2) and three of four B/Victoria NA sequences successfully recovered, none had the common strain-specific mutations associated with reduced inhibition by NAIs (oseltamivir and zanamivir).

Of the 64 virus isolates (41 B, 15 H1N1pdm09 and eiht H3N2) sent to the WIC 61 had sufficient NA activity to resistance to the inhibitors oseltamivir and zanamivir to be assessed in sialidase inhibition assays (Annex 18 a to 18b). Only the A/Switzerland/882/2017 (H3N2) virus was classified as having reduced inhibition by oseltamivir, the NA gene of which had the substitutions S334R and P386S. At present, the WIC does not have a clear cut explanation of the role of these two substitutions in the NA enzymatic activity as other analyzed viruses exhibiting these two mutations were normally inhibited by oseltamivir. None of the analysed viruses had a reduced inhibition by zanamivir. In contrast to the WIC, we did not observe a reduced inhibition by oseltamivir when testing the A/Switzerland/882/2017 (H3N2) isolate at the NRCI. The assay was performed three times in duplicate.

4.5.2. Non-Sentinella isolates

During the 2017/18 influenza season, the NRCI was asked to test five patient-paired isolates (one pre- and post-treatment sample from each patient) of hospitalized patients, four A(H1N1)pdm09 from the Geneva University Hospitals and one from the Interlaken Regional Spital. These patients were under oseltamivir treatment for a confirmed influenza infection, but did not show an improvement in the clinical signs and/or virus clearance. Among the samples, two A(H1N1)pdm09 isolates showed a highly-reduced inhibition by oseltamivir. Both source patients were immunocompromised. The substitution H275Y in the NA gene, known to be associated with a highly-reduced phenotype, was present in both post-oseltamivir treated isolates. All isolates showed normal inhibition by zanamivir.

NAIs sensitivity

One A(H3N2) isolate of 64 tested in the context of influenza seasonal surveillance exhibited a reduced inhibition by oseltamivir at the WIC. All isolates showed normal inhibition by zanamivir

Two isolates originating from immunocompromised hospitalized patients acquired the **H275Y** substitution, which is known to be associated with highly-reduced inhibition by oseltamivir.

5. WHO recommendation for the composition of influenza virus vaccines for the 2018/19 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.[13]

The A(H3N2) and B Victoria vaccine components will be updated for the next influenza season. The vaccine strains recommended for the 2018/19 Northern hemisphere influenza vaccine by the WHO experts are:

Table 2. Recommended influenza vaccine composition for the 2018/19 influenza season.

	Virus strains
A(H1N1)pdm09	A/Michigan/45/2015-like virus
A(H3N2)	A/Singapore/INFIMH-16-0019/2016-like virus
B/Yamagata/16/1988 lineage	B/Phuket/3073/2013-like virus
B/Victoria/2/1987 lineage	B/Colorado/06/2017-like virus*

**Only B strain included in the trivalent vaccine*

6. Human infection with animal influenza viruses

A(H1N1) and A(H3N2) influenza strains are responsible for the seasonal human influenza outbreaks observed worldwide. However, transmission of influenza viruses of animal origin to humans, notably avian and porcine, can potentially lead to severe epidemics and, in a worst-case scenario, to pandemics. Even if non-human influenza strains appear to require a close contact with infected animals for spread and do not (or at least not efficiently) sustain human-to-human transmission as yet, they can be responsible for confined outbreaks. In addition, recombination events between porcine/avian and human viruses due to concomitant circulation with seasonal influenza A strains could lead to the human adaptation of avian strains. To allow the early identification and rapid containment of new potential animal-to-human transmission events, several countries, including Switzerland, have introduced the regular screening of animals (mainly poultry/wild birds and farm pigs) for the presence of the respective influenza strains.

6.1. Swine-to-human influenza virus transmission 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, Switzerland). 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 who present ILI symptoms. The presence of porcine influenza A viruses in human samples is then assessed using a rRT-PCR specially designed by the CDC[14] to detect influenza A virus of human and animal origin, both avian and porcine. During the 2017/18 influenza season, three samples were sent to the NRCI for swine flu testing. Both samples were negative for human influenza but one was found positive for porcine influenza (Table 4). This last sample was reported to the FOPH according to Swiss regulations. An updated report is provided as Annex 21.

Table 3. Pig breeders influenza rtRT-PCR results.

Sample ID	Age	Sex	Result	Origin	Sender	Sample date
****8182	2.8	F	NEG	Zürich	SUISAG-Zürich	30.08.2017*
******6552**	**48**	**M**	**Porcine IA**	**Berne**	**SUISAG-Sempach**	**28.12.2017**
****6093	38	F	Human IB	Luzern	SUISAG-Sempach	10.02.2018

IA/IB:influenza A/B, NEG: negative. *Analyzed prior to the start of the 2017/18 influenza season.

Of note, influenza A viruses known to be genetically similar to viruses circulating in swine (porcine strains), but isolated from human cases, are identified as “variant” viruses and denoted with a letter “v”, such as A(H3N2)v, A(H1N1)v and A(H1N2)v. Since 2005, the systematic reporting of all human infections with variant viruses is mandatory in the USA. As of June 2018, 468 (434 A(H3N2)v, 21 A(H1N1)v and 13 A(H1N2)v) human cases of variant influenza have been reported within several States.[15]

6.2. Avian influenza A subtypes in humans

At the NRCI, we received a single sample for a suspicion of avian influenza from a German patient (sampled 02.03.2018, male, 49 years old), hospitalized in an intensive care unit in Basel (Switzerland). Unfortunately, we lacked information about the clinical picture, including potential travel to China or exposure to infected poultry.

The sampled was negative for influenza A and B screening by PCR, as well as H5N1-, H7N9- and H3N2v- specific PCRs.

As of June 2018, a total of 860 laboratory-confirmed human cases of A(H5N1), including 454 deaths, have been reported to WHO (Figure 15). Only one case, (unfortunately fatal) been identified since our 2016/17 annual report.

Country	2003-2009*		2010-2014**		2015		2016		2017		2018		Total	
	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths
Azerbaijan	8	5	0	0	0	0	0	0	0	0	0	0	8	5
Bangladesh	1	0	6	1	1	0	0	0	0	0	0	0	8	1
Cambodia	9	7	47	30	0	0	0	0	0	0	0	0	56	37
Canada	0	0	1	1	0	0	0	0	0	0	0	0	1	1
China	38	25	9	5	6	1	0	0	0	0	0	0	53	31
Djibouti	1	0	0	0	0	0	0	0	0	0	0	0	1	0
Egypt	90	27	120	50	136	39	10	3	3	1	0	0	359	120
Indonesia	162	134	35	31	2	2	0	0	1	1	0	0	200	168
Iraq	3	2	0	0	0	0	0	0	0	0	0	0	3	2
Lao People's Democratic Republic	2	2	0	0	0	0	0	0	0	0	0	0	2	2
Myanmar	1	0	0	0	0	0	0	0	0	0	0	0	1	0
Nigeria	1	1	0	0	0	0	0	0	0	0	0	0	1	1
Pakistan	3	1	0	0	0	0	0	0	0	0	0	0	3	1
Thailand	25	17	0	0	0	0	0	0	0	0	0	0	25	17
Turkey	12	4	0	0	0	0	0	0	0	0	0	0	12	4
Viet Nam	112	57	15	7	0	0	0	0	0	0	0	0	127	64
Total	**468**	**282**	**233**	**125**	**145**	**42**	**10**	**3**	**4**	**2**	0	0	**860**	**454**

* 2003-2009 total figures. Breakdowns by year available on subsequent tables.
** 2010-2014 total figures. Breakdowns by year available on subsequent tables.

Total number of cases includes number of deaths.
WHO reports only laboratory cases.
All dates refer to onset of illness.

Source: WHO/GIP, data in HQ as of 28 May 2018

World Health Organization

Figure 15. Influenza A/H5N1. Cumulative number of laboratory-confirmed H5N1 cases and deaths from 2003 to 2018. (http://www.who.int/influenza/human_animal_interface/2018_05_28_tableH5N1.pdf?ua=1).

Since February 2014, 19 cases of HPAI A(H5N6), including six deaths, have been identified in Mainland China.[16] A(H5N6) strains have been shown to reassort rapidly.[17,18] Nevertheless, no changes in human-to-human transmissibility have been reported for the most recent isolates.

Since February 2013, six "waves" of A(H7N9) infection cases have been reported with the fifth being the more severe in terms of the number of cases and deaths (Table 4).

Table 4. A(H7N9) epidemic waves, as at March 2018

Waves (period)	1 (02/2013-09/2013)	2 (10/2013-09/2014	3 (10/2014-09/2015)	4 (09/2015-10/2016)	5 (10/2016-10/2016)	6 (10/2017-current)
Cases (deaths)	135 (43)	320 (134)	223 (98)	120 (45)	766 (248)	3 (1)

Adapted from[16]

As at March 2018, 1567 laboratory-confirmed A(H7N9) human cases, including 569 deaths, have been reported to WHO.[16] All cases were of Chinese origin and most were isolated in China (Figure 16). A(H7N9) cases were caused by both low and high pathogenicity influenza A (L/HPAI, respectively) strains without major differences in pathogenicity in humans. According to previous years, new sporadic cases of A(H7N9) are expected to be detected in the next months.

Figure 16. H7N9 cases. Human cases are depicted in the geographic location where they were reported. For some cases, exposure may have occurred in a different geographic location. Imported cases in Canada (2) and Malaysia (1) are not represented.
(http://www.fao.org/ag/againfo/programmes/en/empres/H7N9/situation_update.html)

Forty-three laboratory-confirmed human cases of A(H9N2) infections have been reported since 1998, mainly in Mainland China (36) but also in Egypt (four) and Bangladesh (three). A(H9) viruses are generally considered to cause only mild diseases, but at least one death has been reported to be associated with this avian influenza subtype.[16] No human-to-human transmission of A(H9N2) viruses has been documented so far.

In 2018, the first human case of influenza LPAI A(H7N4) infection was reported by China in a 68-years-old patient exposed to live poultry.[16] In addition, influenza A(H7N4) viruses were also found in birds present in her backyard.

7. Avian influenza A in animals[16,19]

L/HPAI viruses naturally circulate within wild birds. Some LPAI, as well as an increasing proportion of HPAI viruses periodically cause moderate to large outbreaks in poultry. LPAI A(H7N9) was first detected in China in 2013 and continues to circulate, principally in Mainland China. In 2017, the LPAI A(H7N9) mutated into a HPAI virus. At present both LPAI and HPAI are co-circulating in wild birds, poultry and in an increasing number of human cases, but this remains restricted to Chinese provinces. Of note, in 2017, the Chinese government has took several measures to prevent/limit A(H7N9) virus spread in the country, such as setting up extensive surveillance, nationwide vaccination programmes in poultry, and closure of live bird markets and farms in infected province. In March 2017, an HPAI A(H7N9), genetically distinct from the Asian viruses, was identified in poultry in the USA.

Several LPAI and HPAI A(H5) subtypes are regularly reported in many countries. HPAI A(H5N1) continues to circulate and cause outbreaks in poultry in Africa and Asia. HPAI A(H5N8) viruses are present in Africa, Asia, Europe and Middle-East in wild birds and/or poultry. Since 2016, 121 positive cases of A(H5N8) were identified in wild birds in Switzerland. Two genetically distinct groups of HPAI A(H5N6) viruses, the Asian and the newly-emerged European lineages, are currently co-circulating in wild birds and/or poultry. The European A(H5N6) lineage is likely to be a reassortment of the circulating A(H5N8) viruses. Such viruses have been detected in South Korea (poultry), the Netherlands (poultry), Finland, Sweden, Switzerland and in the UK (wild birds). So far only A(H5N6) viruses from the Asian lineage have caused human infections.

Other avian influenza strains responsible for ongoing and/or recent outbreaks in poultry and/or wild birds are H7N3 in the Americas and H5N2 in Asia and Russia. No human infection with these viruses have been reported and the transmission risk to humans in general is considered to be low. Individuals at risk for all avian strains are mainly those in direct contact with infected birds/poultry or their carcasses.

8. Discussion

During 2017/18, Switzerland experienced a prolonged influenza season with an epidemic phase that lasted 15 weeks compared to 11 weeks in 2016/17 and 12 weeks in 2015/16. Surprisingly, two peaks in the MC-ILI per 100.000 inhabitants could be observed at weeks 2 and 4/2018. Such a phenomenon had not been observed in Switzerland since the 2003/04 season. According to the European Centre for Disease Prevention and Control, high influenza activity could be observed from weeks 52/2017 to 12/2018, which was longer than previous seasons.[20] The ILI activity was variable across the European Union/European Economic Area (EU/EEA) with many countries reporting moderate levels compared to previous seasons and few higher levels.[20,21] The same observation could be made in terms of hospitalization and intensive care admission rates. In the USA, influenza activity started to increase earlier than in Europe and was characterized by abnormally high levels of influenza-associated mortality and record-breaking hospitalizations rates compared to previous seasons.[22] Of note, and in contrast to many other regions, A(H3N2) was the dominant strain in this country. This was also the case in the EU/EEA and in Australia during the 2016/17 Northern hemisphere and 2017 Southern hemisphere influenza seasons, respectively, during which the influenza activity and the associated burden was also considered to be particularly high.

Similar to most EU/EEA and East African countries, influenza B Yamagata was predominant in Switzerland (65.7%), followed by A(H1N1)pdm09 (23.3%) and A(H3N2) strains (5.2%).[20,21] In a majority of Asian countries, A(H1N1)pdm09 viruses were often dominant, followed by B Yamagata strains. B Yamagata lineage viruses largely outnumbered those of the B Victoria lineage, while proportions of co-circulating influenza A subtypes were country-dependent. Overall, the 2017/18 influenza season in the EU/EEA was characterized by a high severity associated with a dominance of influenza B viruses, a long duration of influenza activity and all-cause excess mortality, which was very similar to the 2012/13 season.[21] Most severe influenza infections (hospitalized influenza cases in intensive care units or other wards) and severe acute respiratory infections reported in the EU/EEA) occurred in patients >15 years carrying an influenza B strain.[20,21] However, 53% of the influenza viruses detected in intensive care units were of type A, mainly A(H1N1)pdm09. [20]

Compared to 2016/17, 313 additional individuals were tested for influenza by the NRCI (n=1292) during the 2017/18 influenza season. As expected from previous seasons, the female/male ratio was close to 1 (1.12), similar to the number of positive versus negative individuals of each sex. The total number of persons tested per age group this year was comparable to past seasons. As expected almost one-half (n=652/1292) of tested patients were aged 30-64 years, but only 10% (133/1261) were known to have received the 2017/18 influenza vaccine. Of note, but still insufficient, 36% of elderly patients (≥65 years old) were vaccinated against influenza. This rate is slightly higher than the 2017 estimated 32% (n=642) by the FOPH for the >64 years old Swiss population. Influenza vaccination rates in the latter population are close to those observed in Germany, but much lower than in some other EU/EEA counties (e.g. France, Italy and Spain).[23] Nevertheless, most countries worldwide are far below the 75% vaccination rate recommended by the WHO for the "at risk" population.[24] The extrapolated rate of vaccination against influenza for the Swiss general population was only 18% in 2016 according the FOPH and we do not expect it to be higher in 2017/18.

In 2017/18, an overall positivity rate of 57.6% was observed for the analyzed samples. The highest prevalence of positive samples (69%) was observed in the 5-14 years group followed by the 30-64 years group (60%). The ratio of positive/negative samples during this season was comparable to 2015/16 and 2016/17 for the 5-14 years, the 15-29 years and the ≥65 years groups. The positivity rate for the 30-64 years and the 0-4 years groups was higher (60% and 49%, respectively) this year compared to 2015/16 and 2016/17 (42% and 35%; 49% and 31%, respectively). Of note, both 2015/16 and 2017/18 influenza seasons were dominated by influenza B viruses but of different lineages. However, while in 2015/16 there was a higher percentage of B Victoria (59.4%) than A(H1N1)pdm09 (34.4%) viruses isolated in the 0-4 years group, in 2017/18 we observed 22.5% of B Yamagata and 67.5% of A(H1N1)pdm09. The opposite was seen for the 30-64 years group for the corresponding periods (2015/16: 41.7% and 50.3%; 2017/18 67.3% and 22.5% for influenza B Victoria/Yamagata and A(H1N1)pdm09, respectively). As B viruses are generally shown to affect younger age groups[25], such a high rate of influenza B Yamagata positive samples in the 30-64 years group was rather unexpected. The tendency towards older age groups being mainly infected by influenza B Yamagata viruses was also observed in EU/EEA countries where this strain was abundant, particularly for severe influenza B cases.[20,21]

As mentioned previously, Influenza B Yamagata viruses were dominant this season in Switzerland and most were recognized by some of the antisera raised against the B/Phuket/3073/2013 virus, which corresponds to the quadrivalent vaccine strain. This was consistent with unpublished and published antigenic data for other European[20] and Asian countries as well as for the USA.[13] In particular, only the B Victoria B/Brisbane/60/2008 was present in the trivalent vaccine. Serologic studies using human serum panels from individuals vaccinated with the quadrivalent vaccine shown that the titers obtained against most of the recent viruses of the B Yamagata lineage were similar to those raised against the cell cultivar B/Phuket/3073/2013-like reference viruses.[13] At the genetic level, all B Yamagata viruses analyzed belonged to the genetic clade 3, as does the B/Phuket/3073/2013 virus. Moderate variability is a known limitation of HI assay, but it was particularly high this season when performing influenza B Yamagata isolate characterization. The same phenomenon was observed by the WHO Collaborating Centre in London. The reason for this occurrence remains unknown, but the antisera lot, reference strains' production (cell-culture versus egg), and the RBC origin are known to impact on the HI assay results.

Only a few B/Victoria/2/1987-like viruses were observed in 2017/18 in Switzerland, but most were antigenically and genotypically closer to a recent subgroup of viruses, increasingly circulating in Europe and the USA, characterized by the deletion of amino acids 162 and 163 in the HA gene, the B/Colorado/6/2017-like. Most of the viruses belonging to this subgroup were not (or only poorly) recognized by the antisera against vaccine strain B/Brisbane/60/08-like viruses. The majority of viruses, for which the 162-163 deletion was absent, were well recognized by the antiserum targeting cell culture-propagated B/Brisbane/60/2008-like strains.[13] However, when human serum panels were used, titers against recent non-deleted viruses were reduced to some extent compared to HI titers against egg- or cell-propagated reference viruses. In very young children vaccinated with the B/Brisbane/60/2008-like vaccine strain, Hi titers against double and triple deleted (deletion of amino acids 162 to 164) B/Victoria/2/1987-like viruses were highly reduced.[13] For this reason, the B/Victoria/2/1987-like component of the influenza vaccine was updated from B/Brisbane/60/2008-like to B/Colorado/6/2017-like for the 2018/19 Northern hemisphere season. The latter will be the B strain component of the trivalent "version" of the influenza vaccine. Triple deleted B/Victoria/2/1987-like viruses were not observed in Switzerland this year and were mainly detected in China and China Hong Kong Special Administrative Region.[13]

The second most abundant influenza strain circulating in 2017/18 in Switzerland was A(H1N1)pdm09. Similar to the majority of A(H1N1)pdm09 observed worldwide,[13] Swiss isolates were antigenically similar to the vaccine strain A/Michigan/45/2015. They also belonged to the corresponding genetic clade 6B.1. Studies using serum panels of individuals (all age groups) vaccinated with either a 2017/18 trivalent or quadrivalent influenza vaccine showed rather reduced HI titers against circulating cell-culture propagated viruses when compared to HI titers of the reference strain A/Michigan/45/2015.[13]

Concerning the A(H3N2) viruses, all isolates collected in Switzerland were to some extent antigenically related to cell-grown A/Hong-Kong/4801/2014-like reference strains concordant with worldwide[13] observations. In contrast, antiserum raised against egg-propagated A/Hong-Kong/4801/2014 poorly recognized recent circulating viruses, while antiserum raised against egg-grown A/Singapore/INFIMH-16-0019/2016 virus performed better against those viruses. On the basis of all the antigenic and genetic data available, the A(H3N2) vaccine component will be updated, for the 2018 Southern and 2018/19 Northern hemisphere influenza seasons, i.e., A/Hong Kong/4801/2014 is replaced by A/Singapore/INFIMH-16-0019/2016[13]. At the genetic level, the Swiss A(H3N2) isolates were distributed in several subclades, mainly 3C.2a2 within the 3C.2a clade. A similar pattern was observed worldwide but the proportions of viruses found in the different subclades was region-dependent.[13]

Of note, an A(H1N2) reassortment event between a A(H1N1)pdm09 strain (HA and NS genes, clade 6B.1) and an A(H3N2) (all other genes, clade 3C.2a) virus has recently been identified in the Netherlands.[26] This subtype is not considered as being a major threat for the human population as its eight genes are of human origin and are present in viruses currently circulating in the population. This virus is genetically distinct from the A(H1N2) viruses observed in Switzerland during the 2002/03 influenza season.

Similarly to recent influenza seasons, all influenza isolates tested at the NRCI for antiviral resistance in 2017/18 were resistant to M2 protein inhibitors (adamantanes). One A(H3N2) isolate exhibited a phenotypic reduced inhibition by oseltamivir as measured by the WIC but we were unable to reproduce their result in three independent experimental duplicates. The reason for this discrepancy is unknown. However, we postulate that even if the assay was performed on the same clinical isolate, the culture conditions may have differed between the WIC and the NRCI and thus somehow accounted for a differential quasispecies selection. Of note, the substitutions observed in the NA of this isolate were not known to be associated with reduced inhibition by oseltamivir and other viruses bearing the same mutations were classified as being sensitive to oselatamivir. All isolates were sensitive to zanamivir. Two A(H1N1)pdm09 isolates from hospitalized immunocompromised patients were found to have a highly-reduced inhibition by oseltamivir profile. This was fully concordant with the presence for the H275Y substitution in the NA gene. The emergence of resistance mutations in treated patients, particularly in the presence of an immunosuppressant condition is a well documented event.[27]

Less than 1% of the tested seasonal influenza viruses (14/1431 A(H1N1)pdm09, 8/2202 A(H3N2), and 14/1720 B/Victoria (n=6) and Yamagata (n=8)) worldwide showed phenotypic or genotypic signs of abnormal (reduced or highly reduced) inhibition by oseltamivir and/or zanamivir. As expected, almost all A(H3N2) and all A(H1N1)pdm09 isolates analyzed worldwide harboured the S31N mutation associated with resistance to M2 inhibitors.[13]

Similar to the previous year, a human infection with an avian-like swine influenza A(H1N1)v was identified in Switzerland. The sample originated from a farm employee. According to Dr.med.vet. Claudia Bachofen (personal communication; Vetsuisse-Faculty, University of Zürich) at least one farm pig was also positive for swine influenza A(H1N1).

Similar to the 2016/17 season, several avian influenza strains continue to circulate worldwide among wild birds and poultry, mainly A/H5 and A/H7 types, leading to sporadic outbreaks in humans. A low number of human influenza A(H7N9) cases has been observed during the ongoing epidemic wave. Compared to previous years, the Chinese government has made a major investment in avian influenza surveillance, prevention and control measures.

As of May 2018, influenza activity is either at inter-seasonal levels (Northern hemisphere region), or low or increasing in the Southern hemisphere. Influenza A strains accounted for the most detected cases, often A(H1N1)pdm09.

2017/18 season overview

Influenza B/Yamagata/16/1988-like viruses were dominant in Switzerland and in other African, Asian and European regions, followed by influenza A(H1N1)pdm09 or A(H3N2) viruses depending on the country. A(H3N2) viruses were dominant in the USA. B/Victoria/2/1987-like strains were scarce in general.

The majority of the Swiss A(H3N2) viruses had a normal inhibition by oseltamivir, except one with phenotypic reduced inhibition but no know resistance mutations. Substitution H275Y could be identified in the post-oseltamivir treatment samples of two immunocompromised hospitalized patients.

Sporadic human infections with LPAI and HPAI, mainly caused by A(H5) and A(H7) subtypes, continue to be observed, particularly in Mainland China.

Various avian LPAI and HPAI viruses circulate in domestic and wild birds worldwide.

9. Other activities of the National reference Centre for Influenza

9.1. Sharing of influenza cell-cultured isolates and or reference strains

Shared material: *MDCK/1 isolates B/Switzerland/3850/2018 (Yamagata), A/Switzerland/3631/2018 (H1N1)pdm09 A(H3N2) and A/Switzerland/9507/2018 A(H3N2)*

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

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

Shared material: *RNA from isolates A/Switzerland/8337/2018 (H1N1)pdm09 and A/Switzerland/6796/ 2018 A(H3N2)*

With Whom: *Professor Ronald Dijkman, Vetsuisse Faculty, Department of Infectious Diseases and Pathobiology, Institute of Virology and Immunology, University of Berne.*

Purpose: *"In 2017, a seasonal reassortment of influenza A virus was identified in the Netherlands, A(H1N2), consisting of HA and NS genes of human seasonal A(H1N1)pdm09 influenza virus and M, NA, NP, PA, PB1 and PB2 genes of human seasonal A(H3N2) influenza virus. A(H1N2) viruses have been described among avian, swine and human populations. However direct cross-species transfer of swine A(H1N2) is rare. The emergence of H1N2 reassortants containing genes from the H1N1pdm09 virus is a serious concern as the H1N1pdm09 virus is quite adapted to human-to-human transmission and presents an ideal background for the spread of novel strains. Unfortunately, genetic material or virus isolate to characterize the A(H1N2) on well-differentiated human airway epithelial cells – a surrogate model for the human respiratory tract – is not available. Therefore, by generating reverse genetic strains of seasonal A(H1N1)pdm09 and A(H3N2) influenza viruses, we can recreate an A(H1N2) strain in order to characterize the influence of the recent genetic reassortment on its replication kinetics and host innate immune response in comparison to the parental seasonal strains within the primary target tissue."*

9.2. Ongoing projects or publications

<u>1. Poster abstract submitted for the North American Primary Care Research 2018 meeting Group. *(accepted).*</u>

"Attack rate of influenza among primary care workers in Switzerland – a pilot surveillance study"

Martin Sébastien[1*], Maeder Muriel Nirina[1], Gonçalves Ana Rita[2], Pedrazzini Baptiste[1], Perdrix Jean[1], Rochat Carine[3], Senn Nicolas[1], Mueller Yolanda[1*]

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<u>***Abstract provided by Dr Matin Sébastien and Dr Mueller Yolanda.**</u>

“Context: Although primary care practices play a key role during annual influenza epidemics, their role in the transmission chain has rarely been explored. A better understanding of influenza epidemiology among primary care workers could guide future recommendations to prevent transmission in this setting.

Objective: This pilot study aims at evaluating the feasibility of a work-based online surveillance system for influenza among workers of primary care practices.

Study Design: Observational prospective pilot cohort study, conducted between week 40 in 2017 and week 16 in 2018.

Setting: One walk-in clinic and two selected primary care practices. *Participants:* Staff working in the practices enrolled in the pilot study, aged ≥ 18 years and having a work contract covering the study period.

Intervention: Symptoms of influenza-like illness (ILI) were recorded in a weekly online survey completed by the participants. Patients with symptoms also self-collected a nasopharyngeal swab following written instructions. Samples were tested for influenza A and B viruses by RT-PCR on a weekly basis and, twice during the season, for a panel of respiratory pathogens.

Main and Secondary Outcomes: Main outcomes were the adhesion to online survey by primary care workers, the weekly survey response rate and the fully completed questionnaire rate. Secondary outcomes were the assessment of ILI attack rate and the confirmed influenza cases over the entire influenza season as well as the influenza vaccination coverage.

Results: 36 out of 68 workers agreed to participate. 94% of the weekly online questionnaires were completed until week 15. There were 10 ILI reported by 6 out of the 36 participants among which only one was confirmed to be due to Influenza virus.

Conclusions: the study confirmed the feasibility of a work-based online surveillance system for influenza among primary care practices workers. The attack rate of influenza appeared to be low in this population.”

2. Poster submitted to the to the “Joint annual meeting 2018 of the Swiss Societies for Infectious Diseases (SSI), Hospital Hygiene (SSHH), Tropical Medicine and Parasitology (SSTMP) and Tropical and Travel Medicine (SSTTM), 2018”. *(accepted).*

“Feasibility of prospective influenza surveillance among primary care workers in Switzerland”

Martin Sébastien[1], Maeder Muriel Nirina[1], Gonçalves Ana Rita[2], Pedrazzini Baptiste[1], Perdrix Jean[1], Rochat Carine[3], Senn Nicolas[1], Mueller Yolanda[1]

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***Abstract provided by Dr Matin Sébastien and Dr Mueller Yolanda.**

“Aims*:* Although primary care practices play a key role during annual influenza epidemics, their role in the transmission chain has rarely been explored. A better understanding of influenza epidemiology among primary care workers could guide future recommendations to prevent transmission in this setting. This pilot study aims at evaluating the feasibility of a work-based online surveillance system for influenza among workers of primary care practices.

***Methods**:* Observational prospective pilot study, conducted between week 40 in 2017 and week 16 in 2018 in one walk-in clinic and two selected primary care practices. Staff working in the practices, aged ≥ 18 years and having a work contract covering the study period, were invited to record symptoms of influenza-like illness (ILI) in a weekly online survey. Patients with symptoms also self-collected a nasopharyngeal swab following written instructions. Samples were tested for influenza A and B viruses by RT-PCR on a weekly basis and, twice during the season, for a panel of respiratory pathogens (FTDresp21). Main outcomes were the adhesion to online survey by primary care workers, the weekly survey response rate and the fully completed questionnaire rate. Secondary outcomes were the assessment of ILI attack rate and the confirmed influenza cases over the entire influenza season as well as the influenza vaccination coverage.

Results: Out of 69 eligible, 39 (56.5%) consented to the study, 19/49 (38.8%) in the walk-in clinic and 20/20 (100%) in both private practices, corresponding to 23 physicians and 16 medical assistants. 36 (92.3%) finally provided data in the online survey, completing a median of 27 weekly questionnaires out of 29 (IQR 23 – 28.5).
Out of 79 symptomatic episodes (mean 2.2 per participant), 10 fitted the ILI case definition (7 participants). Among the 8 swabbed ILI, one was confirmed to be due to influenza A virus H1N1 (AR 2.8%), in addition to 2 rhinoviruses and one coronavirus OC43.
In total, 20 swabs were taken, a median of 3 days after start of symptoms (IQR 1 – 5), and received in the lab a median of 2 days later (IQR 1 – 3). Out of 16 (4 missing data), 7 (43.8%) were autoswabs, mostly done by physicians (85.7%).

Conclusion: the study confirmed the feasibility of a work-based online surveillance system for influenza among primary care practices workers. The attack rate of symptomatic influenza appeared to be low in this population."

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

Stéphanie Anchisi, Ana Rita Gonçalves, Béryl Mazel-Sanchez, Samuel Cordey, and Mirco Schmolke

The NRCI team contributed to a Springer Nature book chapter currently *in press*.

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

Ana Rita Gonçalves and Laurent Kaiser

The NRCI team submitted a review article on influenza to the Swiss Medical Forum (submission date 06.06.2018, under review).

Geneva, 12 July 2018

Ana Rita Gonçalves Cabecinhas, PhD

Samuel Cordey, PhD

Mrs Patricia Suter-Boquete

Professor Laurent Kaiser, MD

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Annex 1: Weekly report of influenza virus detection and virus characteristics (2017/18)

Weeks	Dates		‰ ILI	Samples received	Influenza A				Influenza B				Total virus (n)	% pos
			Sentinel Surveillance, Season 2017-18		Undet.	A (H1N1) pdm09	A (H3N2) seasonal	Total	Undet.	Bvic	Byam	Total		
40	1-Oct-17	7-Oct-17	1.4	**5**	0	0	0	**0**	0	0	0	**0**	**0**	**0.00**
41	8-Oct-17	14-Oct-17	1.8	**8**	0	0	1	**1**	0	0	0	**0**	**1**	**12.50**
42	15-Oct-17	21-Oct-17	2.1	**14**	0	0	0	**0**	0	0	0	**0**	**0**	**0.00**
43	22-Oct-17	28-Oct-17	2.2	**4**	0	0	0	**0**	0	0	0	**0**	**0**	**0.00**
44	29-Oct-17	4-Nov-17	2.3	**14**	0	0	0	**0**	0	0	0	**0**	**0**	**0.00**
45	5-Nov-17	11-Nov-17	2.1	**15**	0	0	1	**1**	0	0	0	**0**	**1**	**6.67**
46	12-Nov-17	18-Nov-17	2.1	**17**	0	0	0	**0**	0	0	0	**0**	**0**	**0.00**
47	19-Nov-17	25-Nov-17	2.2	**9**	0	0	0	**0**	0	0	2	**2**	**2**	**22.22**
48	26-Nov-17	2-Dec-17	4.1	**26**	0	0	0	**0**	0	0	3	**3**	**3**	**11.54**
49	3-Dec-17	9-Dec-17	4.6	**19**	0	1	0	**1**	0	0	3	**3**	**4**	**21.05**
50	10-Dec-17	16-Dec-17	7.8	**54**	0	0	2	**2**	0	0	21	**21**	**23**	**42.59**
51	17-Dec-17	23-Dec-17	14.9	**86**	0	8	2	**10**	0	0	44	**44**	**54**	**62.79**
52	24-Dec-17	30-Dec-17	47	**85**	0	10	1	**11**	2	0	56	**58**	**69**	**81.18**
1	31-Dec-17	6-Jan-18	60.6	**77**	0	6	2	**8**	4	2	43	**49**	**57**	**74.03**
2	7-Jan-18	13-Jan-18	43.7	**141**	0	9	4	**13**	2	1	74	**77**	**90**	**63.83**
3	14-Jan-18	20-Jan-18	35.6	**63**	1	9	2	**12**	1	2	27	**30**	**42**	**66.67**
4	21-Jan-18	27-Jan-18	42.8	**80**	1	9	2	**12**	6	0	37	**43**	**55**	**68.75**
5	28-Jan-18	3-Feb-18	37.2	**88**	1	19	2	**22**	4	0	37	**41**	**63**	**71.59**
6	4-Feb-18	10-Feb-18	33.9	**75**	1	14	1	**16**	3	1	28	**32**	**48**	**64.00**
7	11-Feb-18	17-Feb-18	35.3	**57**	1	12	1	**14**	3	1	19	**23**	**37**	**64.91**
8	18-Feb-18	24-Feb-18	32.2	**73**	0	16	3	**19**	0	0	27	**27**	**46**	**63.01**
9	25-Feb-18	3-Mar-18	33.3	**50**	0	12	3	**15**	0	0	16	**16**	**31**	**62.00**
10	4-Mar-18	10-Mar-18	25.5	**43**	0	12	4	**16**	2	0	11	**13**	**29**	**67.44**
11	11-Mar-18	17-Mar-18	22.9	**54**	0	14	2	**16**	0	0	18	**18**	**34**	**62.96**
12	18-Mar-18	24-Mar-18	16.2	**43**	0	12	2	**14**	1	0	8	**9**	**23**	**53.49**
13	25-Mar-18	31-Mar-18	12.8	**45**	2	7	2	**11**	1	0	8	**9**	**20**	**44.44**
14	1-Apr-18	7-Apr-18	6	**27**	0	2	1	**3**	0	0	5	**5**	**8**	**29.63**
15	8-Apr-18	14-Apr-18	4.7	**16**	0	1	0	**1**	0	0	2	**2**	**3**	**18.75**
16	15-Apr-18	21-Apr-18	2.5	**8**	0	1	1	**2**	0	0	1	**1**	**3**	**37.50**
				1296	**7**	**174**	**39**		**29**	**7**	**490**		**746**	
						220				**526**				

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

Undet.: Not determined or insufficient viral load

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

BVic: Influenza B Victoria lineage

BYam: Influenza B Yamagata lineage

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

			Antisera			
		Reference viral isolates	A/California/7/09	A/Michigan/45/15	A/St. Petersburg/27/11	A/Hong Kong/3934/11
		A/California/7/09	**64**	128	128	128
		A/Michigan/45/15*	64	**128**	128	64
		A/St Petersburg/27/11	64	64	**64**	64
Isolates	HA titre	A/Hong Kong/3934/11	64	128	64	**64**
		Typisation				
8198[@]	64	A/California/7/09-like	256	256	256	256
6079[@]	64	A/Michigan/45/15-like	64	128	128	64
0683[@]	64	A/California/7/09-like	128	128	128	128
2656[@]	64	A/California/7/09-like	128	128	128	128
4062[@]	64	A/California/7/09-like	128	128	128	128
5320[@]	64	A/Michigan/45/15-like	512	512	512	256
7707[@]	64	A/California/7/09-like	128	128	128	128
6136[@]	64	A/California/7/09-like	256	512	256	128
4883	64	A/Michigan/45/15-like	64	128	nd	nd
7078	64	A/California/7/09-like	128	128	128	128
0671	128	A/Hong Kong/3934/11-like	128	128	32	64
2217	128	A/Hong Kong/3934/11-like	64	128	32	64
3126	128	A/St Petersburg/27/11-like	32	64	32	32
4802	128	A/Michigan/45/15-like	256	256	128	128
7482	64	A/California/7/09-like	256	256	256	256
1730	128	A/Michigan/45/15-like	64	128	32	64
0808	128	A/St Petersburg/27/11-like	32	64	32	64
0950	128	A/St Petersburg/27/11-like	32	64	32	32

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

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

			Antisera			
		Reference viral isolates	A/California/7/09	A/Michigan/45/15	A/St. Petersburg/27/11	A/Hong Kong/3934/11
		A/California/7/09	**64**	128	128	128
		A/Michigan/45/15*	64	**128**	128	64
		A/St Petersburg/27/11	64	64	**64**	64
Isolates	HA titre	A/Hong Kong/3934/11	64	**128**	64	**64**
		Typisation				
3515	128	A/California/7/09-like	512	512	256	256
3583	128	A/Michigan/45/15-like	64	256	64	64
3467	256	A/St Petersburg/27/11-like	32	64	32	32
5960	128	A/St Petersburg/27/11-like	32	64	32	64
9891	128	A/St Petersburg/27/11-like	32	32	32	32
3014	64	A/St Petersburg/27/11-like	32	64	32	64
3405	128	A/St Petersburg/27/11-like	32	64	32	64
3632	64§	A/Michigan/45/15-like	256	256	128	128
1338	64	A/California/7/09-like	64	128	128	128
4505	64	A/Michigan/45/15-like	64	128	64	64
7790	64	A/California/7/09-like	64	128	128	128
7992	64	A/Michigan/45/15-like	256	256	128	128
5775	128	A/California/7/09-like	128	128	64	128
8130	64	A/California/7/09-like	128	128	128	128
0176	64	A/California/7/09-like	128	128	128	128
0377	64	A/Michigan/45/15-like	64	128	64	128
7689	32	A/California/7/09-like	256	256	256	128
8219	64	A/California/7/09-like	128	128	128	128

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

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

			Antisera			
		Reference viral isolates	A/California/7/09	Michigan/45/15	A/St. Petersburg/27/11	A/Hong Kong/3934/11
		A/California/7/09	**64**	128	128	128
		A/Michigan/45/15*	64	**128**	128	64
		A/St Petersburg/27/11	64	64	**64**	64
Isolates	HA titre	A/Hong Kong/3934/11	64	**128**	64	**64**
		Typisation				
6602	**128**	**A/California/7/09-like**	**512**	**256**	**256**	**256**
8337	**64**	**A/California/7/09-like**	**512**	**256**	**256**	**512**
6244	**32**	**A/California/7/09-like**	**512**	**256**	**256**	**256**

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

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

			Antisera			
		Reference viral isolates	**A/Texas/50/12**	**A/Switzerland/9715293/13**	**A/Hong Kong/4801/14**	**A/Slovenia/3188/15**
		A/Texas/50/12	**512**	256	128	256
		A/Switzerland/9715293/13	256	**128**	128	256
		A/Hong Kong/4801/14	64	64	**64**	64
Isolates	**HA titer**	**A/Slovenia/3188/15**	32	32	32	**32**
		Typisation				
0882[@]	**32**	**A/Hong Kong/4801/14-like**	32	32	64	64
2159	**64**	**A/Hong Kong/4801/14-like**	128	128	64	128
		Reference viral isolates	***A/Switzerland/9715293/13***	***A/Hong Kong/4801/14***	***A/Slovenia/3188/15****	***A/Singapore/INFIM-16-0019/16****
		A/Switzerland/9715293/13	**64**	64	64	64
		A/Hong Kong/4801/14	64	**128**	64	64
		A/Slovenia/3188/15	32	32	**32**	32
		A/Singapore/INFIM-16-0019/16	32	32	32	**32**
Isolates	**HA titer**	**Typisation**				
4325	**64**	**A/Singapore/INFIM-16-0019/16-like**	32	32	32	64
9638	**64**	**A/Hong Kong/4801/14-like**	64	128	128	128
6278	**64**	**A/Switzerland/9715293/13-like**	64	64	64	64
8060	**64**	**A/Switzerland/9715293/13-like**	64	64	64	64
8143[@]	**64**	**A/Singapore/INFIM-16-0019/16-like**	32	64	64	32
8180	**64**	**A/Switzerland/9715293/13-like**	64	64	64	64
1511	**64**	**A/Hong Kong/4801/14-like**	1024	128	nd	64
2840	**128**	**A/Switzerland/9715293/13-like**	64	64	nd	64

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

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

			Antisera			
		Reference viral isolates	*A/Switzerland/97 15293/13*	*A/Hong Kong/4801/14*	*A/Slovenia/3188/15 **	*A/Singapore/INFIM-16-0019/16**
		A/Switzerland/9715293/13	**64**	64	64	64
		A/Hong Kong/4801/14	64	**128**	64	64
		A/Slovenia/3188/15	32	32	**32**	32
		A/Singapore/INFIM-16-0019/16	32	32	32	**32**
Isolates	**HA titer**	**Typisation**				
8061	**128**	**A/Hong Kong/4801/14-like**	64	128	nd	64
1570	**128**	**A/Switzerland/9715293/13-like**	64	64	nd	64
0762	**128**	**A/Switzerland/9715293/13-like**	32	64	64	64
3052	**64§**	**A/Hong Kong/4801/14-like**	64	256	256	128
1515	**64**	**A/Hong Kong/4801/14-like**	64	256	128	128
5541	**64**	**A/Switzerland/9715293/13-like**	64	64	64	64
5432	**32**	**A/Switzerland/9715293/13-like**	256	128	128	128
9508	**64**	**A/Hong Kong/4801/14-like**	64	128	64	64
6796	**32**	**A/Hong Kong/4801/14-like**	128	128	128	128
8327	**32**	**A/Hong Kong/4801/14-like**	256	512	256	256
8138	**64**	**A/Switzerland/9715293/13-like**	64	64	64	64

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

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

			Antisera			
		Reference viral isolates	**B/Wisconsin/1/10**	**B/Novosibirsk/1/12**	***B/Massachusetts/2/12***	**B/Phuket/3073/13**
		B/Wisconsin/1/10	**256**, ***64***	128, *64*	*32*	512, *64*
		B/Novosibirsk/1/12	32, *256*	**128**, ***256***	*64*	64, *16*
		B/Massachusetts/2/12	*128*	*64*	***256***	*128*
		B/Phuket/3073/13	256, *256*	64, *128*	*256*	**256**, ***128***
Isolates	**HA titer**	**Typisation**				
0226@	**128**	***B/Novosibirsk/1/12-like***	<16	128	32	*<16*
1042@	**64**	***B/Phuket/3073/13-like***	32	32	nd	*32*
7069@	**32**	***B/Phuket/3073/13-like***	32	32	nd	*32*
0968@	**64§**	***B/Phuket/3073/13-like***	32	32	nd	*32*
0323	**128§**	***B/Phuket/3073/13-like***	16	32	nd	*32*
0558@	**128§**	**B/Novosibirsk/1/12-like**	64	128	nd	64
0747@	**128**	**B/Novosibirsk/1/12-like**	nd	64	nd	64
7707@	**128**	**B/Novosibirsk/1/12-like**	64	64	nd	64
7720	**128**	**B/Novosibirsk/1/12-like**	32	64	nd	64
8089@	**256**	**B/Novosibirsk/1/12-like**	32	64	nd	32
8142@	**128§**	**B/Novosibirsk/1/12-like**	32	32	nd	32
8293	**256**	**B/Novosibirsk/1/12-like**	32	32	nd	32
7965	**128§**	**B/Novosibirsk/1/12-like**	32	32	nd	32
7993	**128**	**B/Novosibirsk/1/12-like**	32	32	nd	32
8112	**128§**	**B/Novosibirsk/1/12-like**	32	32	nd	32
2048	**128§**	**B/Novosibirsk/1/12-like**	<16	32	nd	32
2242	**128§**	**B/Novosibirsk/1/12-like**	<16	32	nd	32

HA titers were established in MDCK-SIAT (S/1) or §MDCK cells (MD/1). **HI titers should be multiplied by 8**. *Grey: 2016/17 antigenic table titers.* @also sent to the WIC. Vaccine strain. 0226 isolate was tested with 2016/17 reagents only.

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

			Antisera			
		Reference viral isolates	B/Wisconsin/1/10	B/Novosibirsk/1/12	*B/Massachusetts/2/12*	B/Phuket/3073/13
		B/Wisconsin/1/10	**256**	128	*32*	512, *64*
		B/Novosibirsk/1/12	32	**128**	*64*	64, *16*
		B/Massachusetts/2/12	*128*	*64*	***256***	*128*
		B/Phuket/3073/13	256	64	*256*	**256, *128***
Isolates	**HA titer**	**Typisation**				
5995@	**64**	**B/Novosibirsk/1/12-like**	<16	32	nd	<16, *256*
6909@	**64**	**B/Novosibirsk/1/12-like**	<16	64	nd	<16
7218@	**64**	**B/Novosibirsk/1/12-like**	<16	128	nd	<16
8231@	**64**	**B/Novosibirsk/1/12-like**	<16	64	nd	<16, *32*
4296@	**64**	**B/Novosibirsk/1/12-like**	<16	128	nd	<16
5936@	**64**	**B/Novosibirsk/1/12-like**	<16	128	nd	<16
0726@	**64**	**B/Novosibirsk/1/12-like**	<16	128	nd	<16
4150@	**64**	**B/Novosibirsk/1/12-like**	<16	128	nd	<16
5423@	**128**	**B/Novosibirsk/1/12-like**	<16	128	nd	<16
5614@	**128**	**B/Novosibirsk/1/12-like**	<16	128	nd	<16
9903@	**64**	**B/Novosibirsk/1/12-like**	<16	128	nd	<16, *256*
0051@	**64**	**B/Novosibirsk/1/12-like**	<16	256	nd	<16
7742@	**64**	**B/Novosibirsk/1/12-like**	<16	512	nd	<16
7653@	**64**	**B/Wisconsin/1/10-like**	256	512	nd	256
6093@	**64**	**B/Novosibirsk/1/12-like**	<16	128	nd	32
4079	**128**	**B/Novosibirsk/1/12-like**	64	128	nd	64
6370	**256§**	**B/Novosibirsk/1/12-like**	32	64	nd	<16

HA titers were established in MDCK-SIAT (S/1) or §MDCK cells (MD/1). **HI titers should be multiplied by 8**. *Grey: 2016/17 antigenic table titers.* @also sent to the WIC.
Vaccine strain.

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

			Antisera			
		Reference viral isolates	**B/Wisconsin/1/10**	**B/Novosibirsk/1/12**	***B/Massachusetts/2/12***	**B/Phuket/3073/13**
		B/Wisconsin/1/10	**256**	128	*32*	512, *64*
		B/Novosibirsk/1/12	32	**128**	*64*	64, *16*
		B/Massachusetts/2/12	*128*	*64*	***256***	*128*
		B/Phuket/3073/13	256	64	*256*	**256, *128***
Isolates	**HA titer**	**Typisation**				
9613	**256[§]**	**B/Novosibirsk/1/12-like**	16	64	nd	<16
8025	**256[§]**	**B/Novosibirsk/1/12-like**	32	64	nd	32
8049	**256[§]**	**B/Novosibirsk/1/12-like**	16	32	nd	32
8125	**256[§]**	**B/Novosibirsk/1/12-like**	16	64	nd	32
2469[@]	**256[§]**	**B/Novosibirsk/1/12-like**	16	64	nd	32
0615	**64[§]**	**B/Novosibirsk/1/12-like**	<16	32	nd	16
0652[@]	**64**	**B/Novosibirsk/1/12-like**	<16	128	nd	32
8274[@]	**128**	**B/Novosibirsk/1/12-like**	32	32	nd	32
0873	**64**	**B/Wisconsin/1/10-like**	256	1024	nd	512
2282	**128**	**B/Phuket/3073/13-like**	128	64	nd	128
2979	**128**	**B/Novosibirsk/1/12-like**	32	32	nd	32
3091	**128**	**B/Novosibirsk/1/12-like**	32	32	nd	32
1300	**128**	**B/Novosibirsk/1/12-like**	64	64	nd	64
8653	**128**	**B/Novosibirsk/1/12-like**	64	64	nd	32
5719	**128**	**B/Novosibirsk/1/12-like**	64	64	nd	64
8737	**128**	**B/Novosibirsk/1/12-like**	64	64	nd	64
1646	**128**	**B/Novosibirsk/1/12-like**	32	64	nd	32

HA titers were established in MDCK-SIAT (S/1) or [§]MDCK cells (MD/1). **HI titers should be multiplied by 8**. *Grey: 2016/17 antigenic table titers.* [@]also sent to the WIC. Vaccine strain.

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

			Antisera			
		Reference viral isolates	**B/Wisconsin/01/10**	**B/Novosibirsk/1/12**	***B/Massachssetts/2/12***	**B/Phuket/3073/13**
		B/Wisconsin/1/10	**256**	128	*32*	512, *64*
		B/Novosibirsk/1/12	32	**128**	*64*	64, *16*
		B/Massachssetts/2/12	*128*	*64*	***256***	*128*
		B/Phuket/3073/13	256	64	*256*	**256**, ***128***
Isolates	**HA titer**	**Typisation**				
9008	**256[§]**	**B/Novosibirsk/1/12-like**	32	32	nd	32
0648	**256**	**B/Novosibirsk/1/12-like**	32	64	nd	64
0989	**128**	**B/Novosibirsk/1/12-like**	32	64	nd	64
3318	**256**	**B/Novosibirsk/1/12-like**	64	64	nd	64
3413	**128**	**B/Novosibirsk/1/12-like**	64	64	nd	64
3543	**128**	**B/Novosibirsk/1/12-like**	32	64	nd	32
3019	**256**	**B/Novosibirsk/1/12-like**	64	64	nd	64
3136	**128**	**B/Novosibirsk/1/12-like**	32	64	nd	32
5675	**128**	**B/Novosibirsk/1/12-like**	64	64	nd	64
9376	**64**	**B/Novosibirsk/1/12-like**	64	128	nd	128
1430	**64**	**B/Novosibirsk/1/12-like**	32	32	nd	32, *64*
1607	**128**	**B/Novosibirsk/1/12-like**	32	32	nd	32, *64*
3191	**128**	**B/Novosibirsk/1/12-like**	32	32	nd	32, *64*
5010	**128**	**B/Novosibirsk/1/12-like**	32	32	nd	32, *64*
1076	**64**	**B/Novosibirsk/1/12-like**	32	32	nd	32
1919	**128**	**B/Novosibirsk/1/12-like**	64	64	nd	64
1433	**64**	**B/Novosibirsk/1/12-like**	64	64	nd	64

HA titers were established in MDCK-SIAT (S/1)or [§]MDCK cells (MD/1). **HI titers should be multiplied by 8**. *Grey: 2016/17 antigenic table titers.* [@]also sent to the WIC. Vaccine strain.

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

			Antisera			
		Reference viral isolates	**B/Wisconsin/01/10**	**B/Novosibirsk/1/12**	***B/Massachssetts/2/12***	**B/Phuket/3073/13**
		B/Wisconsin/1/10	**256**	128	*32*	512, *64*
		B/Novosibirsk/1/12	32	**128**	*64*	64, *16*
		B/Massachssetts/2/12	*128*	*64*	***256***	*128*
		B/Phuket/3073/13	256	64	*256*	**256, *128***
Isolates	**HA titer**	**Typisation**				
2882	**128**	**B/Novosibirsk/1/12-like**	64	32	nd	64
7895	**64**	**B/Novosibirsk/1/12-like**	32	32	nd	32
3850	**64**	**B/Novosibirsk/1/12-like**	32	32	nd	64
7017	**128**	**B/Novosibirsk/1/12-like**	32	32	nd	64
8117	**32**	**B/Wisconsin/1/10-like**	128	256	nd	128
7853	**32**	**B/Novosibirsk/1/12-like**	64	128	nd	128
8278	**32**	**B/Wisconsin/1/10-like**	512	1024	nd	512
1877	**32**	**B/Wisconsin/1/10-like**	128	256	nd	256

HA titers were established in MDCK-SIAT cells (S/1). **HI titers should be multiplied by 8**. *Grey: 2016/17 antigenic table titers.* @also sent to the WIC. Vaccine strain.

Annex 5: Hemagglutination inhibition of influenza B Victoria lineage viruses

			Antisera			
		Reference viral isolates	**B/Brisbane/60/08**	**B/Hong Kong/514/09**	**B/Johannesburg/3964/12**	**B/Norway/2409/17**
		B/Brisbane/60/08	**128**	64	64	64
		B/Hong Kong/514/09	32	**32**	32	64
		B/Johannesburg/3964/12	256	64	**128**	32
		B/Norway/2409/17	<16	<16	<16	**128**
Isolates	**HA titer**	**Typisation**				
9952@	**64**	**B/Norway/2409/17-like**	<16	<16	<16	64
4139	**64**	**B/Hong Kong/514/09-like**	32	16	16	64
4727	**128**	**B/Hong Kong/514/09-like**	32	16	16	64
5355	**64**	**B/Brisbane/60/08-like**	64	64	64	256

HA titers were established in MDCK-SIAT cells (S/1). **HI titers should be multiplied by 8**. *Grey: 2016/17 antigenic table titers.* @also sent to the WIC. Vaccine strain.

Annex 6a: Antigenic analyses of influenza A(H1N1)pdm09 viruses, WIC

Viruses	Other information		Collection date	Passage history	Post-infection ferret antisera										
					A/Mich	A/Cal	A/Bayern	A/Lviv	A/Astrak	A/St. P	A/St. P	A/HK	A/Sth Afr	A/Slov	A/Israel
					45/15	7/09	69/09	N6/09	1/11	27/11	100/11	5659/12	3626/13	2903/2015	Q-504/15
		Passage history			Egg	Egg	MDCK	MDCK	MDCK	Egg	Egg	MDCK	Egg	Egg	MDCK
		Ferret number			NIB F42/16*1	F06/16*1	F09/15*1	F14/13*1	F22/13*1	F26/14*1	F24/11*1	F30/12*1	F03/14*1	F02/16*2	F08/16*2
		Genetic group			6B.1				5	6	7	6A	6B	6B.1	6B.2
REFERENCE VIRUSES															
A/Michigan/45/2015		6B.1	2015-09-07	E3/E3	1280	1280	640	320	1280	320	2560	1280	1280	2560	1280
A/California/7/2009 Clone38-32			2009-04-09	E3/E3	640	640	640	320	640	320	2560	640	640	1280	640
A/Bayern/69/2009	G155E		2009-07-01	MDCK5/MDCK1	<	<	160	160	40	<	40	40	40	<	<
A/Lviv/N6/2009	G155E, D222G		2009-10-27	MDCK4/SIAT1/MDCK3	80	80	1280	1280	80	80	160	160	80	320	80
A/Astrakhan/1/2011		5	2011-02-28	MDCK1/MDCK5	640	640	640	320	1280	320	2560	640	640	1280	640
A/St. Petersburg/27/2011		6	2011-02-14	E1/E3	640	640	640	640	640	320	2560	640	640	1280	640
A/St. Petersburg/100/2011		7	2011-03-14	E1/E5	640	640	320	320	640	320	1280	640	640	1280	640
A/Hong Kong/5659/2012		6A	2012-05-21	MDCK4/MDCK2	160	160	80	80	320	80	640	320	160	320	160
A/South Africa/3626/2013		6B	2013-06-06	E1/E3	640	320	320	640	640	320	1280	320	640	1280	320
A/Slovenia/2903/2015	clone 37	6B.1	2015-10-26	E4/E2	640	640	320	320	1280	320	2560	640	640	2560	1280
A/Israel/Q-504/2015		6B.2	2015-12-15	C1/MDCK2	320	320	320	160	640	160	1280	320	640	1280	640
TEST VIRUSES															
A/Switzerland/892/2017		6B.1	2017-02-21	MDCK1	640	640	320	320	640	320	2560	640	640	2560	640
* Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used)					Vaccine										
1 < = <40	2017-11-15														

Annex 6b: Antigenic analyses of influenza A(H1N1)pdm09 viruses, WIC

Viruses	Other information		Collection date	Passage history	Post-infection ferret antisera											
					A/Mich	A/Cal	A/Bayern	A/Lviv	A/Astrak	A/St. P	A/St. P	A/HK	A/Sth Afr	A/Slov	A/Israel	A/Paris
					45/15	7/09	69/09	N6/09	1/11	27/11	100/11	5659/12	3626/13	2903/2015	Q-504/15	1447/17
		Passage history			Egg	Egg	MDCK	MDCK	MDCK	Egg	Egg	MDCK	Egg	Egg	MDCK	MDCK
		Ferret number			NIB F42/16[*1]	F06/16[*1]	F09/15[*1]	F14/13[*1]	F22/13[*1]	F26/14[*1]	F24/11[*1]	F30/12[*1]	F03/14[*1]	F02/16[*1]	F08/16[*1]	F03/18[*2]
		Genetic group			6B.1				5	6	7	6A	6B	6B.1	6B.2	
REFERENCE VIRUSES																
A/Michigan/45/2015		6B.1	2015-09-07	E3/E3	1280	640	320	320	640	320	2560	640	640	1280	1280	1280
A/California/7/2009	Clone38-32		2009-04-09	E3/E3	1280	1280	640	640	1280	640	2560	640	640	1280	1280	2560
A/Bayern/69/2009	G155E		2009-07-01	MDCK5/MDCK1	40	40	320	320	40	40	80	40	40	80	40	320
A/Lviv/N6/2009	G155E, D222G		2009-10-27	IDCK4/SIAT1/MDCK3	80	160	640	640	80	80	160	80	80	160	80	640
A/Astrakhan/1/2011		5	2011-02-28	MDCK1/MDCK6	640	640	640	320	640	320	2560	640	640	1280	640	1280
A/St. Petersburg/27/2011		6	2011-02-14	E1/E3	1280	640	640	640	640	640	2560	640	640	1280	1280	2560
A/St. Petersburg/100/2011		7	2011-03-14	E1/E4	320	320	320	320	640	320	1280	640	640	1280	640	
A/Hong Kong/5659/2012		6A	2012-05-21	MDCK4/MDCK2	320	640	160	160	640	320	640	640	320	640	320	1280
A/South Africa/3626/2013		6B	2013-06-06	E1/E3	640	640	320	320	640	320	1280	320	640	1280	640	1280
A/Slovenia/2903/2015	clone 37	6B.1	2015-10-26	E4/E2	640	640	320	320	640	320	2560	640	320	1280	640	1280
A/Israel/Q-504/2015		6B.2	2015-12-15	C1/MDCK2	640	640	320	320	640	320	2560	640	640	1280	1280	1280
A/Paris/1447/2017				MDCK1/MDCK3	640	320	320	160	320	320		320	320	640	640	1280
TEST VIRUSES																
A/Switzerland/707/2018		6B.1	2018-01-05	SIAT1/MDCK1	640	640	320	320	640	320	ND	640	640	1280	1280	2560
A/Switzerland/320/2018		6B.1	2018-01-05	SIAT1/MDCK1	1280	640	320	320	640	320	ND	640	640	1280	1280	2560
A/Switzerland/6136/2018		6B.1	2018-01-04	SIAT1/MDCK1	640	640	640	320	640	320	ND	640	640	1280	1280	2560
A/Switzerland/6079/2017		6B.1	2017-12-29	SIAT1/MDCK1	640	640	320	160	640	320	ND	640	640	1280	640	2560
A/Switzerland/4062/2017		6B.1	2017-12-29	SIAT1/MDCK1	640	320	320	160	640	320	ND	320	320	1280	640	2560
A/Switzerland/683/2017		6B.1	2017-12-29	SIAT1/MDCK1	1280	640	640	320	640	640	ND	640	1280	2560	1280	2560
A/Switzerland/656/2017		6B.1	2017-12-21	SIAT1/MDCK1	640	640	320	320	640	320	ND	640	640	1280	1280	2560
A/Switzerland/198/2017		6B.1	2017-12-19	SIAT1/MDCK1	640	320	320	160	320	160	ND	320	320	1280	640	2560
A/Switzerland/8198/2017			2017-12-19	MDCK1	640	640	320	320	640	320	ND	640	640	1280	1280	2560
A/Switzerland/3191/2017		6B.1	2017-12-21	MDCK2	640	320	320	160	640	320	1280	640	640	1280	640	ND
A/Switzerland/2656/2017		6B.1	2017-12-21	MDCK1	640	640	320	320	640	320	1280	640	640	2560	1280	ND
A/Switzerland/3330/2017		6B.1	2017-12-20	MDCK1	640	640	640	320	1280	320	2560	1280	640	2560	1280	ND
A/Switzerland/0007/2017		6B.1	2017-12-05	MDCK1	640	320	320	160	640	320	1280	640	640	1280	640	ND

Assay	HI (Turkey RBC)	[1]	< = <40		Vaccine
RBC	Turkey	[2]	< = <80	2018-03-07	
Virus	A(H1N1)pdm09	ND	Not Done		
*	Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used)				

Annex 7a: Antigenic analyses of influenza A(H3N2), WIC

Viruses	Other information	Passage history Ferret number Genetic group	Collection date	Passage history	Post-infection ferret antisera								
					A/Stock 6/14 SIAT F14/14*1 3C.3a	A/Switz 9715293/13 SIAT F18/15[1] 3C.3a	A/HK 5738/14 MDCK F30/14*1 3C.2a	A/HK 4801/14 MDCK F43/15*1 3C.2a	A/HK 4801/14 Egg F42/15*1 3C.2a	A/Oman 2585/16 SIAT NIB F50/16*1 3C.2a1	A/Nor 4436/16 SIAT F03/17*1 3C.2a1	A/Greece 4/17 SIAT F27/17*1 3C.2a1	A/Singapore 0019/16 Egg 10-4 F41/17*1 3C.2a1
REFERENCE VIRUSES													
A/Stockholm/6/2014		3C.3a	2014-02-06	SIAT1/SIAT2	320	160	160	160	80	320	160	320	320
A/Switzerland/9715293/2013		3C.3a	2013-12-06	SIAT1/SIAT3	320	160	160	80	40	160	160	160	80
A/Hong Kong/5738/2014		3C.2a	2014-04-30	MDCK1/MDCK2/SIAT3	160	80	160	640	160	320	320	320	640
A/Hong Kong/4801/2014		3C.2a	2014-02-26	MDCK4/MDCK3	320	160	160	320	80	320	320	320	640
A/Hong Kong/4801/2014	isolate 1	3C.2a	2014-02-26	E6/E2	80	40	320	640	1280	640	320	640	2560
A/Singapore/INFIMH-16-0019/2016		3C.2a1	2016-06-14	E5/E2	<	ND	80	320	320	80	80	>320	1280
TEST VIRUSES													
A/Switzerland/882/2017		3C.2a	2017-10-11	SIAT1	320	160	160	320	40	160	320	160	40
									Vaccine NH 2017-18				Vaccine SH 2018

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

[1] < = <40 Guinea Pig RBC with 20nM Oseltamivir 17.11.2017

Annex 7b: Antigenic analyses of influenza A(H3N2), WIC

Viruses	Other information	Passage history / Ferret number / Genetic group	Collection date	Passage history	A/Stock 6/14 SIAT F14/14[*1] 3C.3a	A/HK 5738/14 MDCK F30/14[*1] 3C.2a	A/HK 4801/14 Egg F42/15[*1] 3C.2a	A/Bretagne 1413/17 SIAT F01/18 3C.2a	A/Oman 2585/16 SIAT NIB F50/16[*1] 3C.2a1	A/Nor 4436/16 SIAT F03/17[*1] 3C.2a1	A/Greece 4/17 SIAT F27/17[*1] 3C.2a1	A/Singapore 0019/16 Egg 10-4 F41/17*1 3C.2a1
					Post-infection ferret antisera							
REFERENCE VIRUSES												
A/Stockholm/6/2014		3C.3a	2014-02-06	SIAT1/SIAT2	320	160	80	160	160	320	160	160
A/Hong Kong/5738/2014		3C.2a	2014-04-30	MDCK1/MDCK2/SIAT3	160	160	160	160	320	320	160	320
A/Hong Kong/4801/2014	isolate 1	3C.2a	2014-02-26	E6/E2	80	320	1280	640	640	320	640	2560
A/Bretagne/1413/2017		3C.2a		MDCK1/SIAT4	160	160	80	640	160	320	160	160
A/Singapore/INFIMH-16-0019/2016		3C.2a1	2016-06-14	E5/E1	40	40	320	80	160	160	160	640
TEST VIRUSES												
A/Switzerland/143/2017		3C.2a2	2017-12-15	SIAT1/SIAT2	160	80	40	320	160	320	160	80

Assay HI (Guinea Pig RBC with 20nM Oseltamivir)	*	Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used)	Vaccine SH 2018
RBC Guinea Pig	1	< = <40	NH 2018
Virus Influenza A(H3N2)	ND	Not Done 2018-02-23	

Annex 8a: Antigenic analyses of influenza B viruses (Yamagata lineage), WIC

Viruses		Collection date	Passage history	Post-infection ferret antisera										
				B/Phuket 3073/13	B/Fl 4/06	B/Bris 3/07	B/Estonia 55669/11	B/Mass 02/12	B/Mass 02/12	B/Wis 1/10	B/Stock 12/11	B/Phuket 3073/13	B/Phuket 3073/13	B/HK 3417/14
	Passage history			Egg	Egg	Egg	MDCK	MDCK	Egg	Egg	Egg	MDCK	Egg	Egg
	Ferret number			SH614*1,3	F17/13*1	F38/14*2	F27/13*2	F05/15*2	F16/14*1	F36/15*2	F06/15*2	F27/15*4	NIB F51/16*2	St Judes F715/14*2,4
	Genetic Group			3	1	2	2	2	2	3	3	3	3	3
REFERENCE VIRUSES														
B/Florida/4/2006	1	2006-12-15	E7/E1	1280	640	640	80	80	1280	160	160	40	640	160
B/Brisbane/3/2007	2	2007-09-03	E2/E2	1280	640	640	80	80	1280	80	160	40	640	160
B/Estonia/55669/2011	2	2011-03-14	MDCK2/MDCK3	640	40	40	320	160	80	40	10	40	40	80
B/Massachusetts/02/2012	2	2012-03-13	MDCK1/C2/MDCK4	2560	320	320	320	640	320	320	80	160	320	160
B/Massachusetts/02/2012	2	2012-03-13	E3/E4	640	640	640	160	80	1280	160	160	20	640	320
B/Wisconsin/1/2010	3	2010-02-20	E3/E2	1280	160	160	20	<	160	80	40	40	320	80
B/Stockholm/12/2011	3	2011-03-28	EX/E2	5120	320	160	40	20	320	160	80	80	320	160
B/Phuket/3073/2013	3	2013-11-21	MDCK2/MDCK2	5120	160	160	160	320	320	320	160	320	320	160
B/Phuket/3073/2013	3	2013-11-21	E4/E3	1280	160	80	10	<	160	80	40	20	320	80
B/Hong Kong/3417/2014	3	2014-06-04	E4/E1	640	40	40	10	<	80	40	10	20	80	80
TEST VIRUSES														
B/Switzerland/901/2017	3	2017-04-06	MDCK1	1280	40	20	20	10	40	20	10	40	40	80
													Vaccine#	

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

[1] < = <40 [3] hyperimmune sheep serum 15.11.2017

[2] < = <10 [4] RDE serum pre-absorbed with TRBC

[#] B/Yamagata-lineage virus recommended for use in quadravalent vaccines

Annex 8b: Antigenic analyses of influenza B viruses (Yamagata lineage), WIC

Viruses	Other information	Passage history / Ferret number / Genetic Group	Collection date	Passage history	Post-infection ferret antisera										
					B/Phuket 3073/13	B/Bris 3/07	B/Estonia 55669/11	B/Mass 02/12	B/Mass 02/12	B/Wis 1/10	B/Stock 12/11	B/Phuket 3073/13	B/Phuket 3073/13	B/Maur 1791/17	B/HK 3417/14
		Passage history			Egg	Egg	MDCK	MDCK	Egg	Egg	Egg	MDCK	Egg	MDCK	Egg
		Ferret number			SH614[*1,3]	F38/14[*2]	F27/13[*2]	F05/15[*2]	F16/14[*2]	F36/15[*2]	F06/15[*2]	F27/15[*2]	NIB F51/16[*#]	F04/18[*1]	St Judes F716/14[*2]
		Genetic Group			3	2	2	2	2	3	3	3	3		3
REFERENCE VIRUSES															
B/Brisbane/3/2007	196-198 DKT	2	2007-09-03	E2/E2	2560	1280	160	320	1280	320	320	160	640	20	320
B/Estonia/55669/2011	196-198 NKT	2	2011-03-14	MDCK2/MDCK3	5120	160	640	640	320	160	80	320	80	320	10
B/Massachusetts/02/2012	196-198 NK(T=P)	2	2012-03-13	MDCK1/C2/MDCK3	2560	640	640	320	640	160	160	160	640	80	320
B/Massachusetts/02/2012	196-198 DKT	2	2012-03-13	E3/E3	1280	320	80	40	640	80	80	40	160	<	160
B/Wisconsin/1/2010	196-198 (D>N)KT	3	2010-02-20	E3/E2	2560	320	40	20	640	160	160	80	640	80	80
B/Stockholm/12/2011		3	2011-03-28	E4/E1	1280	160	40	10	320	80	80	40	160	40	40
B/Phuket/3073/2013	196-198 NKT	3	2013-11-21	MDCK2/MDCK3	5120	160	160	160	320	160	160	320	320	320	80
B/Phuket/3073/2013	196-198 DKT	3	2013-11-21	E4/E3	1280	80	40	10	160	80	40	40	320	40	80
B/Mauritius/1791/2017		3		MDCK1/MDCK3	5120	320	320	320	320	320	160	320	320	320	ND
B/Hong Kong/3417/2014	196-198 NKN	3	2014-06-04	E4/E3	2560	80	40	20	80	80	40	20	160	ND	160
TEST VIRUSES															
B/Switzerland/8125/2017		3	2017-12-21	MDCK1MDCK1	1280	160	160	40	320	320	40	320	320	320	ND
B/Switzerland/2734/2017		3	2017-12-20	MDCK1	1280	80	80	20	160	80	20	80	80	160	ND
B/Switzerland/8231/2017		3	2017-12-19	MDCK1	1280	80	40	20	80	80	20	80	160	80	ND
B/Switzerland/7218/2017		3	2017-12-19	MDCK1	1280	80	40	20	80	80	20	80	80	80	ND
B/Switzerland/2469/2017		3	2017-12-19	MDCK1	2560	80	80	20	160	80	20	80	80	160	ND
B/Switzerland/0720/2017		3	2017-12-13	MDCK1	2560	80	40	20	80	80	20	80	80	160	ND
B/Switzerland/8274/2017		3	2017-12-11	MDCK1	1280	80	40	20	80	80	20	80	80	80	ND
B/Switzerland/8142/2017		3	2017-12-11	MDCK1	2560	80	80	20	160	80	20	80	80	160	ND
B/Switzerland/8089/2017		3	2017-12-11	MDCK1	1280	40	40	20	80	40	20	40	40	80	ND
B/Switzerland/0707/2017		3	2017-12-11	MDCK1	1280	80	40	20	80	80	20	80	40	80	ND
B/Switzerland/2696/2017		3	2017-12-20	MDCK2	2560	40	40	20	80	40	40	40	40	80	ND
													Vaccine NH 2015-16 SH2016[#]		

Assay HI (Turkey RBC)

RBC Turkey
Virus Influenza B/Yamagata-lineage
Date 20.02.2018

[1]< = <40
[2]< = <10
[5]< = <20

[3]hyperimmune sheep serum
[4]RDE serum pre-absorbed with TRBC
Not Done

*Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used)
[#]B/Yamagata-lineage virus recommended for use in quadravalent vaccines

Annex 8c: Antigenic analyses of influenza B viruses (Yamagata lineage), WIC

Viruses	Other information		Collection date	Passage history	Post-infection ferret antisera										
					B/Phuket 3073/13	B/Bris 3/07	B/Estonia 55669/11	B/Mass 02/12	B/Mass 02/12	B/Wis 1/10	B/Stock 12/11	B/Phuket 3073/13	B/Phuket 3073/13	B/Maur 1791/17	B/HK 3417/14
		Passage history			Egg	Egg	MDCK	MDCK	Egg	Egg	Egg	MDCK	Egg	MDCK	Egg
		Ferret number			SH614[*1,3]	F38/14[*2]	F27/13[*2]	F05/15[*2]	F16/14[*2]	F36/15[*2]	F06/15[*2]	F27/15[*2]	NIB F51/16[*2]	F04/18[*1]	St Judes F716/14[*2]
		Genetic Group			3	2	2	2	2	3	3	3	3		3
REFERENCE VIRUSES															
B/Brisbane/3/2007	196-198 DKT	2	2007-09-03	E2/E2	2560	1280	160	320	1280	320	320	160	640	20	320
B/Estonia/55669/2011	196-198 NKT	2	2011-03-14	MDCK2/MDCK3	5120	160	640	640	320	160	80	320	80	320	10
B/Massachusetts/02/2012	196-198 NK(T=P	2	2012-03-13	MDCK1/C2/MDCK3	2560	640	640	320	640	160	160	160	640	80	320
B/Massachusetts/02/2012	196-198 DKT	2	2012-03-13	E3/E3	1280	320	80	40	640	80	80	40	160	<	160
B/Wisconsin/1/2010	196-198 (D>N)K	3	2010-02-20	E3/E2	2560	320	40	20	640	160	160	80	640	80	80
B/Stockholm/12/2011		3	2011-03-28	E4/E1	1280	160	40	10	320	80	80	40	160	40	40
B/Phuket/3073/2013	196-198 NKT	3	2013-11-21	MDCK2/MDCK3	5120	160	160	160	320	160	160	320	320	320	80
B/Phuket/3073/2013	196-198 DKT	3	2013-11-21	E4/E3	1280	80	40	10	160	80	40	40	320	40	80
B/Mauritius/1791/2017		3		MDCK1/MDCK3	5120	320	320	320	320	320	160	320	320	320	ND
B/Hong Kong/3417/2014	196-198 NKN	3	2014-06-04	E4/E3	2560	80	40	20	80	80	40	20	160	ND	160
TEST VIRUSES															
B/Switzerland/0747/2017		3	2017-12-06	MDCK1	2560	80	80	20	80	80	40	80	80	80	ND
B/Switzerland/0558/2017		3	2017-12-06	MDCK1	2560	80	80	40	160	80	80	80	80	80	ND
B/Switzerland/7069/2017		3	2017-12-04	MDCK1	2560	80	40	20	80	80	40	80	80	80	ND
B/Switzerland/1042/2017		3	2017-11-30	MDCK1	1280	40	40	20	80	80	40	80	80	80	ND
B/Switzerland/0968/2017			2017-11-30	MDCK1	1280	80	40	20	80	80	40	80	80	80	ND
B/Switzerland/0226/2017		3	2017-11-21	MDCK1	1280	80	40	20	80	80	40	80	80	80	ND
B/Switzerland/3147/2017		3	2017-12-21	MDCK1	2560	160	80	40	80	80	40	80	160	ND	160
B/Switzerland/3106/2017		3	2017-12-21	MDCK1	2560	160	80	80	160	160	80	80	320	ND	160
B/Switzerland/2959/2017		3	2017-12-21	MDCK1	2560	80	80	40	80	80	40	80	160	ND	80
B/Switzerland/2945/2017		3	2017-12-20	MDCK1	2560	160	80	80	80	160	40	80	160	ND	160
B/Switzerland/2911/2017		3	2017-12-20	MDCK1	2560	80	80	40	80	80	40	80	160	ND	160
B/Switzerland/2779/2017		3	2017-12-20	MDCK1	2560	160	80	40	80	80	40	80	160	ND	160
													Vaccine NH 2015-16 SH2016[#]		

Assay HI (Turkey RBC)

RBC Turkey

Virus Influenza B/Yamagata-lineage

Date 20.02.2018

[1] < = <40
[2] < = <10
[5] < = <20

[3] hyperimmune sheep serum
[4] RDE serum pre-absorbed with TRBC
Not Done

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

[#] B/Yamagata-lineage virus recommended for use in quadravalent vaccines

Annex 8d: Antigenic analyses of influenza B viruses (Yamagata lineage), WIC

Viruses	Other information	Genetic Group	Collection date	Passage history	Haemagglutination inhibition titre									
					B/Phuket 3073/13	B/Bris 3/07	B/Estonia 55669/11	B/Mass 02/12	B/Mass 02/12	B/Wis 1/10	B/Stock 12/11	B/Phuket 3073/13	B/Phuket 3073/13	B/Maur 1791/17
		Passage history			Egg	Egg	MDCK	MDCK	Egg	Egg	Egg	MDCK	Egg	MDCK
		Ferret number			SH614[*1,3]	F38/14[*2]	F27/13[*2]	F05/15[*2]	F16/14[*2]	F36/15[*2]	F06/15[*2]	F27/15[*2]	F37/15[*2]	F04/18[*1]
		Genetic Group			3	2	2	2	2	3	3	3	3	
REFERENCE VIRUSES														
B/Brisbane/3/2007	196-198 DKT	2	2007-09-03	E2/E2	2560	640	320	160	1280	320	320	80	320	40
B/Estonia/55669/2011	196-198 NKT	2	2011-03-14	MDCK2/MDCK3	2560	320	640	640	320	320	160	320	160	320
B/Massachusetts/02/2012	196-198 NK(T=P)	2	2012-03-13	MDCK1/C2/MDCK3	1280	320	320	160	640	320	160	80	160	40
B/Massachusetts/02/2012	196-198 DKT	2	2012-03-13	E3/E3	640	320	160	40	640	80	80	20	80	<
B/Wisconsin/1/2010	196-198 (D>N)KT	3	2010-02-20	E3/E2	2560	320	40	20	320	160	160	80	160	80
B/Stockholm/12/2011		3	2011-03-28	E4/E1	1280	160	40	10	160	160	160	40	80	40
B/Phuket/3073/2013	196-198 NKT	3	2013-11-21	MDCK2/MDCK3	5120	160	320	320	320	320	160	320	160	640
B/Phuket/3073/2013	196-198 DKT	3	2013-11-21	E4/E3	1280	160	40	10	160	160	80	40	160	40
B/Mauritius/1791/2017				MDCK1/MDCK3	5120	320	320	640	640	640	160	640	320	640
TEST VIRUSES														
B/Switzerland/20051/2018		3	2018-01-08	SIAT1/MDCK1	5120	320	320	640	640	640	320	640	640	640
B/Switzerland/903/2018		3	2018-01-08	SIAT1/MDCK1	5120	320	160	160	320	320	160	320	160	640
B/Switzerland/423/2018		3	2018-01-08	SIAT1/MDCK1	5120	320	640	1280	640	1280	320	640	640	640
B/Switzerland/653/2018		3	2018-01-05	SIAT1/MDCK1	5120	320	320	320	320	640	160	640	320	640
B/Switzerland/995/2018		3	2018-01-04	SIAT1/MDCK1	5120	320	320	640	320	640	160	320	320	640
B/Switzerland/742/2018		3	2018-01-03	SIAT1/MDCK1	5120	160	160	320	320	320	160	320	320	320
B/Switzerland/936/2018		3	2018-01-02	SIAT1/MDCK1	2560	160	160	80	160	160	80	160	160	320
B/Switzerland/150/2017		3	2017-12-30	SIAT1/MDCK1	2560	160	160	160	160	320	80	160	160	320
B/Switzerland/6093/2017		3	2017-12-29	SIAT1/MDCK1	5120	160	160	160	320	320	160	160	160	320
B/Switzerland/296/2017		3	2017-12-27	SIAT1/MDCK1	5120	320	320	320	320	320	160	320	320	640
B/Switzerland/652/2017		3	2017-12-22	SIAT1/MDCK1	5120	160	80	80	160	320	80	160	160	320
B/Switzerland/614/2017		3	2017-12-22	SIAT1/MDCK1	5120	320	320	640	640	640	160	640	320	640
B/Switzerland/726/2017		3	2017-12-21	SIAT1/MDCK1	5120	320	320	320	320	640	160	320	320	640
B/Switzerland/909/2017		3	2017-12-19	SIAT1/MDCK1	5120	160	160	80	320	320	160	160	160	320
B/Switzerland/231/2017		3	2017-12-19	SIAT1/MDCK1	2560	80	80	40	160	160	80	80	40	160
B/Switzerland/218/2017		3	2017-12-19	SIAT1/MDCK1	5120	160	160	80	160	320	80	160	160	320
													Vaccine NH 2015-16 SH2016[#]	

Assay H (Turkey RBC)

RBC Turkey

Virus Influenza B/Yamagata-lineage

Date 06.03.2018

[1] < = <40

[2] < = <10

[5] < = <20

[3] hyperimmune sheep serum

[4] RDE serum pre-absorbed with TRBC

Not Done

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

[#] B/Yamagata-lineage virus recommended for use in quadravalent vaccines

Annex 9: Antigenic analyses of influenza B viruses (Victoria lineage), WIC

Viruses	Passage history / Ferret number / Genetic group	Collection date	Passage history	Post-infection ferret antisera										
	Passage history			B/Bris 60/08 Egg	B/Mal 2506/04 Egg	B/Bris 60/08 Egg	B/Malta 636714/11 Egg	B/Jhb 3964/12 Egg	B/For V2367/12 MDCK	B/Sth Aus 81/12 Egg	B/HK 514/09 MDCK	B/Ireland 3154/16 MDCK	B/Nord-West 1/16 MDCK	B/Nor 2409/17 MDCK
	Ferret number			Sh 539,540,543,544,570,571,574*1	F41/14[*2]	NIB F52/16[*2]	F29/13[*2]	F04/16[*2]	F09/16[*2]	F25/16[*2]	F09/13[*2]	F15/16[*2]	F16/16[*2]	F40/17[*2]
	Genetic group			1A		1A	1A	1A	1A	1A	1B	1A	1A	1A(Δ2)
REFERENCE VIRUSES														
B/Malaysia/2506/2004		2004-12-06	E3/E6	2560	320	160	160	40	160	160	20	<	<	<
B/Brisbane/60/2008	1A	2008-08-04	E4/E4	2560	160	640	320	160	320	640	80	40	40	<
B/Malta/636714/2011	1A	2011-03-07	E4/E1	1280	80	320	320	80	320	320	40	20	40	<
B/Johannesburg/3964/2012	1A	2012-08-03	E1/E2	5120	320	1280	1280	640	1280	1280	160	80	160	<
B/Formosa/V2367/2012	1A	2012-08-06	MDCK1/MDCK3	5120	40	320	320	80	320	640	80	80	80	<
B/South Australia/81/2012	1A	2012-11-28	E4/E2	2560	160	640	320	160	320	640	80	40	40	<
B/Hong Kong/514/2009	1B	2009-10-11	MDCK1/MDCK2	5120	10	40	160	160	320	40	160	80	160	<
B/Ireland/3154/2016	1A	2016-01-14	MDCK1/MDCK4	5120	<	20	40	10	80	40	80	160	160	<
B/Nordrhein-Westfalen/1/2016	1A	2016-01-04	C2/MDCK2	2560	<	20	40	40	80	<	80	80	80	<
B/Norway/2409/2017	1A(Δ2)		MDCK1/MDCK2	80	<	<	<	<	<	<	<	<	<	80
TEST VIRUSES														
B/Switzerland/952/2018	1A(Δ2)	2018-01-05	SIAT1/MDCK1	80	<	<	<	<	<	<	<	<	<	80
						Vaccine NH 2015-16[#] SH 2016								

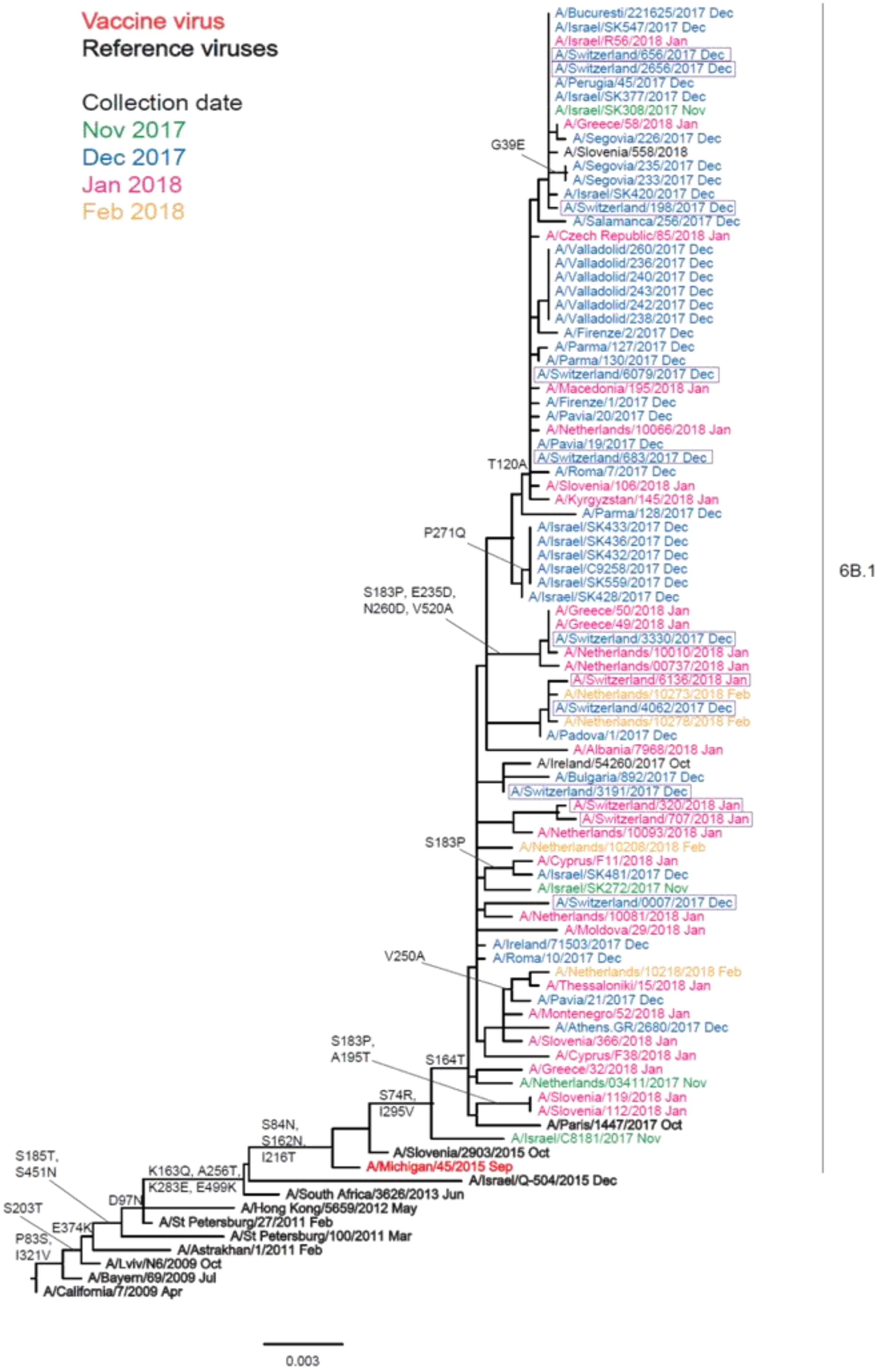
Assay HI (Turkey RBC)

RBC	Turkey	[1]	< = <40	[4]	< = <20
Virus	Influenza B/Victoria-lineage	[2]	< = <10	ND	Not Done
Date	2018-02-06	[3]	hyperimmune sheep serum		

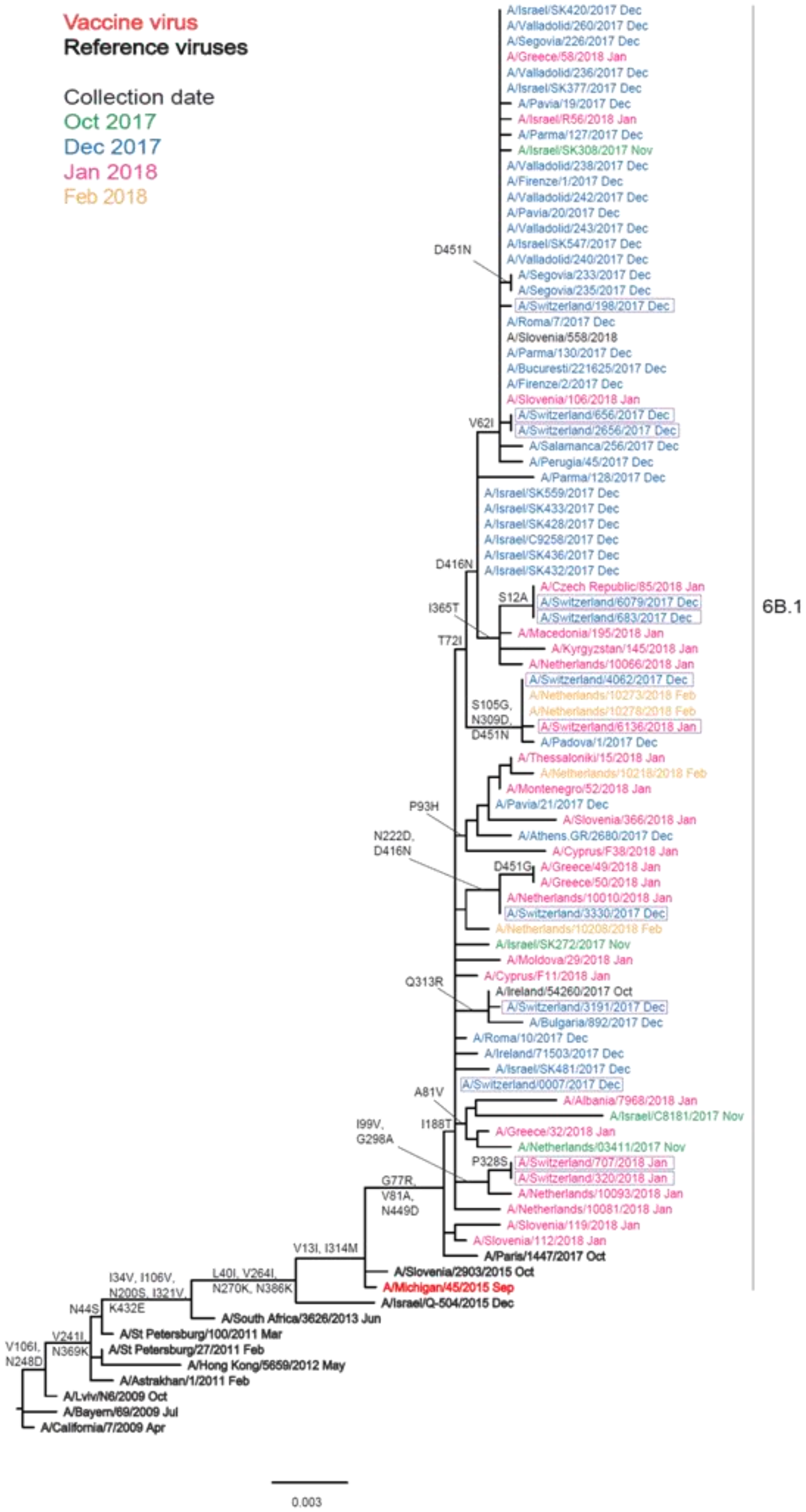
* Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used)

[#] B/Victoria-lineage virus recommended for use in quadravalent vaccines

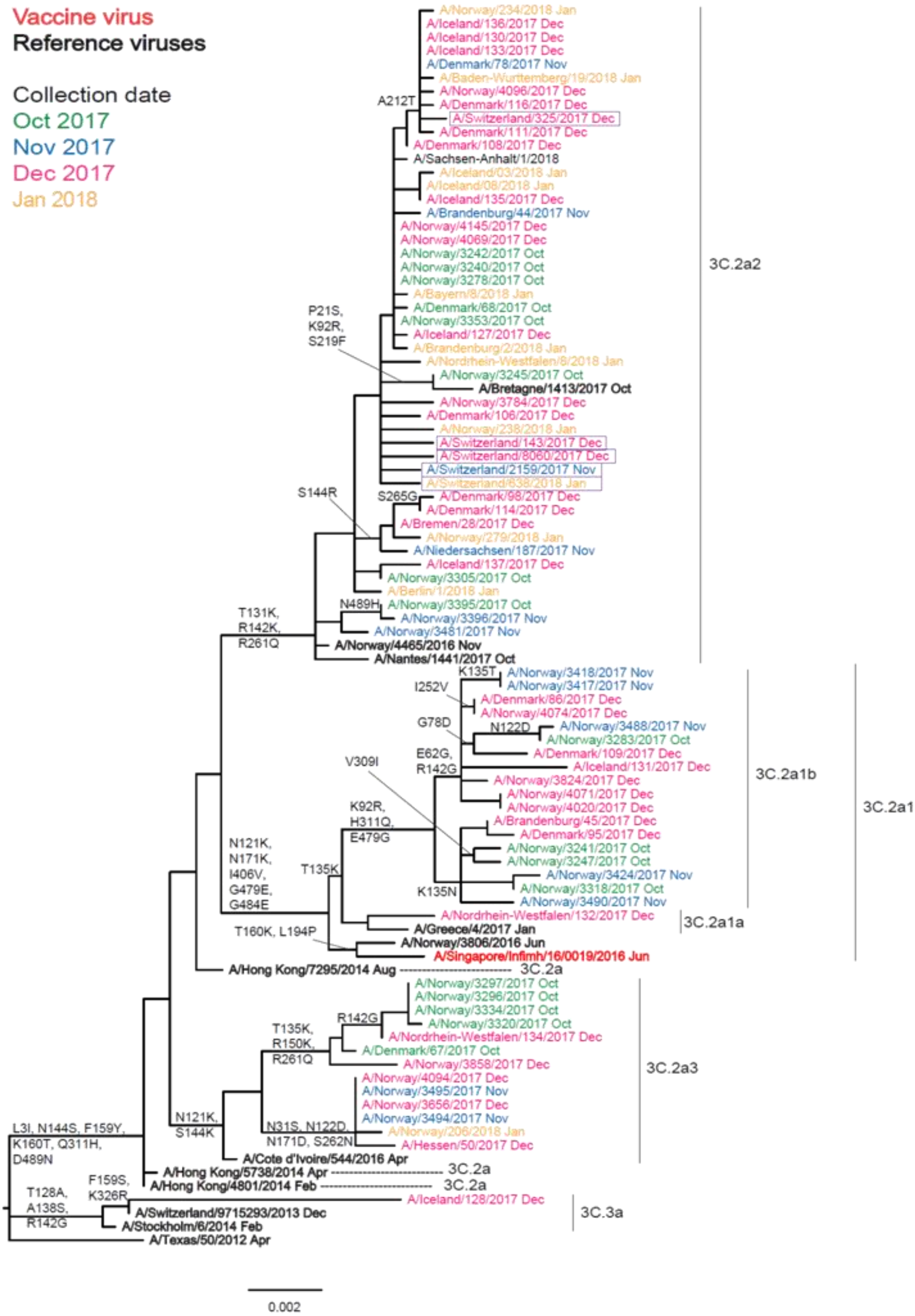
Annex 10: Phylogenetic comparison of influenza A(H1N1)pdm09, Hemagglutinin gene, WIC



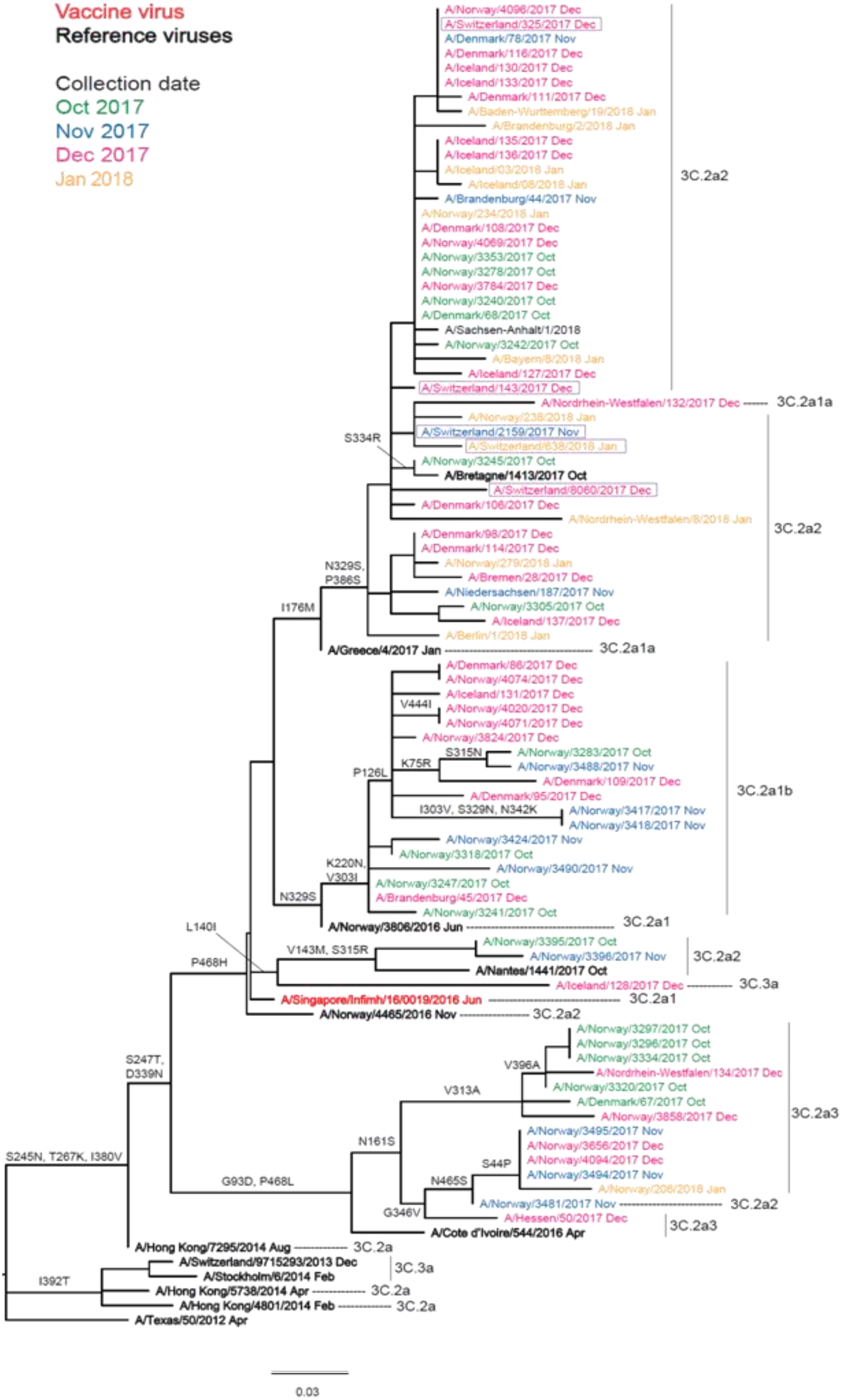
Annex 11: Phylogenetic comparison of influenza A(H1N1)pdm09, Neuraminidase gene, WIC



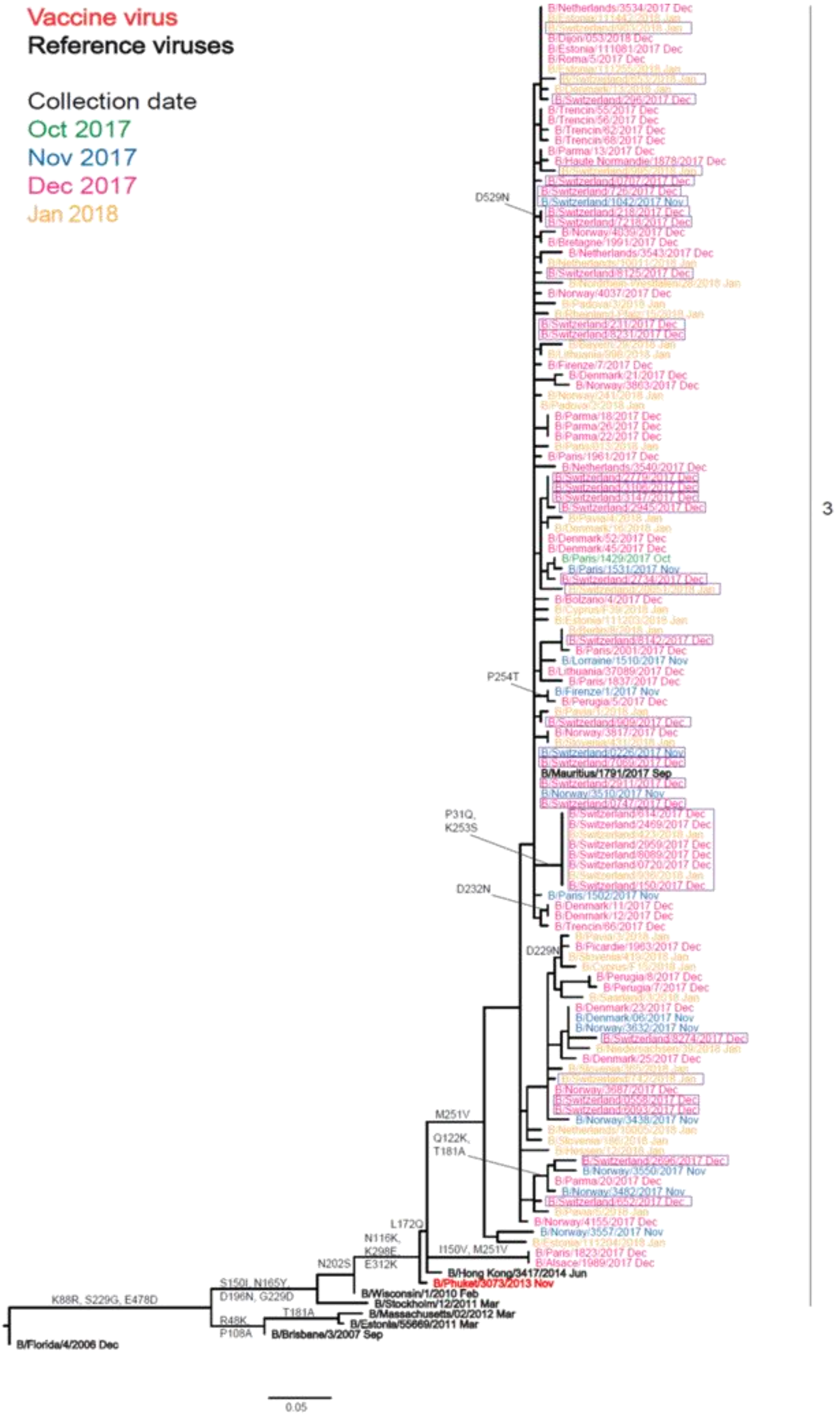
Annex 12: Phylogenetic comparison of influenza A(H3N2), Hemagglutinin gene, WIC



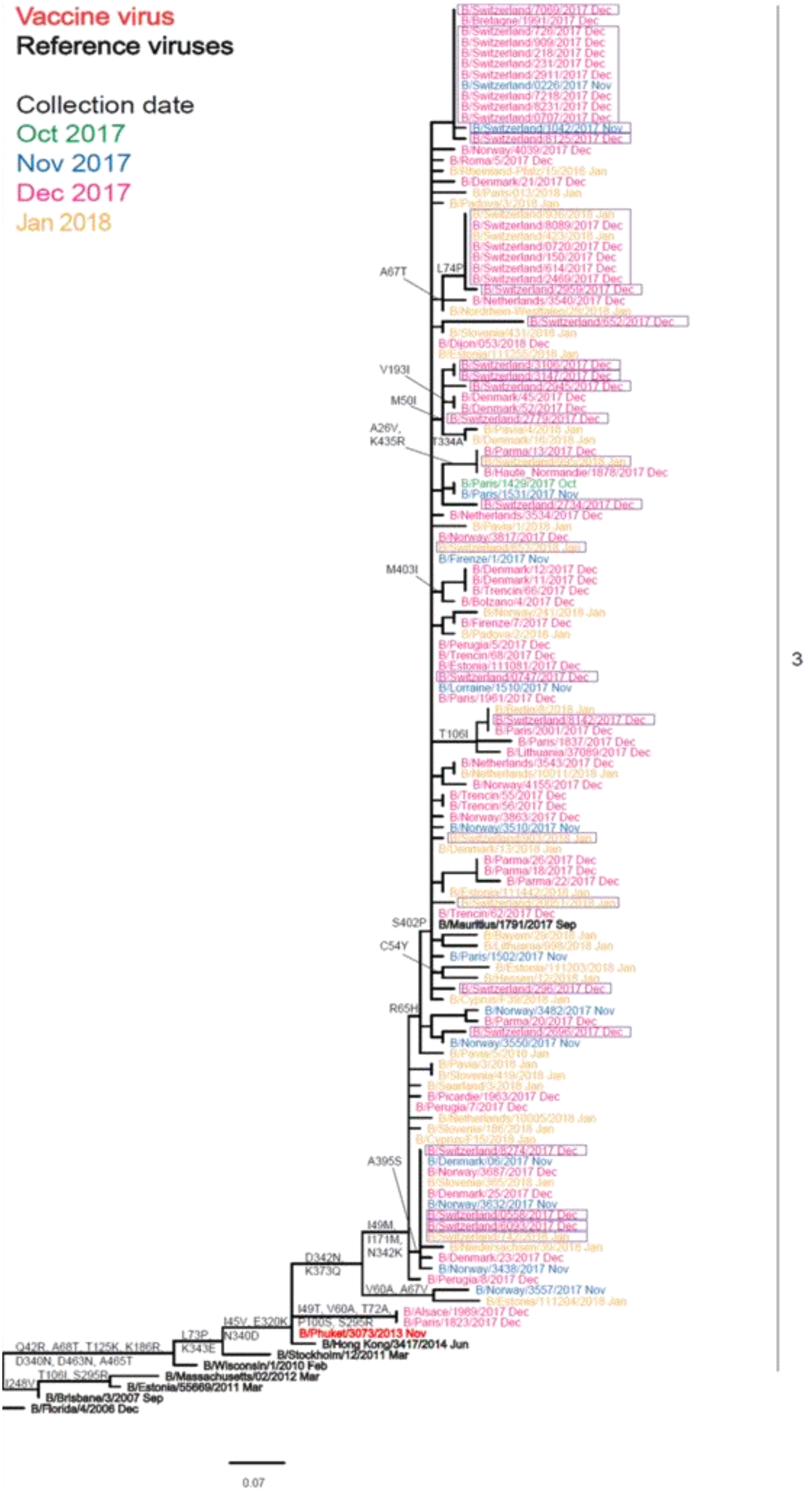
Annex 13: Phylogenetic comparison of influenza A(H3N2), Neuraminidase gene, WIC



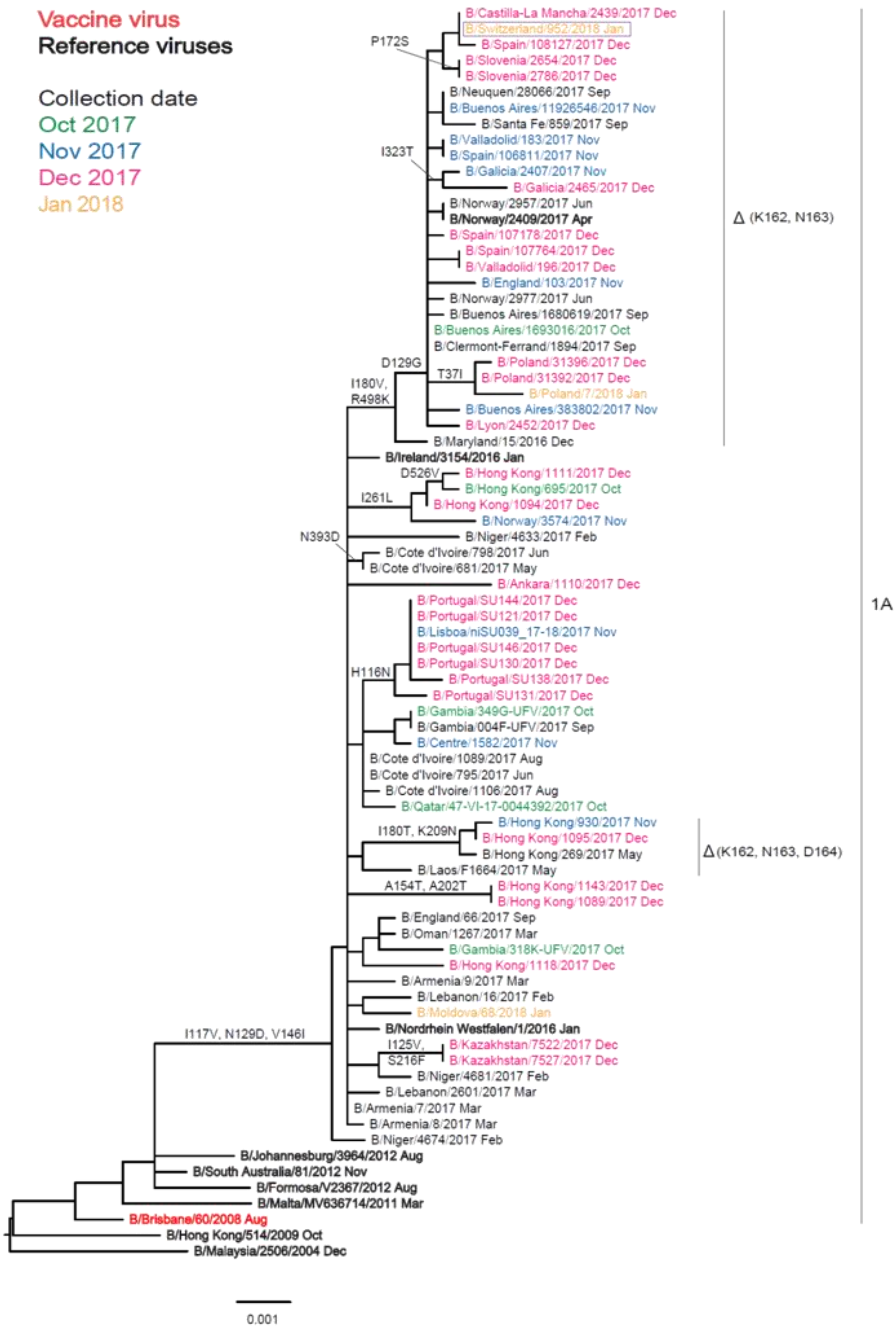
Annex 14: Phylogenetic comparison of influenza B Yamagata, Hemagglutinin gene, WIC



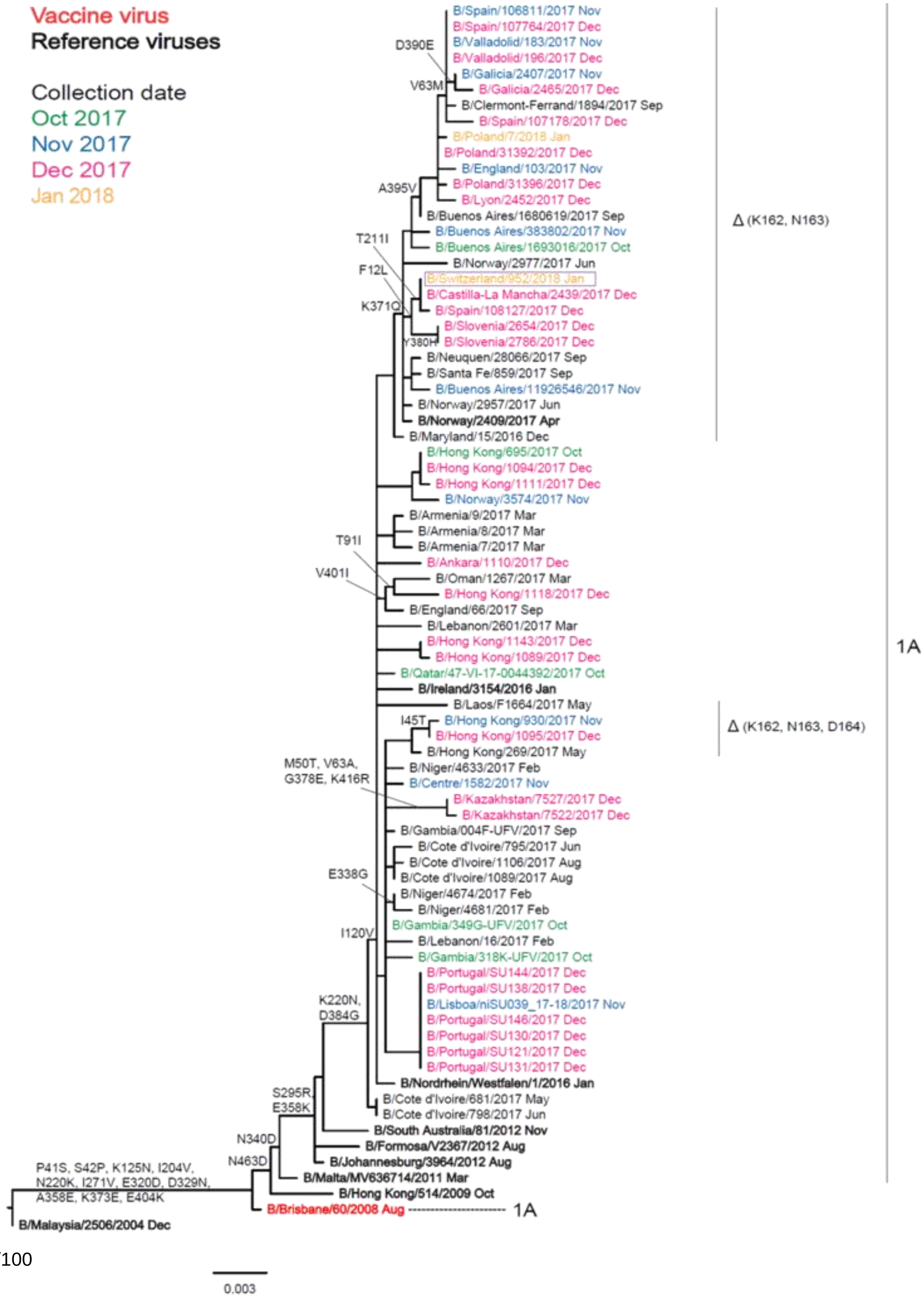
Annex 15: Phylogenetic comparison of influenza B Yamagata, Neuraminidase gene, WIC



Annex 16: Phylogenetic comparison of influenza B Victoria, Hemagglutinin gene, WIC



Annex 17: Phylogenetic comparison of influenza B Victoria, Neuraminidase gene, WIC



Annex 18a: Antiviral sensitivity testing on influenza A viruses, WIC

Virus name	Type/Subtype	OS IC50	OS sensitivity	Zan IC50	Zan sensitivity	Collection date	Centre ID	HI result 1	Date received	Test Date	WIC number	Observed mutations
A/Switzerland/882/2017	H3	4.26	Reduced inhibition	2.26	Normal inhibition	11.10.2017	CHE		02.nov.17	20.11.2017	180109	S334R, P386S
A/Switzerland/475/2017	H3					27.07.2017	CHE	Virus not recovered	02.nov.17		180110	
A/Switzerland/431/2017	H3	0.39	Normal inhibition	0.31	Normal inhibition	25.07.2017	CHE	Na+ Ha-	02.nov.17	20.11.2017	180111	
B/Switzerland/901/2017	BY	33.86	Normal inhibition	2.13	Normal inhibition	06.04.2017	CHE		02.nov.17	13.11.2017	180112	
A/Switzerland/892/2017	H1pdm	1.38	Normal inhibition	0.47	Normal inhibition	21.02.2017	CHE		02.nov.17	13.11.2017	180113	
A/Switzerland/21159465/2017	H3					09.06.2017	CHE	Neut training - not cultured	02.nov.17		180114	
A/Switzerland/20164082/2017	H3					02.03.2017	CHE	Neut training - not cultured	02.nov.17		180115	
A/Switzerland/20007065/2017	H3					13.02.2017	CHE	Neut training - not cultured	02.nov.17		180116	
A/Switzerland/19687744/2017	H3					16.01.2017	CHE	Neut training - not cultured	02.nov.17		180117	

Annex 18b: Antiviral sensitivity testing on influenza A viruses, 04.01.2018 and 24.01.2018 WIC

Collection date	Virus name	Type/Subtype	OS IC50	OS sensitivity	Zan IC50	Zan sensitivity	HI result 1	Centre ID	Date received
05.01.2018	B/Switzerland/952/2018	BV	30.03	Normal inhibition	4.67	Normal inhibition		CHE	24.janv.18
21.12.2017	B/Switzerland/8125/2017	BY	24.23	Normal inhibition	2.05	Normal inhibition		CHE	04.janv.18
21.12.2017	B/Switzerland/3147/2017	BY	28.00	Normal inhibition	0.97	Normal inhibition		CHE	04.janv.18
21.12.2017	B/Switzerland/3106/2017	BY	24.13	Normal inhibition	1.23	Normal inhibition		CHE	04.janv.18
21.12.2017	B/Switzerland/2959/2017	BY	32.60	Normal inhibition	1.47	Normal inhibition		CHE	04.janv.18
20.12.2017	B/Switzerland/2945/2017	BY	24.48	Normal inhibition	1.32	Normal inhibition		CHE	04.janv.18
20.12.2017	B/Switzerland/2911/2017	BY	32.32	Normal inhibition	1.50	Normal inhibition		CHE	04.janv.18
20.12.2017	B/Switzerland/2779/2017	BY	36.54	Normal inhibition	1.00	Normal inhibition		CHE	04.janv.18
20.12.2017	B/Switzerland/2734/2017	BY	20.66	Normal inhibition	1.69	Normal inhibition		CHE	04.janv.18
20.12.2017	B/Switzerland/2696/2017	BY	18.73	Normal inhibition	2.00	Normal inhibition		CHE	04.janv.18
19.12.2017	B/Switzerland/8231/2017	BY	22.04	Normal inhibition	1.70	Normal inhibition		CHE	04.janv.18
19.12.2017	B/Switzerland/7218/2017	BY	19.69	Normal inhibition	1.39	Normal inhibition		CHE	04.janv.18
19.12.2017	B/Switzerland/2469/2017	BY	20.85	Normal inhibition	1.93	Normal inhibition		CHE	04.janv.18
13.12.2017	B/Switzerland/0720/2017	BY	19.62	Normal inhibition	1.25	Normal inhibition		CHE	04.janv.18
11.12.2017	B/Switzerland/8274/2017	BY	20.34	Normal inhibition	1.76	Normal inhibition		CHE	04.janv.18
11.12.2017	B/Switzerland/8142/2017	BY	43.28	Normal inhibition	1.66	Normal inhibition		CHE	04.janv.18
11.12.2017	B/Switzerland/8089/2017	BY	16.53	Normal inhibition	1.74	Normal inhibition		CHE	04.janv.18
11.12.2017	B/Switzerland/0707/2017	BY	27.95	Normal inhibition	3.80	Normal inhibition		CHE	04.janv.18
06.12.2017	B/Switzerland/0747/2017	BY	21.59	Normal inhibition	1.69	Normal inhibition		CHE	04.janv.18
06.12.2017	B/Switzerland/0558/2017	BY	19.75	Normal inhibition	2.10	Normal inhibition		CHE	04.janv.18
04.12.2017	B/Switzerland/7069/2017	BY	32.69	Normal inhibition	2.06	Normal inhibition		CHE	04.janv.18
30.11.2017	B/Switzerland/1042/2017	BY	22.21	Normal inhibition	1.86	Normal inhibition		CHE	04.janv.18
30.11.2017	B/Switzerland/0968/2017	BY	22.64	Normal inhibition	2.19	Normal inhibition		CHE	04.janv.18
21.11.2017	B/Switzerland/0226/2017	BY	27.89	Normal inhibition	2.14	Normal inhibition		CHE	04.janv.18
08.01.2018	B/Switzerland/20051/2018	BY	24.21	Normal inhibition	1.71	Normal inhibition		CHE	24.janv.18
08.01.2018	B/Switzerland/903/2018	BY	17.09	Normal inhibition	2.50	Normal inhibition		CHE	24.janv.18
08.01.2018	B/Switzerland/423/2018	BY	31.26	Normal inhibition	3.96	Normal inhibition		CHE	24.janv.18
05.01.2018	B/Switzerland/653/2018	BY	21.48	Normal inhibition	1.77	Normal inhibition		CHE	24.janv.18
04.01.2018	B/Switzerland/995/2018	BY	28.42	Normal inhibition	2.97	Normal inhibition		CHE	24.janv.18
03.01.2018	B/Switzerland/742/2018	BY	18.00	Normal inhibition	3.00	Normal inhibition		CHE	24.janv.18
02.01.2018	B/Switzerland/936/2018	BY	19.36	Normal inhibition	1.97	Normal inhibition		CHE	24.janv.18
30.12.2017	B/Switzerland/150/2017	BY	21.39	Normal inhibition	2.23	Normal inhibition		CHE	24.janv.18
29.12.2017	B/Switzerland/6093/2017	BY	20.64	Normal inhibition	3.18	Normal inhibition		CHE	24.janv.18
27.12.2017	B/Switzerland/296/2017	BY	24.47	Normal inhibition	2.34	Normal inhibition		CHE	24.janv.18
22.12.2017	B/Switzerland/652/2017	BY	15.38	Normal inhibition	2.71	Normal inhibition		CHE	24.janv.18
22.12.2017	B/Switzerland/614/2017	BY	22.80	Normal inhibition	1.88	Normal inhibition		CHE	24.janv.18
21.12.2017	B/Switzerland/726/2017	BY	15.96	Normal inhibition	2.24	Normal inhibition		CHE	24.janv.18
19.12.2017	B/Switzerland/909/2017	BY	20.96	Normal inhibition	3.77	Normal inhibition		CHE	24.janv.18
19.12.2017	B/Switzerland/231/2017	BY	22.95	Normal inhibition	3.34	Normal inhibition		CHE	24.janv.18
19.12.2017	B/Switzerland/218/2017	BY	19.05	Normal inhibition	3.00	Normal inhibition		CHE	24.janv.18
21.12.2017	A/Switzerland/3191/2017	H1pdm	0.76	Normal inhibition	0.35	Normal inhibition		CHE	04.janv.18
21.12.2017	A/Switzerland/2656/2017	H1pdm	1.54	Normal inhibition	0.69	Normal inhibition		CHE	04.janv.18
20.12.2017	A/Switzerland/3330/2017	H1pdm	1.84	Normal inhibition	0.57	Normal inhibition		CHE	04.janv.18
19.12.2017	A/Switzerland/8198/2017	H1pdm	0.89	Normal inhibition	0.32	Normal inhibition		CHE	04.janv.18
05.12.2017	A/Switzerland/0007/2017	H1pdm	1.65	Normal inhibition	0.49	Normal inhibition		CHE	04.janv.18
05.01.2018	A/Switzerland/707/2018	H1pdm	0.77	Normal inhibition	0.30	Normal inhibition		CHE	24.janv.18
05.01.2018	A/Switzerland/320/2018	H1pdm	0.85	Normal inhibition	0.34	Normal inhibition		CHE	24.janv.18
04.01.2018	A/Switzerland/6136/2018	H1pdm	1.07	Normal inhibition	0.34	Normal inhibition		CHE	24.janv.18
29.12.2017	A/Switzerland/6079/2017	H1pdm	0.76	Normal inhibition	0.39	Normal inhibition		CHE	24.janv.18
29.12.2017	A/Switzerland/4062/2017	H1pdm	0.94	Normal inhibition	0.40	Normal inhibition		CHE	24.janv.18
29.12.2017	A/Switzerland/683/2017	H1pdm	0.93	Normal inhibition	0.42	Normal inhibition		CHE	24.janv.18
21.12.2017	A/Switzerland/656/2017	H1pdm	0.76	Normal inhibition	0.42	Normal inhibition		CHE	24.janv.18
19.12.2017	A/Switzerland/198/2017	H1pdm	0.87	Normal inhibition	0.37	Normal inhibition		CHE	24.janv.18
04.01.2018	A/Switzerland/6136/2018	H1pdm	0.00	Insufficient Titre	0.00	Insufficient Titre		CHE	24.janv.18
21.12.2017	A/Switzerland/8060/2017	H3	0.40	Normal inhibition	0.34	Normal inhibition	Na+ Ha-	CHE	04.janv.18
08.11.2017	A/Switzerland/2159/2017	H3	0.53	Normal inhibition	0.36	Normal inhibition	Na+ Ha-	CHE	04.janv.18
06.01.2018	A/Switzerland/638/2018	H3	0.34	Normal inhibition	0.33	Normal inhibition	Na+ Ha-	CHE	24.janv.18
29.12.2017	A/Switzerland/325/2017	H3	0.25	Normal inhibition	0.28	Normal inhibition	Na+ Ha-	CHE	24.janv.18
15.12.2017	A/Switzerland/143/2017	H3	0.00	Insufficient Titre	0.00	Insufficient Titre		CHE	24.janv.18
28.12.2017	A/Switzerland/552/2017	other					Virus not recovered	CHE	24.janv.18

Annex 19: List of reference antisera provided by the WIC for the2017/18 season

Reference antisera	Source
A/Hong Kong/4801/2014	F41/15
A/Slovenia/3188/2015	Ferret 18/16
A/Hong Kong/5738/2014	F38/15
A/Switzerland/9715293/2013 clone128	Ferret 29/15
A/Singapore/INFIMH-16-0019/2016	F41/17
B/Brisbane/60/2008	FNIB 73/15
B/Hong Kong/514/2009	Ferret 9/13
B/Johannesburg/3964/2012	Ferret 04/16
B/Norway/2409/2017	Ferret 20/17
B/Wisconsin/1/2010	F10/13
B/Novosibirsk/1/2012	Ferret 31/12
B/Phuket/3073/2013	NIB F51/16
A/California/7/2009 clone 38-32	Ferret 07/16
A/Michigan/45/2015	NIB F42/16
A/Hong kong/3934/2011	Ferret 21/11
A/St Petersburg/27/2011	Ferret 23/11

Annex 20: Sequencing primers used during the 2017/18 season

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

Annex 21: Swine influenza Report to the Swiss Federal office of public Health *(updated in May.2018)*

Human infection by a swine influenza virus

On December 2017, a human nasopharyngeal swab specimen was sent to the Swiss National Centre for Influenza (NCI) by a veterinarian from the SUISAG “SchweineGesundheitsDienst” (SGD) Sempach-West. The specimen revealed to be positive for an influenza virus of swine origin.

1. Case description

A 48-year-old male (initials, M.C.) working in a farm in the county of Berne, Switzerland, presented acute respiratory symptoms (moderate cough, myalgia, bronchitis and headache) starting 8 days prior to the nasopharyngeal swab sampling. The swab was performed on December 28 by a veterinarian in charge of animal surveillance. Three pigs from the same farm were also reported to be sick and one tested positive for influenza A virus (subtype N1; information transmitted by Dr Claudia Bachofen from the Virologisches Institut, Vetsuisse-Fakultät, Zürich University). The other two pigs were negative for influenza. The human clinical sample was shipped to the NCI on December 28 (2017), received and labeled as *****6552* on January 4 (2018), and analyzed on January 5 (2018). There was no report of close contacts or relatives being also ill.

2. Analysis

2.1. rRT-PCR analyses

The nasopharyngeal swab specimen was screened for influenza using specific rRT-PCR assays (Table 1). A generic influenza A combination[1] targeting matrix protein (MP) gene sequences of influenza A viruses of both animals and humans and a combination detecting neuraminidase protein 1 (N1) sequences, were positive. rRT-PCRs targeting the human Hemagglutinin 1 (H1) H1N1pdm2009 and the human Hemagglutinin 3 (H3) were negative. A rRT-PCR targeting the MP but used for the screening of human influenza A strains (H1 and H3) prior the emergence of the H1N1pdm2009 strain was positive, but with a low threshold cycle (Ct) value (41.3 Ct, close to the detection limit), suggesting that the detected strain was not a former seasonal H1 strain. All these results pointed toward an animal strain.

rRT-PCR					
Target	**Influenza A MP**	**Influenza A MP**	**Pandemic influenza A/H1pdm2009**	**Seasonal influenza A/H3**	**Universal N1**
Specificity	Animal/ human	Human (used for seasonal Influenza A detection before 2009)	Human	Human	Animal/ human
Sample *6552***	**Detected (31Ct)**	**(41.3 Ct)**	**Not detected**	**Not detected**	**Detected (Ct 30)**

Table 1: *rRT-PCR assays used to screen the nasopharyngeal specimen ****6552.*

2.2. Viral culture

Viral culture of sample ****6552 gave only a poor yield. This was not unexpected for an influenza strain of porcine origin with a weak initial viral load.

2.3. Sequencing

A combination of plublished[2] and in-house designed RT-PCRs were used for the complete genome amplification and sequencing. Five of eight genes were completely (NS) or partially (HA, NP, NA and M) recovered from the initial clinical sample (Table 2).

	Gene fragments sequenced				
A/Berne/****6552/2017	HA	NP	NA	M	NS
Length (bp)	859/1701	757/1497	687/1410	722/982	838/838
Region (bp)	70-928	71-827	6-692	59-780	1-838

Table 2: *Summary of gene sequences obtained for influenza A/Berne/****6552/2017 (H1N1)v virus. The first nucleotide corresponds to the start of the coding region. bp; base pairs. A/swine/Netherlands/Dalfsen-12/2012 was used as reference, KR700020, KR700021, KR700022, KR700023 and KR700024 respectively).*

2.3.1. Blast analysis

A blast analysis with publically-available influenza sequences obtained from the NCBI database website were downloaded on the Smartgene® platform and allowed to confirm that the five sequences were of swine origin (Figure 1). On the basis of the NA gene, both the variant strain and the virus recovered from the influenza A positive swine (information provide by Dr. Claudia Bachofen) are closely related to the A/swine/Netherlands/Dalfsen-12/2012 reference (both with 97% NA identity).

However, direct comparison (alignment) between the variant and the swine NA was not be performed.

Query sequence - locus HA												
Dataset	Seq. length	Creation date	No Unilab	Strain name / Strain ID (auto fill)	Antigenic IHA typisation	Host category	Subtype HA	Subtype NA	Country of collection [iso_3166-1]	Internal NCI remark	s4-HA length	s4-HA mutations
G Influenza Samples	859	11.01.2018	23086552	A/human/Berne/23086552-1/2017 (H1N1)		human	1	1	CH	pig breeder	859	16A, 19I, 35T, 36N, 43S, 45N, 47K, 51Q, 54N, 57V, ...

Similar sequences found								
Dataset	Official strain name	s4-HA AC	Length	Seq. length	Identities	Mismatches	Match length	Score
IDNS Influenza References (1)	A/Swine/Bavaria/11801/2013 (H1N1)	KU320704	871	871	795 (98.15%)	15	810	1487
IDNS Influenza References (1)	A/Swine/Bavaria/85301/2012 (H1N1)	KU320699	923	923	794 (97.90%)	17	811	1473
IDNS Influenza References (1)	A/Swine/Bavaria/30701/2012 (H1N1)	KU320695	878	878	796 (97.79%)	18	814	1471
IDNS Influenza References (1)	A/Swine/Bavaria/31391/2012 (H1N1)	KU320696	952	952	799 (97.56%)	20	819	1465
IDNS Influenza References (1)	A/Swine/Bavaria/35303/2012 (H1N1)	KU320698	919	919	794 (97.66%)	19	813	1461
IDNS Influenza References (1)	A/Swine/Germany/Wunnenberg-IDT13220/2011 (H1N1)	KR699726	1701	1701	825 (96.04%)	34	859	1435
IDNS Influenza References (1)	A/Swine/Germany/Reinberg-IDT14457-1/2012 (H1N1)	KR700366	1701	1701	825 (96.04%)	34	859	1433
IDNS Influenza References (1)	A/Swine/Netherlands/Dalfsen-12/2012 (H1N1)	KR700020	1701	1701	823 (95.81%)	36	859	1417

Query sequence - locus NP											
Dataset	Seq. length	Creation date	No Unilab	Strain name / Strain ID (auto fill)	Antigenic IHA typisation	Host category	Subtype HA	Subtype NA	Country of collection [iso_3166-1]	Internal NCI remark	s5-NP length
UG Influenza A Samples	757	11.01.2018	23086552	A/human/Berne/23086552-1/2017 (H1N1)		human	1	1	CH	pig breeder	757

Similar sequences found								
Dataset	Official strain name	s5-NP AC	Length	Seq. length	Identities	Mismatches	Match length	Score
IDNS Influenza References (1)	A/Swine/Germany/Wunnenberg-IDT13220/2011 (H1N1)	KR699727	1497	1497	743 (98.15%)	14	757	1400
IDNS Influenza References (1)	A/Swine/Germany/Reinberg-IDT14457-1/2012 (H1N1)	KR700367	1497	1497	743 (98.15%)	14	757	1400
IDNS Influenza References (1)	A/Swine/Germany/Barle-IDT13149/2011 (H1N2)	KR699711	1497	1497	742 (98.02%)	15	757	1392
IDNS Influenza References (1)	A/Swine/Netherlands/Rheezerveen-66/2011 (H1N2)	KR699997	1497	1497	742 (98.02%)	15	757	1392
IDNS Influenza References (1)	A/Swine/Germany/Visbek-IDT13347/2011 (H3N2)	KR701400	1497	1497	739 (98.01%)	15	754	1386
IDNS Influenza References (1)	A/Swine/Germany/Belecke-IDT12963/2011 (H1N2)	KR699703	1497	1497	741 (97.89%)	16	757	1384
IDNS Influenza References (1)	A/Swine/Germany/Dulmen-IDT14394/2011 (H1N2)	KR700359	1497	1497	741 (97.89%)	16	757	1384
IDNS Influenza References (1)	A/Swine/Germany/Steinfeld-IDT12115/2010 (H1N2)	KR699656	1497	1497	741 (97.89%)	16	757	1384
IDNS Influenza References (1)	A/Swine/Netherlands/Dalfsen-12/2012 (H1N1)	KR700021	1497	1497	740 (97.75%)	17	757	1376

Query sequence - locus NA												
Dataset	Seq. length	Creation date	No Unilab	Strain name / Strain ID (auto fill)	Antigenic IHA typisation	Host category	Subtype HA	Subtype NA	Country of collection [iso_3166-1]	Internal NCI remark	s6-NA length	s6-NA mutations
G Influenza Samples	687	11.01.2018	23086552	A/human/Berne/23086552-1/2017 (H1N1)		human	1	1	CH	pig breeder	687	9I, 11S, 13I, 16I, 17N, 19I, 21S, 23M, 34V, 40I, 4 ...

Similar sequences found								
Dataset	Official strain name	s6-NA AC	Length	Seq. length	Identities	Mismatches	Match length	Score
IDNS Influenza References (1)	A/Swine/Germany/Reinberg-IDT14457-1/2012 (H1N1)	KR700368	1410	1410	668 (97.23%)	19	687	1213
IDNS Influenza References (1)	A/Swine/Netherlands/Dalfsen-12/2012 (H1N1)	KR700022	1410	1410	668 (97.23%)	19	687	1213

Query sequence - locus M											
Dataset	Seq. length	Creation date	No Unilab	Strain name / Strain ID (auto fill)	Antigenic IHA typisation	Host category	Subtype HA	Subtype NA	Country of collection [iso_3166-1]	Internal NCI remark	s7-M length
UG Influenza A Samples	722	11.01.2018	23086552	A/human/Berne/23086552-1/2017 (H1N1)		human	1	1	CH	pig breeder	722

Similar sequences found								
Dataset	Official strain name	s7-M AC	Length	Seq. length	Identities	Mismatches	Match length	Score
IDNS Influenza References (1)	A/Swine/Germany/Wunnenberg-IDT13220/2011 (H1N1)	KR699729	982	982	714 (98.89%)	8	722	1368
IDNS Influenza References (1)	A/Swine/Germany/Bakum-IDT12292/2010 (H1N2)	KR699682	982	982	714 (98.89%)	8	722	1368
IDNS Influenza References (1)	A/Swine/Germany/Ellerbrock-IDT14696/2012 (H1N1)	KR700392	982	982	714 (98.89%)	8	722	1368
IDNS Influenza References (1)	A/Swine/Germany/Lohne-IDT12877/2011 (H1N2)	KR699697	982	982	713 (98.75%)	9	722	1360
IDNS Influenza References (1)	A/Swine/Germany/Steinfeld-IDT12115/2010 (H1N2)	KR699658	982	982	713 (98.75%)	9	722	1360
IDNS Influenza References (1)	A/Swine/Germany/Reinberg-IDT14457-1/2012 (H1N1)	KR700369	982	982	712 (98.61%)	10	722	1352
IDNS Influenza References (1)	A/Swine/Netherlands/Rheezerveen-66/2011 (H1N2)	KR699999	982	982	712 (98.61%)	10	722	1352
IDNS Influenza References (1)	A/Swine/Netherlands/Dalfsen-12/2012 (H1N1)	KR700023	982	982	712 (98.61%)	10	722	1352

Query sequence - locus NS											
Dataset	Seq. length	Creation date	No Unilab	Strain name / Strain ID (auto fill)	Antigenic IHA typisation	Host category	Subtype HA	Subtype NA	Country of collection [iso_3166-1]	Internal NCI remark	s8-NS length
JG Influenza A Samples	838	11.01.2018	23086552	A/human/Berne/23086552-1/2017 (H1N1)		human	1	1	CH	pig breeder	838

Similar sequences found								
Dataset	Official strain name	s8-NS AC	Length	Seq. length	Identities	Mismatches	Match length	Score
IDNS Influenza References (1)	A/Swine/Germany/Wunnenberg-IDT13220/2011 (H1N1)	KR699730	838	838	816 (97.37%)	22	838	1489
IDNS Influenza References (1)	A/Swine/Germany/Reinberg-IDT14457-1/2012 (H1N1)	KR700370	838	838	816 (97.37%)	22	838	1487
IDNS Influenza References (1)	A/Swine/Germany/Ellerbrock-IDT14696/2012 (H1N1)	KR700393	838	838	816 (97.37%)	22	838	1487
IDNS Influenza References (1)	A/Swine/Germany/Visbek-IDT13347/2011 (H3N2)	KR701403	838	838	814 (97.14%)	24	838	1471
IDNS Influenza References (1)	A/Swine/Netherlands/Dalfsen-12/2012 (H1N1)	KR700024	838	838	814 (97.14%)	24	838	1471

Figure 1. *Blast analysis of the hemagglutinin HA, the nucleoprotein NP, the neuraminidase NA, the matrix M and the non-structural protein NS sequences of A/Berne/****6552/2016 sequence (H1N1)v influenza virus.*

2.3.2. Phylogenetic analysis

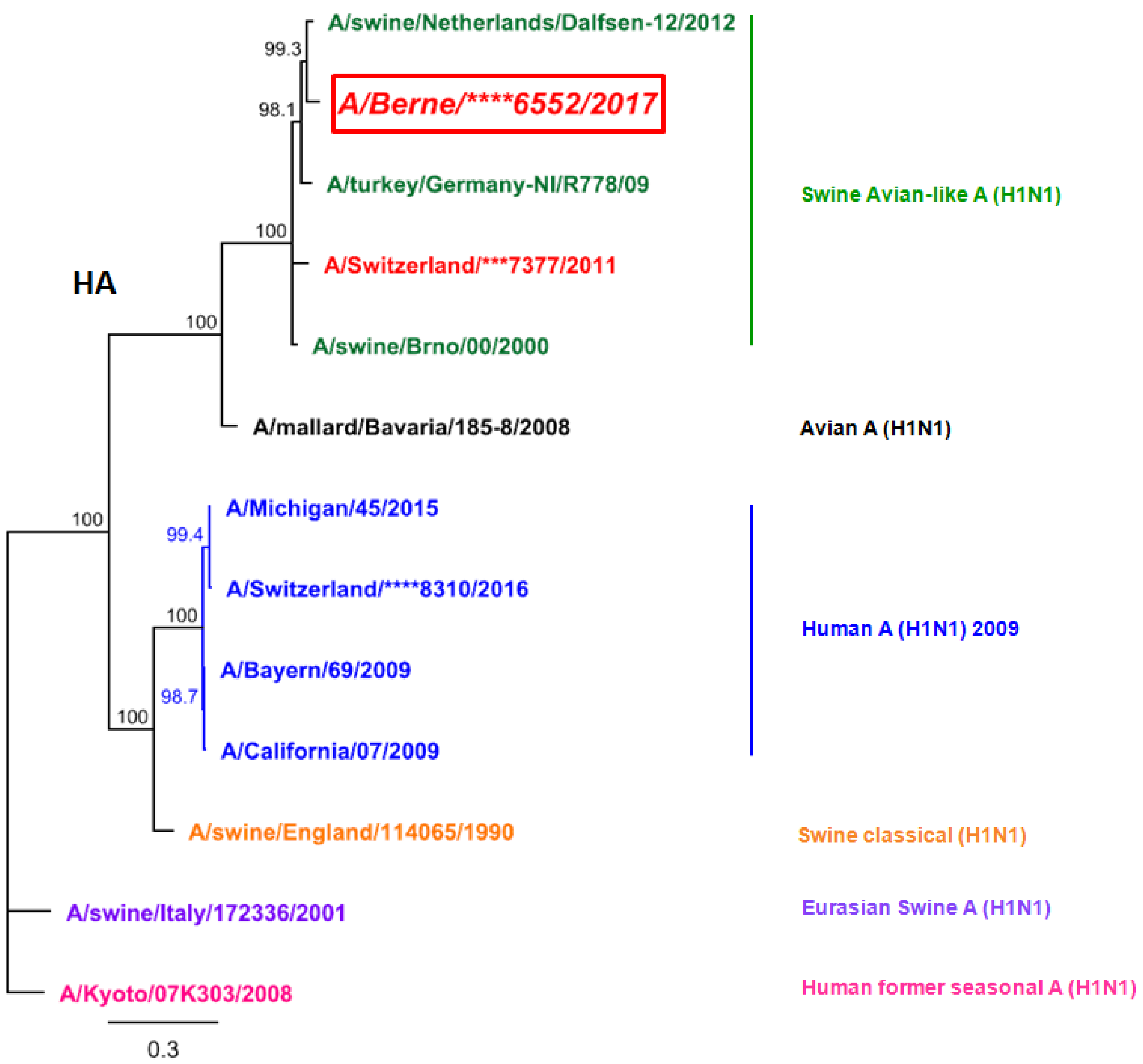


***Figure 2**. **ML phylogenetic tree for the HA gene**. Segment of H1 Subtype avian, human, avian-like and classical Eurasian swine influenza viruses were defined. Red squared: analyzed sample strain. Red: 2011 Swiss human sample of porcine origin. Green: "avian-like" swine strain. Blue: human A(H1N1)pdm09 strains. Orange: classical swine strain. Pink: human former seasonal strain. Violet: Eurasian swine strain.*

3. Conclusion

The influenza strain detected in the upper respiratory tract of a Swiss pig farm employee from the county of Berne was confirmed to be of swine origin. In addition, the identified NA sequence seems to be concordant with the one recovered from the sampled animal. The Blast results as well as the phylogeny of the HA, and NA (not shown in this update) and sequences confirms that the A/Berne/****6552/2017 H1N1 strain belongs to the European "avian-like" H1N1 swine influenza A cluster, which predominates in European pigs since 1979[4-5]. This case, in addition to cases already observed in 2003[3], 2009, 2010, 2011, and 2016 confirm that sporadic animal-to-human transmission occurs in Switzerland.

4. References

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Annex of “Swine influenza Report to Federal office of public Health”

A/Berne/6552/2017_HA (H1N1)v, 859bp

TACCATGCTAACAATTCCACAGACACCGCAGACACAATATTGGAGAAGAATGTGACTGTTACACATTCAGTTAACTTACTAGAAACCAACCATA
ATGGAAAACTCTGTAGCCTGAATGGAAAGGCCCCCTTACAACTGGGGAACTGCAATGTAGCAGGATGGATTCTGGGCAATCCAGAATGTGACTT
GCTGCTCACAGCGGATTCGTGGTCATACATAATCGAGACCCCAGATTCAAAAAATGGAACATGCTACCCCGGAGAATTCACTGATTATGAAGAG
TTAAGGGAGCAACTGAGTACAGTTTCTTCATTTGAAAGATTTGAAATTTTCCCGAAAGCAACCTCATGGCCAAATCATGATACAACCAGAGGTA
CCACAGTTGCGTGCTCCCACTCTGGAACCAACAGCTTTTATCGGAACTTGCTATGGATAGTAAAAAAAGGAAACGCTTATCCTAAACTCAGAAA
GTCATACACAAACAATAAAGGGAAAGAAGTGCTTGTAGTCTGGGGAGTGCACCATCCTCCAACTGACAGTGACCAACAAACCCTCTACCAGAAC
AATCATACATACATTTCAGTTGGATCATCAAAATACTACCAAAGATTCACACCAGAAATAGTAACCAGACCTAAAATTAGAGAACAAGCAGGTA
GAATGAATTACTATTGGACACTTTTAGATCAAGGAGACACCATAACCTTTGAAGCCACGGGAAACTTAATAGCACCATGGCACGCATTTGCATT
GAATAAGGGCTCTAATTCTGGAGTTATAATGTCAGATGCTCATGTTCACAATTGCTCTACAAAGTGCCAGACTCCTCATGGGGCTTTGAAAAGC
AATCTTCCTTTTC

A/Berne/6552/2017_NP (H1N1)v, 757bp

AAATCAGAGCATCTGTTGGGAGAATGGTTGAAGGAATTGGGCGATTCTACATACAGATGTGTACTGAACTCCAACTCAGTGACTATGAAGGGAG
GTTGATCCAAAATAGTATAACGATAGAGAGAATGGTCCTCTCTGCATTTGACGAGAGAAGAAACAAATACTTGGAAGAACATCCCAGTGCGGGA
AAAGATCCAAAGAAAACTGGAGGTCCAATCTACAAAAAGAGAGATGGAAAATGGCTGAGAGAGCTGATTCTGTATGACAAAGATGAGATCAGGA
GAATCTGGCGCCAAGCAAACAATGGTGATGATGCTACTGCTGGTCTCACTCACCTGATGATTTGGCATTCCAACCTGAATGACGCCACATATCA
GAGAACAAGAGCTTTAGTGCGCACTGGGATGGATCCCAGAATGTGCTCTCTGATGCAAGGTTCAACCCTCCCAAGGAGATCTGGAGCTGCTGGT
GCCGCAGTGAAGGGGGGTTGGGACACTAGTAATGGAGCTGATTCGAATGATAAAGAGGGGTATCANTGATCGGAATTTCTGGAGAGGANAGAATG
GACGAAGAACAAGAATTGCATATGAGAGAATGTGCAACATCCTCAAAGGGAAATTCCAAACAGCAGCGCAACGAGCAATGATGGACCAGGTGCG
AGAAAGCANAAATCCAGGAAATGCTGANATTGAAGATCTCATCTTTTTGGCACGATCAGCACTCNTCCTGAGAGGATCAGTGGCTCATAAATCC
TGCCT

A/Berne/6552/2017_NA (H1N1)v, 687bp

TCCAAATCAGAAGATAATAATCATTAGCTCGATCTGCATGATAAATGGAATTGCTAGCTTGATGTTACAAATTGGGAACATAATCTCAATATGG
GTTAGCCACTCAATTCAAATTGGGAACCAAAACCAGACTGAAACATGCAATCAAAGTGTCATTANTTATGAAAACAAAACTTGGGTAAATCAGA
CATATGTCAATATCAGCAATATCAATTTTGTTGCTAAACAGGCAGTGATTTCTCTAAATTTAGCGGGCAGTTCTCCTCTCTGCCCTGTTAGTGG
TTGGGCTATATACAGTAAAGACAACAGTGTAAGAATTGGTTCCAGGGGGGATGTGTTTGTCATAAGAGAGCCATTCATCTCATGCTCCCACTTG
GAATGTAGAACCTTCTTCTTGACCCAAGGGGCCCTACTAAACGACAAACATTCCAATGGAACCATTAAAGACAGAAGTCCCTATCGAACCCTGA
TGAGCTGTCCTATTGGCGAAGTCCCCTCTCCGTACAACTCAAGATTTGAGTCAGTTGCTTGGTCAGCAAGCGCTTGCCATGATGGCACCAATTG
GATGACAATTGGTATTTCTGGGCCAGACAATGGGGCAGTAGCTGTATTGAAATACAATGACGTAATTACAGATACTATCAAGAGTTGGAGAAAC
AACATATTGAGAACACAAGAGTCTGAATG

A/Berne/6552/2017_M (H1N1)v, 722bp

TCAAAGCCGAGATCGCGCAGAGACTGGAAGGGGTTTTTGCAGGGAAGAACACAGATCTTGAGGCTCTCATGGAATGGCTAAAGACAAGACCAAT
TCTGTCACCTCTGACTAAGGGAATTCTGGGATTTGTGTTCACGCTCACCGTGCCCAGTGAGCGAGGACTGCAGCGTAGACGCTTTGTTCAAAAT
GCCCTAAATGGAAATGGGGACCCTAACAACATGGATAGAGCAGTCAAATTGTACAAGAAGCTAAAAAGGGAAATAACGTTCCATGGGGCCAAGG
AAGTGTCACTAAGCTACTCAACTGGTGCTCTTGCCAGTTGCATGGGCCTCATATACAATAGGATGGGAACAGTAACCACAGAAGCTGCGTTCGG
CCTGGTGTGTGCCACTTGTGAGCAGATCGCTGACTCACAACATCGGTCACACAGACAAATGGCCACTACCACTAATCCACTAATCAGGCATGAA
AACAGAATGGTACTGGCTAGCACTACAGCTAAGGCTATGGAACAGATGGCTGGATCGAGTGAACAGGCAGCAGAGGCCATGGAGGTTGCCAGTC
AGACAAGGCAGATGGTGCATGCAATGAGAACAATTGGGACACATCCCAGCTCCAGTGCCGGTCTGAAAGATGATCTTCTTGAAAATTTGCAGGC
TATCAGAAACGGATGGGAGTGCAAATACAGCGGTTCAAGTGATGCTATCGCCACTGCAGCAAA

A/Berne/6552/2017_NS (H1N1)v, 838bp

ATGGACTCAAACACTGTGTCAAGCTTTCAGGTAGATTGCTTCCTTTGGCATGTCCGAAAAAGGTTTGCAAACTGGGGGCTTGGCGATGCCCCAT
TTCTCGATAGGCTTCGCCGAGACCAGAAGTCTCTAAGAGGAAGAGGCAGCACTCTTGGTCTTGAAATCGAACCGGCAACTCGTGTGGGGAAACA
AATAGTAAAGCGTATTCTGGAGGAAGAATGCGATGAAGCACTTAAAATAACTGTCGCTTCTGTGCCTGCTTCACGCTATCTGACTGACATGACT
CTTGAAGAGATGTCAAGGGACTGGTTCATGCTCATGCCCAAGCAAAAAGTGGCAGGTCCCTTTTGCATCAGAATGGACCAGGCAATAATGGATA
AAAACATCACATTGAAGGCAAATTTCAGTGTGATTTTTGATCGACTGGAAACTCTGATACTCCTTAGAGCTTTCACAGAAGAAGGGGCAATTGT
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GATAATGCAGTTCGAATCTCTGAAGCTCTACAGAGATTCGCTTGGAGAAGCACTAATGAGAACAGGAGATCTTCATTCCCTCCAAAACAGAAAC
GGAAAATGGCGAGAGCAACTGGGCCAGAAGTTTGAAGAAATAAGATGGCTAATTGAAGAAGTACGACACAGATTAAAAATAACAGAGAATAGCT
TTGAACAGATAACGTTTATGCAAGCATTACAACTACTGCTTGAAGTGGAACAAGAGATAAGGACATTCTCGTTTCAGTTTATTTAA