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# Influenza virus surveillance in Switzerland
# Season 2016 – 2017

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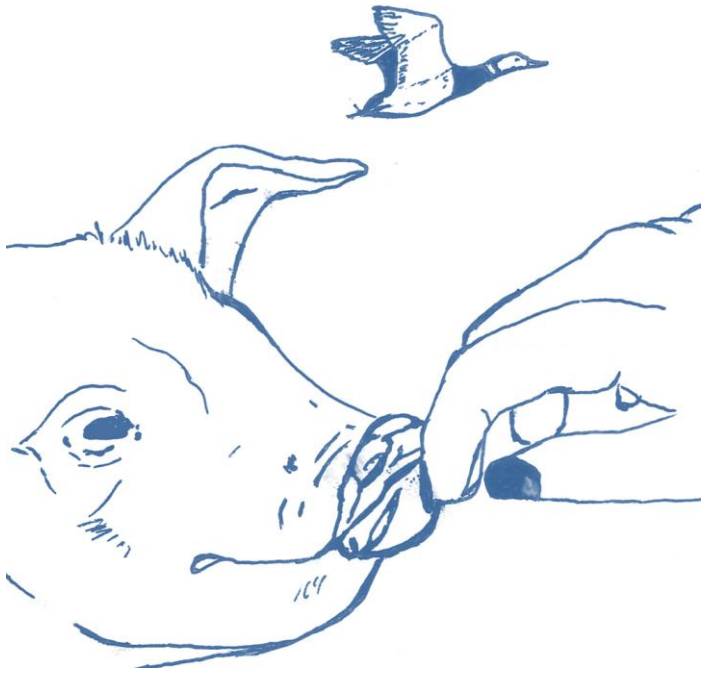

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# Acronyms and Abbreviations

**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
**HAI**: hemagglutination inhibition
**H/LPAI:** highly/low pathogenic avian influenza
**HUG**: University of Geneva 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
**NRCI**: National Reference Centre for Influenza
**NS**: non-structural
**OIE**: World Organization for Animal Health
**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 d’influenza a débuté plus tôt que les saisons précédentes cette année en Suisse. Similairement, le pic de l'épidémie est survenu plus tôt au cours de la semaine 2/2017. En dépit d'un début précoce, la durée (11 semaines) de la phase épidémique était similaire aux saisons précédentes. En Suisse, la saison de grippe 2016/17 était comparable en termes de consultations médicales pour un état grippal et de types de virus en circulation à la saison 2014/15.

Neuf-cent-huitante-deux prélèvements nasopharyngés ont été analysés cette saison pour la présence du virus de la grippe et 49% étaient positifs. Une forte prédominance de virus de la grippe A a été observée par rapport à ceux de la grippe B, ces dernier étant plus abondants vers la fin de la saison. La plupart des virus de la grippe A isolés appartenaient au sous-type A (H3N2).

La majorité des A(H3N2) isolés en 2016/17 appartenaient au groupe génétique 3C.2a et au sous-groupe génétique 3C.2a1. Ceux-ci ont été bien reconnus par des antisérums dirigés contre la souche vaccinale A/Hong Kong/4801/14 (3C.2a). Peu de viruses de la grippe A(H1N1 09) et de la grippe B des lignées B/Yamagata/16/88 et B/Victoria/2/87 ont circulé en Suisse cette saison. Fait intéressant et rare en Suisse, un virus A(H1N1)v a été isolé cette saison à partir du prélèvement nasopharyngé d’un employé agricole. Les virus A(H1N1 09) étaient généralement antigéniquement similaires à la souche A/California/7/2009 contenue dans le vaccin 2016/17. Mais certains virus étant distincts de cette dernière, une substitution de la souche A/California/7/2009 par la souche A/Michigan/45/2015 a été décidée pour le futur vaccin 2017/18. Les virus de la lignée B/Yamagata/16/88 étaient plus abondants que ceux de la lignée B/Victoria/2/87 en Suisse. Les deux lignées étaient antigéniquement et génétiquement comparables à leur souche vaccinale respective.

Tous les virus de la grippe A testés pour une résistance aux adamantanes en Suisse ont présenté la mutation de résistance S31N. En revanche, aucun n'était résistant aux inhibiteurs de la neuraminidase.

# Zusammenfassung

Die Influenza Epidemie begann in der Schweiz dieses Jahr früher als in den vorangegangenen Jahren. Vergleichsweise wurde das Maximum der Epidemie dieses Jahr auch früher erreicht, genauer gesagt im Laufe der Woche 2/2017. Ungeachtet des früheren Beginns war die Dauer der Epidemie (11 Wochen) vergleichbar mit denjenigen vorangegangener Jahre. In der Schweiz war die Grippesaison 2016/17 vergleichbar mit der Saison 2014/15 in Bezug auf die Häufigkeit der Arztkonsultationen für grippeartige Erkrankungen und der Art der Viren welche zirkulierten.

Neunhundert und Zweiundachtzig nasopharyngeal Abstriche wurden in dieser Saison untersucht um Grippeviren nachzuweisen. 49% der Proben waren positiv. Influenza A Viren zeigten eine starke Dominanz gegenüber Influenza B. Letztere waren hingegen Ende der Saison häufiger nachzuweisen. Die meisten der nachgewiesenen Influenza A Viren gehörten zur Untergruppe des Typs Influenza A(H3N2).

Die Mehrheit der in der Saison 2016/17 nachgewiesenen Influenza A (H3N2) Viren gehörten zur genetischen Gruppe 3C.2a und zur genetische Untergruppe 3C.2a1. Diese Stämme wurden gut vom Antiserum erkannt welches gegen den Impfstamm Influenza A/Hong Kong/4801/14 (3C.2a) gerichtet ist. Nur wenige der Stämme Influenza A(H1N1 09), Influenza B/Yamagata/16/88 und B/Victoria/2/87 zirkulierten in dieser Saison in der Schweiz. Die Influenza A(H1N1 09) Viren waren antigenetisch verwandt mit dem Stamm A/Kalifornien/7/2009 welcher im Impfstoff 2016/17 enthalten ist. Jedoch zeigten einige dieser Stämme Veränderungen, sodass beschlossen wurde, den Stamm A/Kalifornien/7/2009 durch den Stamm A/Michigan/45/2015 für den Impfstoff der nördlichen Hemisphäre für die Saison 2017/18 zu verwenden. In der Schweiz waren Influenza B Viren der Linie B/Yamagata/16/88 zahlreicher als diejenigen der Linie B/Victoria/2/87. Die Viren dieser zwei Linien waren antigenisch und genetisch vergleichbar mit den entsprechenden im Impfstoff enthaltenen Stämmen.

Alle Influenza A Viren welche auf eine Resistenz gegen Amantadine untersucht wurden, wiesen die Resistenz Mutation S31N auf. Dagegen wies keiner der geprüften Stämme eine Resistenz gegen Neuraminidase Inhibitoren auf.

Eine interessante und seltene Beobachtung ist, dass ein Virus vom Typ A (H1N1)v diese Saison aus einem Nasen-/Rachenabstrich eines Landwirtschaftsarbeiters nachgewiesen wurde.

## Summary

The 2016/17 influenza outbreak started earlier than the in previous years in Switzerland. The epidemic peak also occurred earlier at week 2/2017 but the duration (11 weeks) of the epidemic phase was similar to prior seasons. Apart from the earlier epidemic start, the 2016/17 influenza season in Switzerland was comparable to the 2014/15 season in terms of the overall rate of medical consultations for influenza-like illness and dominant types of circulating viruses.

Of 982 samples screened for influenza, 49% were positive. The outbreak was marked by a large dominance of influenza A over influenza B viruses. Most of the influenza A viruses identified were of A(H3N2) subtype. The majority of A(H3N2) viruses characterized in 2016/17 belonged to clade 3C.2a and subclade 3C.2a1 and were recognized well by antisera raised against A/Hong Kong/4801/14 (3C.2a clade). Few influenza A(H1N1 09) and influenza B viruses of both B/Yamagata/16/88 and B/Victoria/2/87 lineages circulated in Switzerland this season. Influenza B viruses were more abundant towards the end of the season. In general, A(H1N1 09) viruses were generally antigenically similar to A/California/7/2009 the 2016/17 vaccine strain. However, some viruses were distinct from A/California/7/2009 and this observation led to the replacement of the actual vaccine strain by the A/Michigan/45/2015 strain in the 2017/18 Northern hemisphere vaccine. B/Yamagata/16/88 viruses were more abundant than B/Victoria/2/87. Both lineages were antigenically and genetically comparable to their respective vaccine strain.

All influenza A viruses tested for adamantanes resistance in Switzerland exhibited the S31N resistance mutation. In contrast, none was resistant to neuraminidase inhibitors.

Interestingly, an A(H1N1)v virus was isolated this season from a farm employee.

## 1. Introduction

Influenza virus infections are a major medical and economic burden worldwide.[2,3] In Switzerland, the Sentinel surveillance system is a network of primary care medical practitioners who report medical consultations for influenza-like illnesses (MC-ILI) to the Federal Office of Public Health (FOPH). In addition, a subgroup of these practitioners randomly collects respiratory samples from patients diagnosed with ILI and sends them to the National Reference Centre for Influenza (NRCI) in Geneva for virologic characterization. This report summarizes the virological surveillance data from samples processed and analyzed during 2016/17 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.[4,5] Influenza A viruses have a wide host tropism, while influenza B viruses are only found in humans.[6] These two latter 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 are mainly found in swine and cattle.[5]

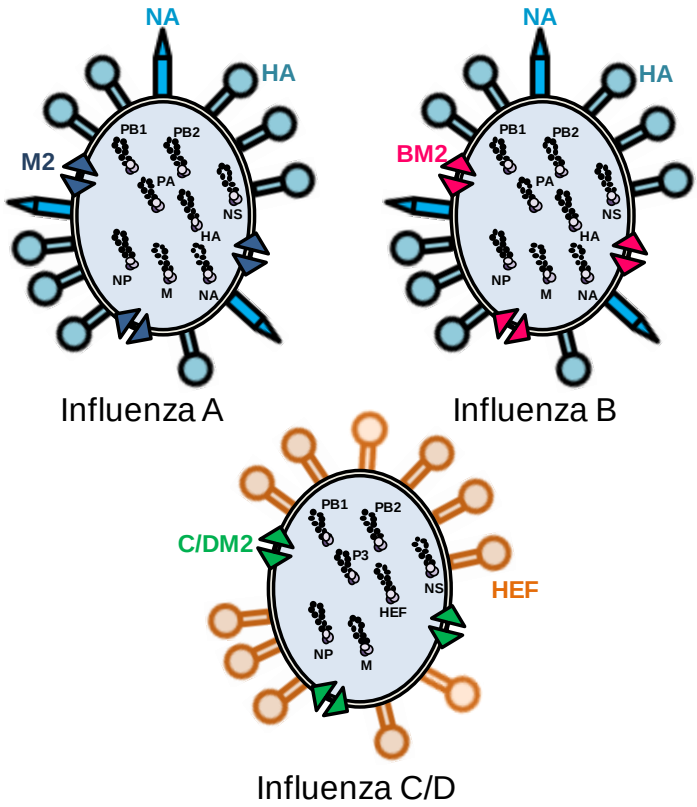


**Figure 1. The structure of influenza viral particles.** Hemagglutinin (HA), neuraminidase (NA), hemagglutinin-esterase-fusion (HEF) and the ion channel M2, BM2 and C/DM2 proteins for influenza A, B and C/D, respectively. Their respective roles are virus attachment for HA and HEF to sialic acids, virion detachment from the cellular surface by cleaving the HA on the virus surface for NA (HEF), and virion acidification required for fusion for M2, NB and CM2. The RNA segments PB1, PB2, PA, HA or HEF, NP, NA (not present in influenza C and D), M and NS are present inside the viral capsid, protected by nucleoproteins. Influenza D is structurally closer to influenza C than to A and B.[1]

# 3. Methodology

## 3.1. Clinical identification of influenza cases

During the 2016/17 influenza season, 155 primary care practitioners participated in the national influenza surveillance network (Figure 2, yellow circles). The number of participants per Sentinel region is related to the population density. Medical practitioners 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[7]. A subgroup of 74 Sentinel practitioners (47.8%) (Figure 2, red circles) collected nasopharyngeal swabs from patients with ILI for subsequent viral detection and characterization. The sampling procedure of specimens is done according to the following rules:

1) During the pre- and post-epidemic phases: when the number of MC-ILI reported by Sentinel practitioners remains below the annual pre-defined epidemic threshold, screening for influenza viruses is performed in all cases that fulfill the 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 2009-2010). It corresponded to ≥64 suspected influenza cases per 100,000 inhabitants for the 2016/17 influenza season.

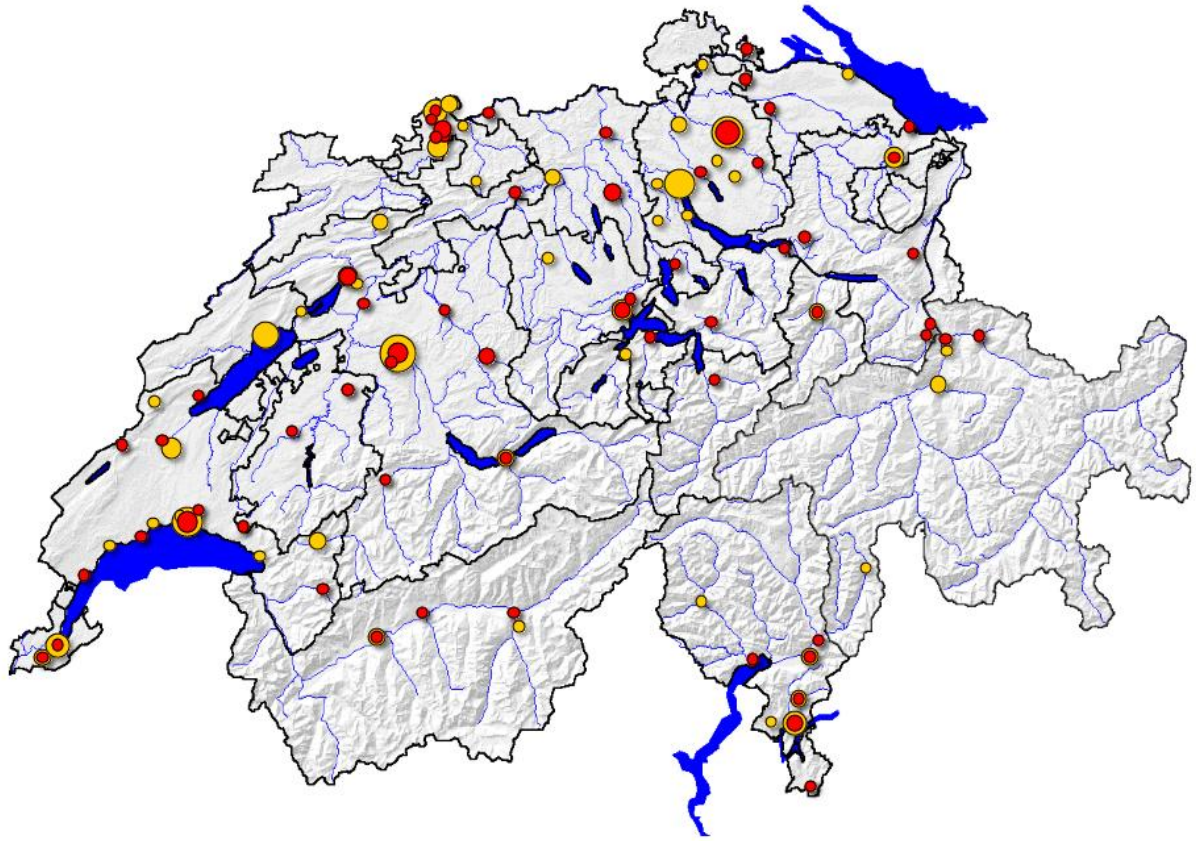

**Figure 2. Geographic distribution of the Swiss Sentinel Network practitioners (2016/17).** Yellow circles: location of participants (155) conducting clinical surveillance. Red circles: participants conducting both clinical surveillance and specimen collection (74). Circle size: participants per community (range 1-8).

### 3.2. 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 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 HA genes in order to discriminate between influenza A H1 and H3 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.

### 3.3. Antigenic and genetic characterization of influenza viruses

A selection of influenza viruses are submitted to phenotypic and genotypic analysis (Figure 3). Briefly, during the pre- and post-epidemic phases all positive samples with sufficient HA titers are phenotypically characterized using the hemagglutination inhibition (HAI) assay, which evaluates the antigenic similarity between reference and circulating influenza strains. During the epidemic phase, the first 5 positive samples per week, with a cycle threshold (Ct) value ≤30 and sufficient HA titers, are analyzed. Similarly, a microneutralization (MN) test can be used for samples that do not (or only poorly) hemagglutinate red blood cells (RBC). Reference antisera and corresponding viral strains used for the HAI and MN were kindly provided by the World Health Organization (WHO) Collaborating Centre Reference Laboratory at the Francis Crick Worldwide Influenza Centre (WIC, London, UK). HAIs 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 (around 5 per week). NA genes are also sequenced and 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.

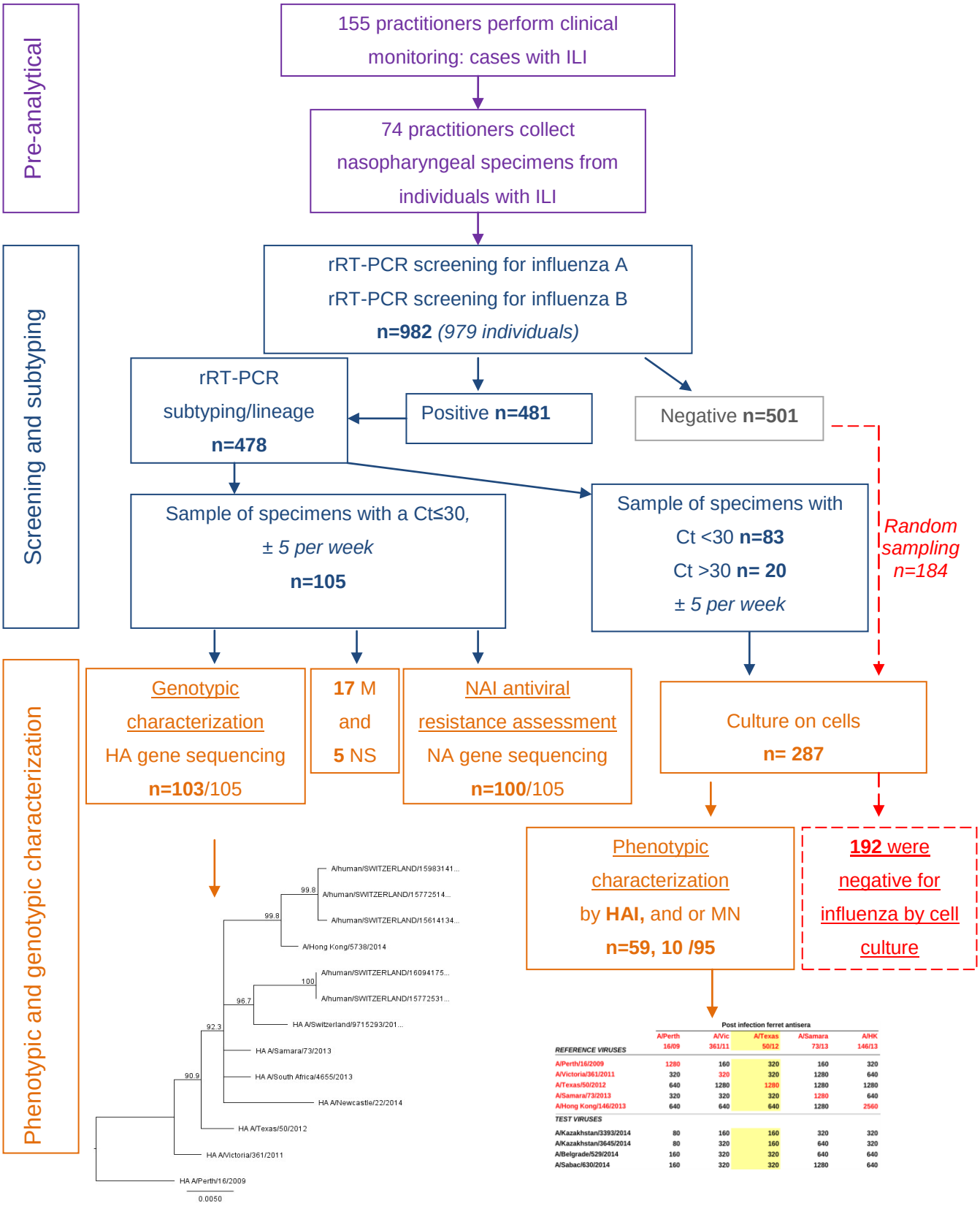


**Figure 3. Flow chart of Sentinel sample collection and processing**. Numbers (n) represent the number of samples submitted to the described step during the 2016/17 season.

### *3.3.1. Cell culture*

As HAI analysis requires a high concentration of influenza virus, a viral amplification step is performed by inoculating the clinical samples on Madin-Darby canine kidney (MDCK) cells and MDCK-sialic acid-enriched (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 lower than 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 HAI assays are dependent on the ability of the viral HA to bind to sialic acids present at the surface of RBCs.

### *3.3.2. 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, HAI is performed as follows. 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 antisera dilution and incubated for 1 h at room temperature. 25 µl of guinea pig RBC are then added to each well. The plates are incubated for 1 h at 4°C. The HAI 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 antisera (antigenic table). The antigenic tables are influenza strain-specific (Figure 4), and are therefore adjusted each year. As the serum is initially diluted 1/8, the titers provided in Figure 4 and Annexes 2a to 2d should be multiplied by 8 to obtain the final titers.

| a. H1N1 09 / antisera | *A/Brisbane/59/07* | A/California/07/09 | A/St Petersburg/27/11 | A/Hong Kong /3934/11 |
|---|---|---|---|---|
| *A/Brisbane/59/07* | *1024* | *<16* | *<16* | *<16* |
| A/California/7/09 | *<16* | 1024 | 64 | 256 |
| A/St Petersburg/27/11 | *<16* | 128 | 128 | 128 |
| A/Hong Kong/3934/11 | *<16* | 256 | 128 | 512 |

| b. H3N2 / antisera | 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 |
| A/Slovenia/3188/15 | 32 | 32 | 32 | 32 |

| c. B / antisera | B/ Brisbane/ 60/08 | B/ Odessa/ 3886/10 | B/ Johannesburg /3964/12 | B/ Wisconsin/ 01/10 | B/ Novosibirsk/1/12 | B/ Massachusetts/02/12 Egg | B/ Phuket/ 3073/13 |
|---|---|---|---|---|---|---|---|
| B/Brisbane/60/08 | 128 | 64 | 128 | <16 | | | |
| B/Odessa/3886/10 | 512 | 512 | 256 | | | | |
| B/Johannesburg/3964 /12 | 32 | 32 | 64 | | | | |
| B/Wisconsin/01/10 | <16 | | | 64 | 64 | 32 | 64 |
| B/Novosibirsk/1/12 | | | | 256 | 256 | 64 | 16 |
| B/Massachusetts/02/ 12 Egg | | | | 128 | 64 | 256 | 128 |
| B/Phuket/3073/13 | | | | 256 | 128 | 256 | 128 |

**Figure 4**. **HAI titers of reference influenza strains tested with the 2016/17 reference antisera**. HAI reaction is performed as described in the methodology section. HAI titers mentioned in tables correspond to the highest dilution where an inhibition is still observed. In red: 2016/17 flu vaccine strains. a, b and c correspond to A(H1N1 09), A(H3N2) and B influenza virus antigenic tables, respectively. The first line and column of each influenza type/subtype table correspond to the antiserum and virus strain tested, respectively.

**Antigenic similarity**

A strain is considered as being antigenically related to a reference strain when its HAI titer is no more than 4-fold below or above the titer obtained with the reference strain.

### *3.3.3. Microneutralization assay*

The MN (neutralization) assay represents an alternative to the HAI assay for antigenic characterization of influenza viruses, specially for viruses that do not agglutinate RBCs. MN allows not only to measure the ability of antibodies to inhibit virus infection to host cells (binding process), as HAI does, but also to assess their potential to block virus-induced cytopathic effects (virus entry, internalization and fusion processes). MN was shown to be more sensitive and specific than HAI, though in a strain-specific manner[8]. It is also more expensive and time-consuming. Therefore, at the NRCI, it is used for isolates that could not be characterized by HAI.

The MN test protocol used at the NRCI was kindly provided by Katja Hoschler in 2012 (Health Protection Agency, London, UK). In brief, a two-fold dilution of reference antisera are prepared on 96-well plates (Figure 5). A standardized amount (10 $TCID_{50}$) of viruses to be characterized is added to the diluted reference sera and the mix incubated for 1h at 37°C 5% $CO_2$. A MDCK-SIAT cell suspension ($5x10^4$ cells/well) is then distributed in each well. After 16 h of incubation at 37°C and 5% $CO_2$, the supernatant is removed and cells are fixed for 20 min with a methanol/$H_2O_2$ (0.6%) solution at room temperature. Viruses are detected using a monoclonal anti-NP antibody and revealed by a secondary antibody HRP-conjugate in presence of the 3,3',5,5'-Tetramethylbenzidine substrate. After stopping the chromogenic enzyme-substrate reaction by the addition of 0.5M HCl, the optical density is measured by a spectrophotometer. Antisera-dependent virus growth inhibition (neutralization) is determined by the ratio between viral suspension with and without antiserum.

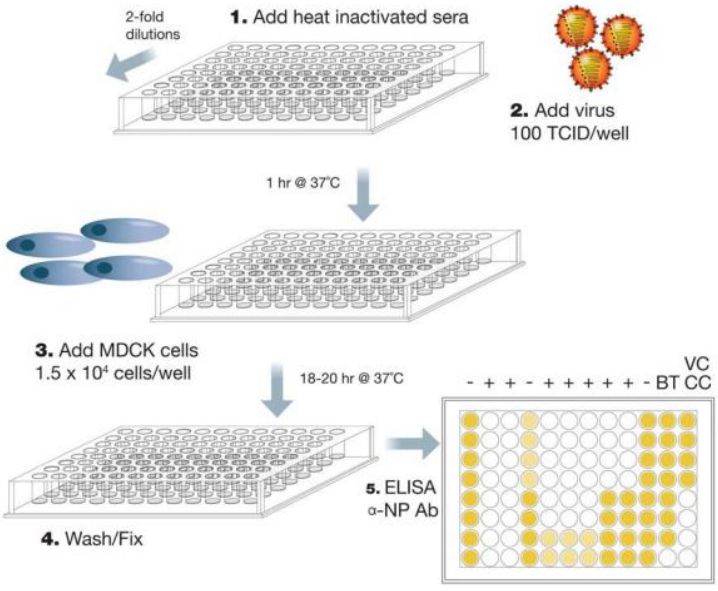


**Figure 5. Schematic representation of a microneutralization assay.** Reproduced from http://www.who.int/influenza/gisrs_laboratory/2010_12_06_serological_diagnosis_of_influenza_by_microneutralization_assay.pdf

### *3.3.4. Influenza genes sequencing*

A subset of the influenza samples isolated at the NRCI are genetically characterized by sequencing the HA 1 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 09) 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 19. 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 genes sequencing but with gene-specific primers (Annex 19).

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 (GISAID) platform (http://platform.gisaid.org, restricted access).

### *3.3.5. 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 resistances 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 resistances 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 be performed if needed and/or upon request using the NA-Fluor™ Influenza Neuraminidase Assay Kit (Thermo Fisher Scientific, Ecublens, Switzerland). Briefly, 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 wavelength is of 355 nm and the emission wavelength was of 460 nm. Data are plotted as log inhibitor concentration against fluorescence inhibition and the IC50s are read from the graph. (Table 2)

# 4. 2016/17 Influenza season results

## 4.1. Detection of influenza in nasopharyngeal samples

The winter influenza surveillance lasted 29 weeks. It started on 3 October 2016 (week 40/2016) and ended on 21 April 2017 (week 16/2017). The epidemic threshold of ≥64 suspected influenza cases per 100,000 inhabitants was exceeded from weeks 50/2016 to 8/2017 with a peak of MC-ILI during week 2/2017. Of the 155 practitioners participating in clinical surveillance, 74 sent a total of 982 nasopharyngeal swabs from 979 patients for influenza screening. Overall, 481 (49%) were positive for influenza by rRT-PCR (Figure 6a; Annex 1). Four hundred and sixty-two of 481 were of type A (96%) and 19/481 (4%) of type B. Four hundred and fifty-three (98.1%) influenza A were A(H3N2), 8/462 (1.7%) were A(H1N1 09), and 1/462 (0.2%) could not be further characterized due to a low viral load. Concerning the influenza B viruses, 15/19 (79%) belonged to the Yamagata lineage, 2/19 (10.5%) were B Victoria, and 2/19 (10.5%) could not be attributed to a specific lineage (Figure 6b).

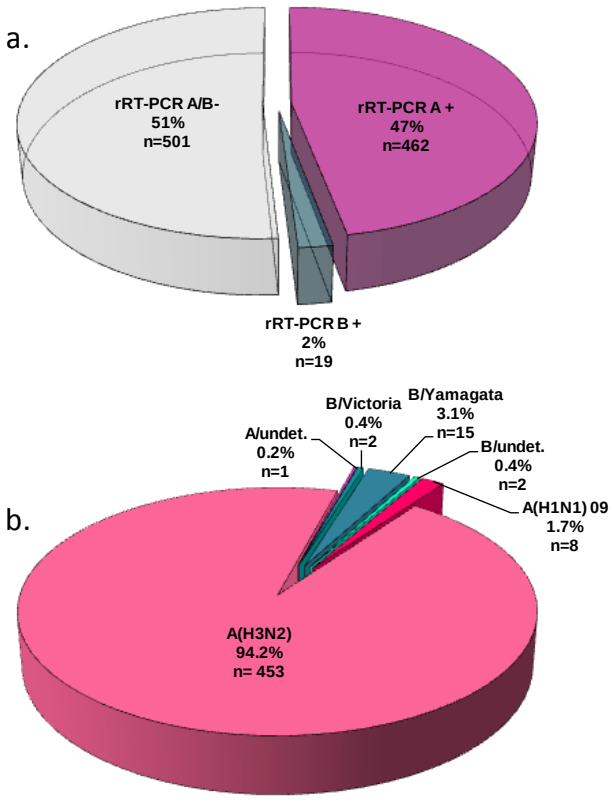


**Figure 6. Distribution of influenza viruses detected in nasopharyngeal specimens collected during the 2016/17 season.** a. Percentage of rRT-PCR A and B-positive versus rRT-PCR-negative specimens (n=982). b. Distribution of the different subtypes (influenza A) and lineages (influenza B) of the viruses in % (n=481).

The number of influenza-positive samples processed started to increase at week 48/2016 and peaked at week 2/2017 (n=56; 60.9% positivity). The positivity rate remained above 60% from weeks 51/2016 to 6/2017 (Figure 7). A(H3N2) viruses predominated during the entire season. A low increase in B Yamagata viruses was observed at the end of the season. A few sporadic cases of B/Victoria and A(H1N1 09) were also observed (Figure 7).

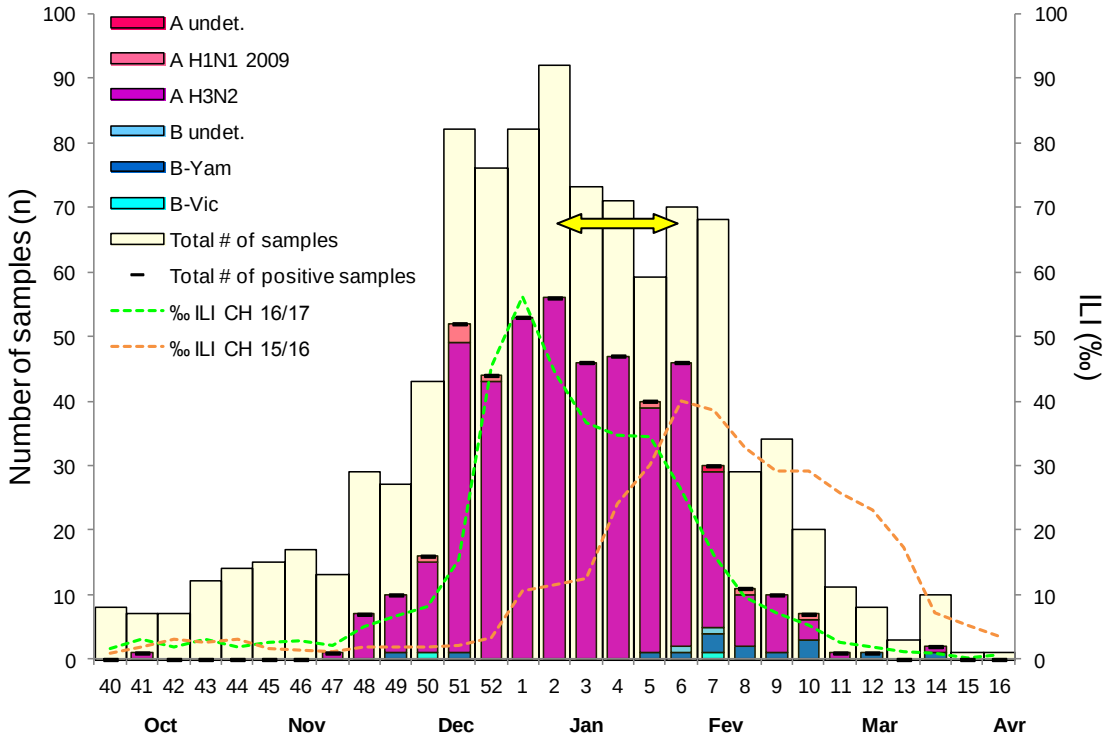


**Figure 7. Schematic illustration of the 2016/17 flu/influenza season.** A undet.: influenza A, but the type could not be determined; A/H1N1 2009: influenza A/H1N1pdm09; A/H3N2 seasonal: influenza A/H3N2 viruses; B undet.: influenza B, but the type could not be determined; B-Yam: influenza B of the Yamagata lineage; B-Vic: influenza B of the Victoria lineage; ILI 16/17 and 15/16: ILI suspected cases registered during the 2016/17 and 2015/16 season (‰); sampling: yellow arrow indicates the weeks when Sentinel practitioners sent 1/5 samples for influenza screening (weeks 2 to 6/2017).

**Influenza outbreak summary**

*Influenza season duration*: **29 weeks**

*Total number of samples*: **982** *(979 individuals)*

*Percentage of positive samples*: **49%** (n=481)

**96% influenza A** of which 98.1% were A(H3N2)

**4% influenza B** of which 79% were B Yamagata

## 4.2. Epidemiology of influenza viruses detected by the Sentinel network

### *4.2.1. Stratification by sex and age*

Influenza-positive and -negative samples were first analyzed according to the sex and age of the "source" individuals. Age groups were defined by the FOPH as follows: 0-4 years; 5-14 years; 15-29 years; 30-64 years; and ≥ 65 years. The 982 samples sent to the NRCI corresponded to 979 patients (three individuals were sampled twice). Information on sex was available for 965/979 individuals and age was available for 974/979.

Among 965 patients, 497 (51.5%) were female (240 influenza-positive and 257 negative) and 468 (48.5%) were male (229 influenza-positive and 239 negative).Four hundred and forty-two (45.4%; n=974) individuals belonged to the 30-64 years group, 190 (19.5 %) to the 15-29 years, 150 (15.4%) to the 5-14 years, 115 (11.8%) to the ≥65 years, and 77 (7.9%) to the 0-4 years group. The highest prevalence of positive samples (63%) was observed in the 5-14 years, followed by the ≥65 years group (57%) (Figure 8). The 0-4 years group exhibited the lowest prevalence of positive samples (31%) (Figure 8).

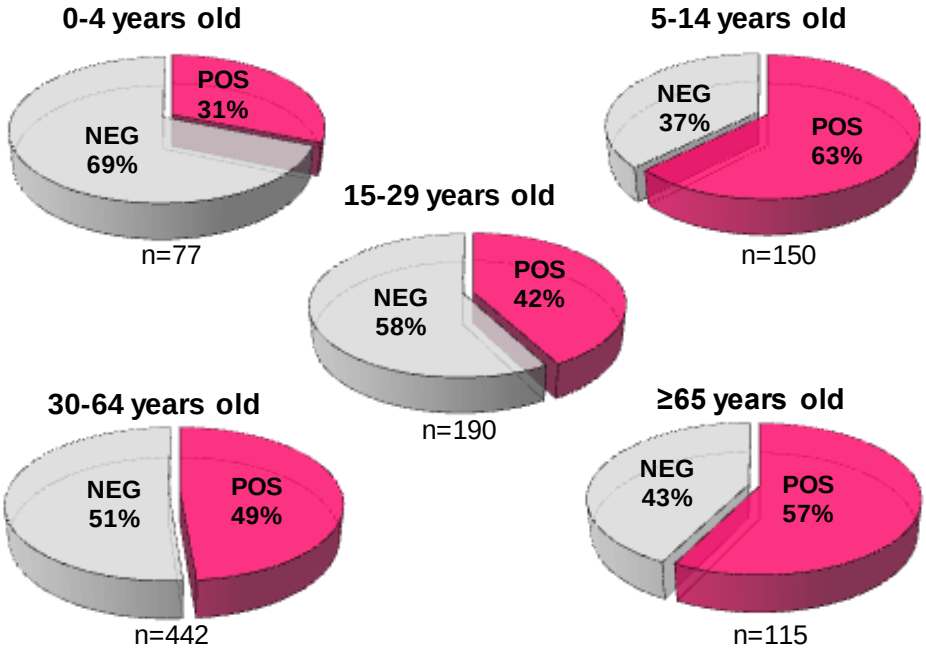


**Figure 8. Influenza prevalence per age group.**

Of the 479 influenza-positive samples considered, 45% originated from the 30-64 years group, 19% from the 5-14 years, 17% from the 15-29 years, 14% from the ≥65

years, and 5% from the 0-4 years group (Figure 9). Among the 495 negative samples analyzed, 46% belonged to the 30-64 years group, 22% to the 15-29 years, 11% to the 5-14 years, 11% to the 0-4 years and 10% to the ≥65-years group (Figure 9).

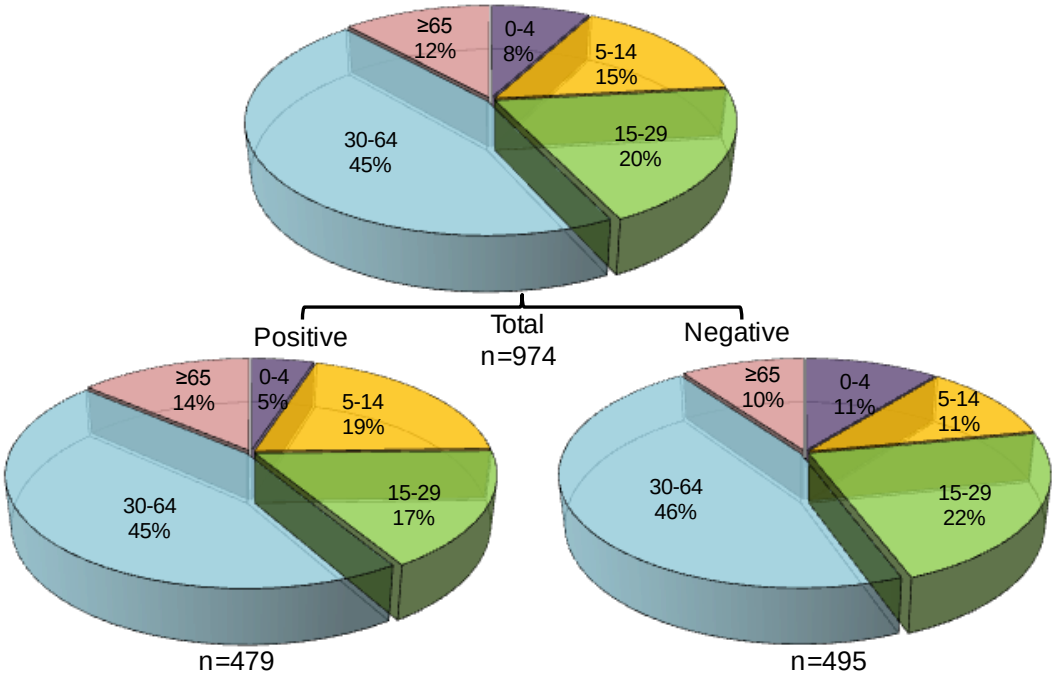


**Figure 9. Proportions of total, influenza positive and negative samples per age groups.**

A(H3N2) viruses were dominant accross all age groups (91.7% for 0-4 years, 96.8% for 5-14 years, 91.3% for 15-29 years, 93% for 30-64 years and 98.5% for ≥65 years; n=451/453 A(H3N2)). Even if much lower, B/Yamagata were the second most abundant viruses circulating this season (8.3% for 0-4 years, 1.1% for 5-14 years, 2.5% for 15-29 years, 4.2% for 30-64 years and 1.5% for ≥65 years). B/Victoria (n=2) and A(H1N1 09) (n=8) viruses were only sporadically detected (Figure 10).

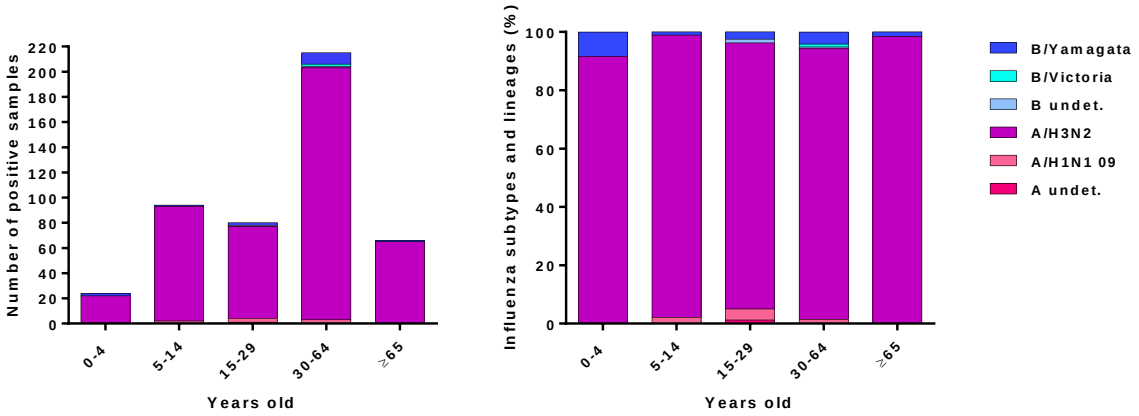


**Figure 10. Distribution of influenza virus subtypes/lineages per age group.** Left panel: total number of positive samples per subtype per age group. Right panel: subtypes/lineages proportions per age group (%). B Vic = B Victoria; B Yam = B Yamagata. Undet.= not able to be subtyped.

### *4.2.2. Stratification by influenza vaccination status*

Information on vaccination status was provided for 945 (96.5%) of the 979 sampled individuals. The status was reported as "unknown" by Sentinel practitioners for four (0.4%; n=979) individuals (one influenza-negative and three A(H3N2) influenza positive) and no vaccination data were provided at all for 30 (3.1%; n=979) patients. Eight hundred and twelve (85.9%; n=945) patients did not receive the 2016/17 influenza vaccine. Among these, 390 (48%; n=812) were positive for influenza (Table 1). Of the 133 (14.1%; n=945) individuals vaccinated against influenza in 2016/17, 63 (47.4%; n=133) had a negative influenza test and 70 (52.6%; n=133) a positive one (Table 1). Of note, 25 of the 133 vaccinated individuals (16 influenza-negative and 9 influenza-positive) were reported as vaccinated, but no vaccination date was provided. In the present report, we have assumed that these individuals received the 2016/17 vaccine. The 70 influenza-positive vaccinees were all infected with A(H3N2) viruses.

**Table 1. Vaccination status among influenza positive and negative individuals**

| **Exposure : influenza during the 2016/17 outbreak; n=945** | *Vaccinated n=133 (%)* | *Not vaccinated n=812 (%)* |
|---|---|---|
| **Yes** | 70 (52.6) | 390 (48) |
| **No** | 63 (47.4) | 422 (52) |

In Switzerland, vaccination is recommended for some specific populations,[13] such as elderly patients (≥65 years), pregnant women, and individuals suffering from several

chronic diseases. During the 2016/17 season, the NRCI received samples from six pregnant females (two were vaccinated, one was infected with an influenza A(H3N2)). Thirty-one patients were reported as chronically ill (7/31 ≥65 years), among which 11 were vaccinated (five negative and six positive for influenza A(H3N2)). Two of the 11 vaccinated patients were ≥65 years and both were positive for influenza. In total, 47 vaccinees (35.3%; n=133) were ≥65 years (32 positive for influenza A and 15 negative).

### 4.3. Antigenic and genetic characterization of influenza viruses

HAI-tested samples were selected as follows: 1) before and after the epidemic period, all cultured samples with a sufficient HA titer were tested; 2) during the epidemic period, each week the first five rRT-PCR positive samples (Ct value <30) with sufficient HA titers were further characterized (Figure 3). One hundred and three influenza-positive samples were cultured on MDCK and MDCK-SIAT cells. Of these, 95 grew on MDCK and/or MDCK-SIAT cells. Eighty-four of 95 were submitted to antigenic characterization by HAI. Eleven of 95 had an unusual HA pattern and were not further characterized. Fifty-nine of 84 were successfully subtyped by HAI. The 25 non-typable samples were all A(H3N2) viruses (Figure 11; Annexes 2-5). Of the 25 non-typable viral samples, 13 were submitted to a MN assay after the end of the season (Annex 2e).

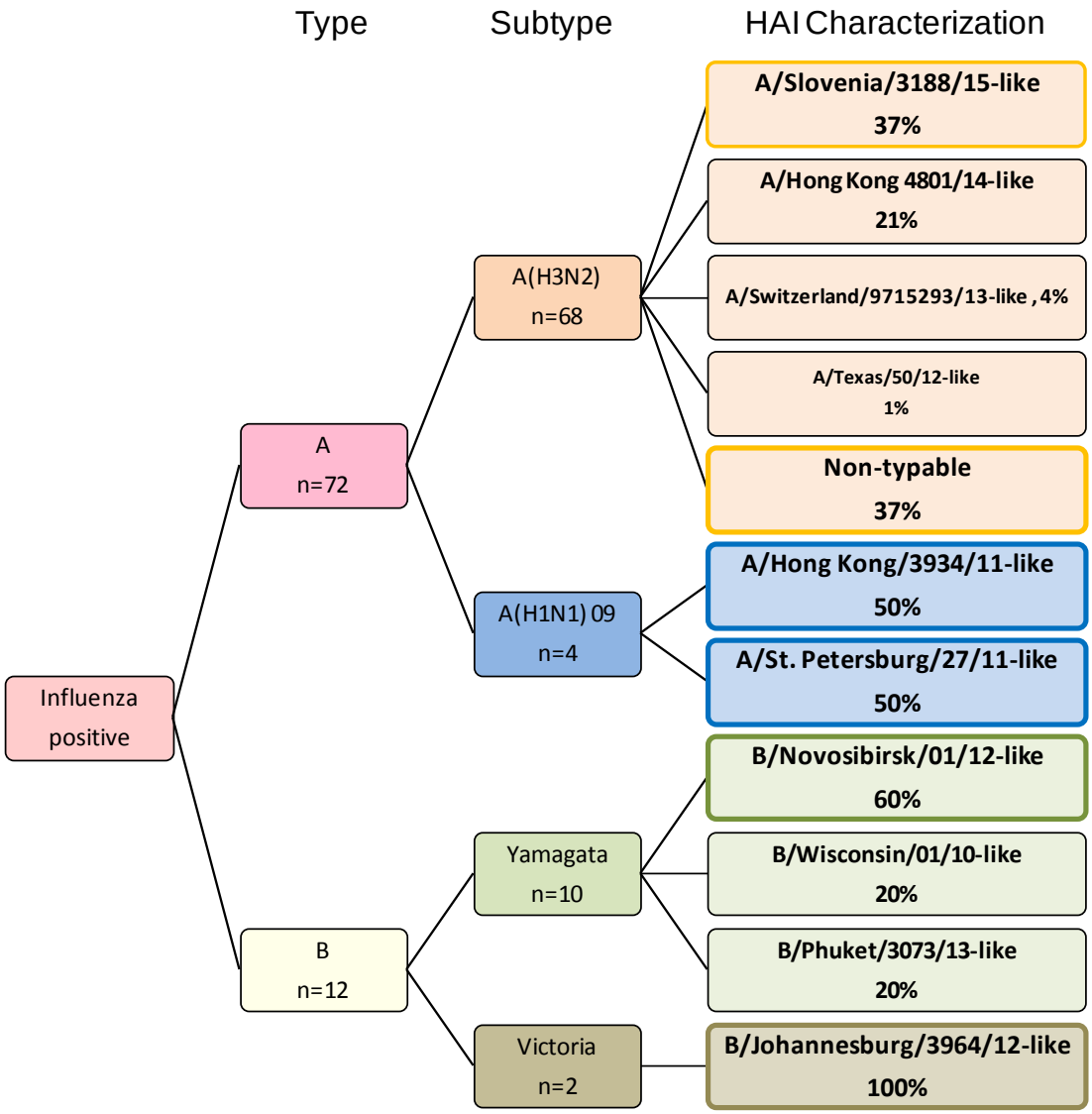


**Figure 11. Antigenic characterization by HAI of selected influenza viruses isolated through the 2016/17 season.**

One-hundred-and-five samples were submitted for genetic characterization by HA and NA gene sequencing. Seventy M and five NS genes were also sequenced. One hundred and three HA sequences were successfully recovered. Among these, 89 belonged to A(H3N2), four to A(H1N1 09), eight to B/Yamagata and two to B/Victoria subtypes/lineages (Figures 12-15). One hundred NA were successfully sequenced: 89 were A(H3N2); four A(H1N 09); six B/Yamagata; and one B/Victoria. All of the 17 (15 A(H3N2) and 2 A(H1N1 09)) M and 5 B NS (3 B/Yamagata and 2 B/Victoria) 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.

Twenty samples (16 A(H3N2), 2 A(H1N1 09), and 2 B) were shared with the WIC for additional characterization. Phenotypic analysis results are available in Annexes 6-9; phylogenic analysis in Annexes 10-17; and antiviral resistance in Annex 18.

#### *4.3.1. Characterization of influenza A(H3N2) viruses*

Similar to the two last influenza seasons, the WIC reported that antigenic characterization of A(H3N2) viruses by HAI 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 clade 3C.2a as well as the newly emerged 3C.2a1 subclade. Titration and antigenic characterization of successfully isolated A(H3N2) viruses was achieved by MN assay (plaque reduction neutralization).

For the first time since the variable agglutination phenomenon was described, we observed a marked increase at NRCI in the number of A(H3N2) isolates that exhibited an “unstable” hemagglutination that did not allow further characterization by HAI (25 non-typable viruses shown in Figure 11). Among the 59 isolates successfully characterized by HAI, 43 (72.9%) were A(H3N2). Fourteen of 43 were classified as A/HongKong4801/14-like viruses (the 2016/17 vaccine strain), 25 as A/Slovenia/3188/15-like, three as A/Switzerland/9715293/13-like, and one A/Texas/50/12-like. All A/Slovenia/3188/15-like and A/Switzerland/9715293/13-like isolates, but not the unique A/Texas/50/12-like were recognized within two to four-fold of the homologous titer by the antiserum raised against A/HongKong4801/14 virus (Figure 11, Annexes 2a to 2d).

Of 13/25 non-typable viruses submitted to MN, 10 were recognized within four-fold of the homologous titer of the A/Slovenia/3188/15 antiserum but only three gave similar titers with the antiserum raised against the vaccine virus A/HongKong4801/14. Nine of 10 were defined as being A/Slovenia/3188/15-like strains and one as A/Switzerland/9715293/13-like. Isolates A/Switzerland/*****161/17, A/Switzerland/*****352/17, and A/Switzerland/*****010/17 were not recognized by any of the reference antisera tested, including the most recent antiserum available at NRCI targeting the A/Slovenia/3188/15 strain. Therefore, they remained non-typable. (Annex 2e). In summary, among all the A(H3N2) isolates (n=68) submitted to either HAI or MN characterization, 15 (22.1%) remained classified as non-typable.

All 16 viruses sent to the WIC were recovered. They were all identified solely on the basis of the sialidase activity of the virus NA as they were unable to agglutinate the guinea pig RBC used at the WIC. Therefore, no HAI were carried out on any of these samples at the WIC. The plaque reduction neutralization results showing that A/Switzerland/*****223/16, A/Switzerland/*****147/16, A/Switzerland/*****699/16, and A/Switzerland/*****103/16 isolates were recognized within two to four-fold of the homologous A/HongKong4801/14 titer can be found in Annexes 6a and 6b. The isolates A/Switzerland/*****442/16 and A/Switzerland/*****514/16 were not well recognized (eight-fold the homologous titer) by the A/HongKong4801/14 antiserum. Interestingly, the isolates A/Switzerland/*****147/16, A/Switzerland/*****699/16, A/Switzerland/*****103/16, A/Switzerland/*****442/16 and A/Switzerland/ *****514/16 isolates had low, but sufficient HA activity to be further characterized by HAI at the NRCI. They all were recognized within two-fold of the homologous titer by the antiserum raised against the A/Hong Kong4801/14 vaccine strain at the NRCI. Discrepancies in HAI/MN results between NRCI and WIC antigenic analyses could be explained by site-specific assay variations and the use of different reagents, notably fixed RBCs (NRCI) and reference sera lots.

At the genetic level, 89 A(H3N2) HA1 (Figure 12) and 89 NA (not shown) sequences were successfully recovered. All fell into the 3C.2a genetic group (A/Hong Kong/4801/14) but 55/89 belonged to the 3C.2a1 subclade (A/Slovenia/3188/15). As typically observed for the highly variable A(H3N2) viruses, we observed several sub-clusters, characterized by specific mutations, within the 3C.2a cluster and 3C.2a1 sub-cluster (Figure 12). Mutation V149A in the NA was found in three isolates and was known to be associated to a mild resistance to zanamivir in the A(H5N1) strain background. In order to exclude a possible, but not yet documented, resistance of these isolates to NAI oseltamivir and zanamivir, a phenotypic resistance assessment was performed. All three isolates were sensitive to oseltamivir and zanamivir (Table 2).

The HA and NA genes of 15 of the 16 isolates sent to the WIC were analyzed. The HA gene of all 15 isolates fell into the 3C.2a subclade, 10 of these belonged to the 3C.2a1 cluster. Viruses were separated into two groups within the 3C.2a1 cluster (Annex 10). Four viruses had the N121K substitution and six were without this substitution. The HA gene of the other five viruses fell into a cluster within 3C.2a,

defined by the substitutions N121K and S144K in HA1 (Annex 10). The NA genes clustered similarly (Annex 11). The genetic characterization and subsequent cluster/sub-cluster attribution for the different Swiss A(H3N2) isolates analyzed in both sites was concordant in-between the WIC and the NRCI (Figure 12; Annex 10)

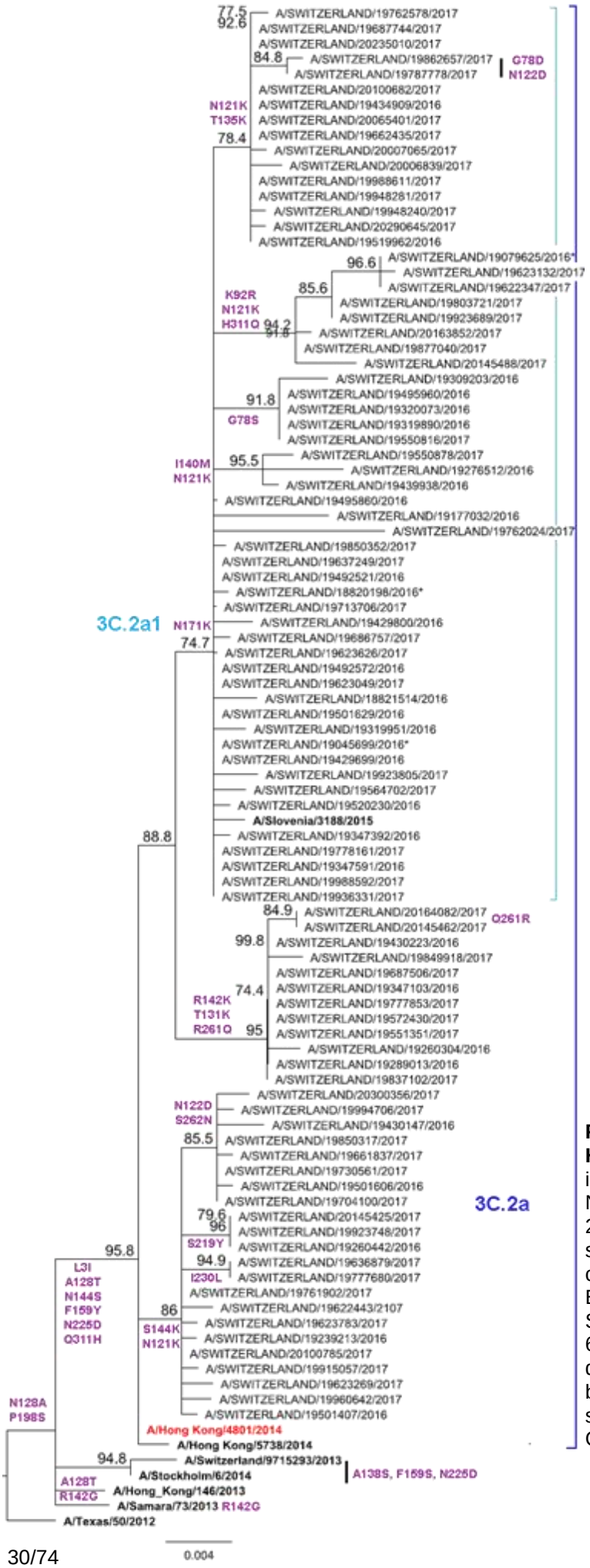


**Figure 12. Phylogenetic analysis of the HA1 gene of A(H3N2)-like viruses.** Black: influenza virus detected in the Sentinel Network during the 2016/17 season. Red: 2016/17 vaccine strain. Bold: reference strains. Purple: some typical mutations characterizing the respective clusters. Blue: A(H3N2) genetic groups/sub-groups. Sequences were aligned using Geneious 6.1.8 MAFFT alignment (v7.017) with default settings. A consensus tree was built from 1000 original trees in ML (70% support threshold) constructed using Geneious 6.1.8 PHYML default settings.

*Comments from the WIC*

*"Seven genetic groups based on the HA gene have been defined for H3N2 viruses, two derived from A/Perth/16/2009 and five from A/Victoria/208/2009; but now the majority of HA genes fall into the A/Victoria/208/2009 genetic clade and predominantly into the genetic subgroup 3C of group 3. This subgroup has three subdivisions: 3C.1, 3C.2 and 3C.3.*

*The vaccine virus A/Texas/50/2012 previously recommended to be used for 2014/2015 belongs to genetic subgroup 3C.1. Amino acid substitutions that define subgroups 3C.2 and 3C.3 are:*

- *3C.2* ***N145S*** *in HA1, and* ***D160N*** *in HA2, e.g. A/Hong Kong/146/2013;*
- *3C.2a also carries* ***N144S*** *(loss of potential glycosylation motif),* ***F159Y***, ***K160T*** *(gain of a potential glycosylation site),* ***N225D***, ***Q311H*** *in HA1, e.g. A/Hong Kong/5738/2014 and A/Hong Kong/4801/2014.*
  - *3C.2a1 subgroup of 3C.2a is defined by the amino acid substitution* ***N171K*** *in HA1 and* ***I77V*** *and* ***G155E*** *in HA2 (e.g. A/Slovenia/3188/2015)."*

**A(H3N2) viruses**

Around two-thirds of the A(H3N2) strains were antigenically related to the vaccine strain A/Hong Kong/4801/14 (3C.2a cluster). An increasing number of isolates were either antigenically or genetically closer to A/Slovenia/3188/15 viruses from the 3C.2a1 subclade of 3C.2a group.

### *4.3.2. Characterization of influenza A(H1N1 09)*

Among the 59/84 isolates successfully characterized by HAI, only four were influenza A(H1N1 09). Three of these influenza viruses were recognized by antiserum directed against the vaccine strain A/California/7/09 at two- to four-fold of the homologous titer. Only one isolate, A/Switzerland/*****226/16, was not well recognized by the A/California/7/09 antiserum (64-fold of the homologous titer). This virus was weakly recognized, but within four-fold the of homologous titer by all the other reference A(H1N1 09) antisera tested. It was identified as A/St-Petersburg/27/11-like (Figure 13; Annex 3). Both A(H1N1 09) viruses, A/Switzerland/*****226/16 and A/Switzerland/*****003/ 16, sent to the WIC were successfully recovered and both were recognized by antisera raised against the 2016/17 and 2017/18 vaccine viruses A/California/7/2009 and A/Michigan/45/2015, respectively (Annex 7).

Sequence analysis of four HA genes of randomly selected NRCI A(H1N1 09) viruses showed that all isolates belonged to clade 6B.1 (Figure 13). The A/Switzerland/*****226/2016 and A/Switzerland/*****003/2016 isolates both carry the V321I substitution in HA1, which places them in a small genetic group with an HA slightly different from that of A/Michigan/45/2015, the 2017/18 influenza vaccine. The NA sequences cultured similarly. NRCI and WIC phylogenetic results were fully concordant for both HA and NA genes (Figure 13; Annexes 7, 12 and 13).

*Comments from the WIC*

> ***"****H1N1 viruses cluster into seven genetic groups, previously described, groups 2 to 8. Most recently viruses have fallen into genetic group 6B.1. The main characteristics of viruses in the 6B.1 group are that the viruses carry the amino acid substitutions* ***S84N****,* ***S162N*** *(introducing a new potential glycosylation site) and* ***I216T*** *in* ***HA1****, e.g. A/Slovenia/2903/2015. H1N1pdm09 viruses were causing problems in many parts of the world in during the 2015/2016 Northern hemisphere influenza season."*

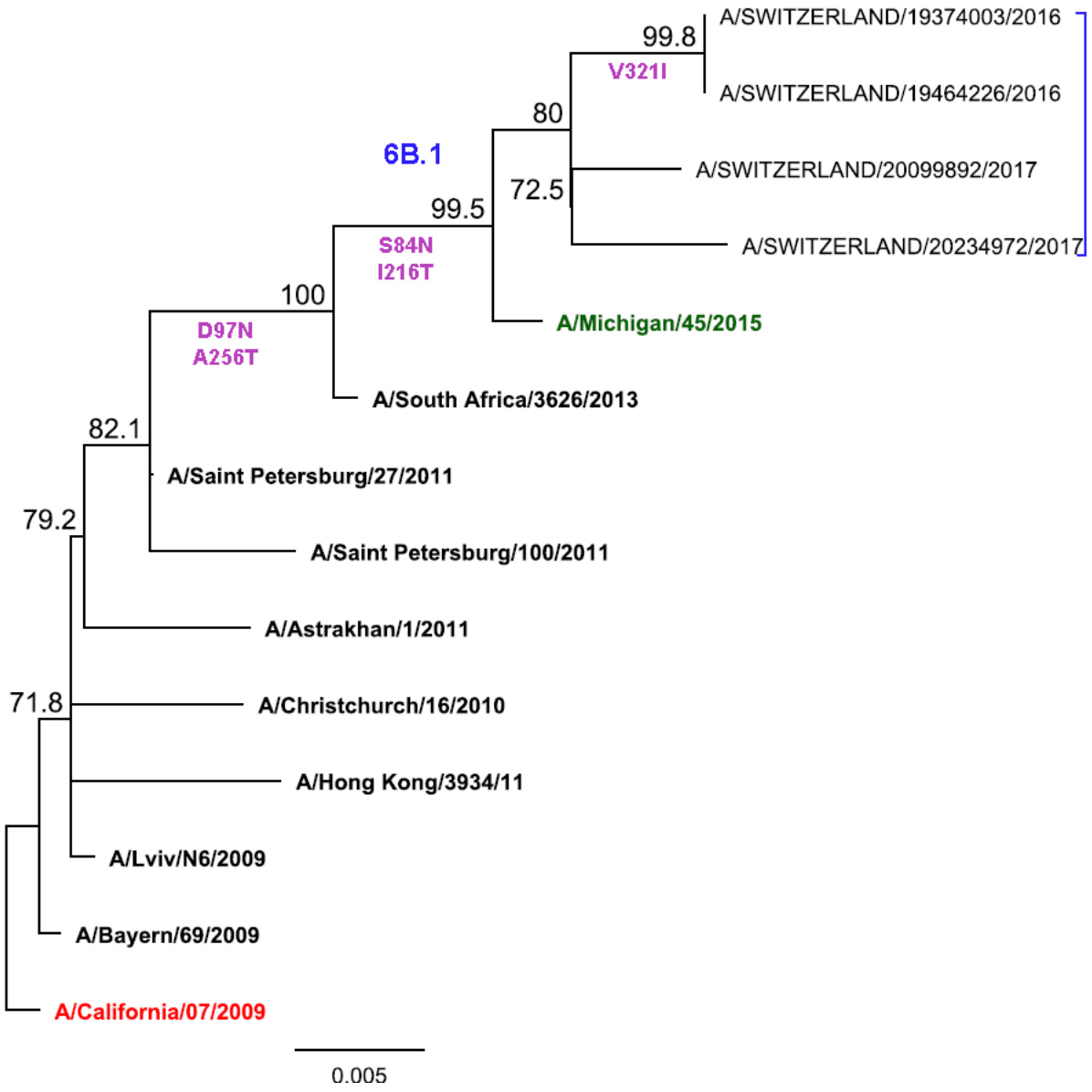


**Figure 13. Phylogenetic analysis of the HA1 gene of A(H1N1 09)-like viruses.** Black: influenza virus detected in the Sentinel Network during the 2016/17 season. Red: 2016/17 vaccine strain. Green: 2017/18 vaccine strain. Bold: reference strains. Blue: A(H1N1 09) genetic cluster. Purple: some typical mutations described by the WIC. Sequences were aligned using Geneious 6.1.8 MAFFT alignment (v7.017) with default settings. A consensus tree was built from 1000 original trees in ML (70% support threshold) constructed using Geneious 6.1.8 PHYML default settings.

**A(H1N1) 09 viruses**

Three of four A(H1N1 09) were recognized within four-fold the of homologous titer by the antiserum raised against the 2016/17 vaccine strain, A/California/07/09.

### *4.3.3. Characterization of influenza B viruses*

#### *4.3.3.1. B/Yamagata/16/88 viruses:*

Ten B/Yamagata viruses were submitted to HAI characterization at the NRCI. Six were identified as B/Novosibirsk/01/12-like strains, two B/Wisconsin/01/10-like and two B/Phuket/3073/13-like. All B/Yamagata viruses, apart from the B/Switzerland/*****969/2017 isolate, were recognized within two to four-fold by the antiserum raised against B/Phuket/3073/13 (the 2016/17 vaccine strain). The B/Switzerland/*****969/2017 virus reacted better with B/Wisconsin/01/10 antiserum. (Annex 4).

The unique influenza B/Yamagata virus sent to the WIC, B/Switzerland/*****131/2016, was successfully recovered. In contrast to what we observed at the NRCI, the B/Switzerland/*****131/2016 isolate recovered at the WIC was not well recognized by antisera raised against either the egg-propagated vaccine virus B/Phuket/3073/2013 or its cell culture-propagated cultivar. B/Switzerland/*****131/2016 was also poorly recognised by the antiserum raised against the formerly recommended vaccine virus B/Wisconsin/1/2010. However, B/Switzerland/*****131/2016 was recognized at a titre within four- and two-fold of the homologous titre of the antiserum, by the antiserum raised against egg-propagated B/Stockholm/12/2011 and the antiserum targeting the egg-propagated B/Hong Kong/3417/2014, respectively (Annexes 4 and 8). Sequence analysis of the HA and the NA genes of B/Yamagata viruses carried out at both the WIC (1 HA and NA, Annexes 14-15) and the NRCI (8 HA, Figure 14 and 6 NA), showed that both genes fell in the genetic clade 3 for all isolates

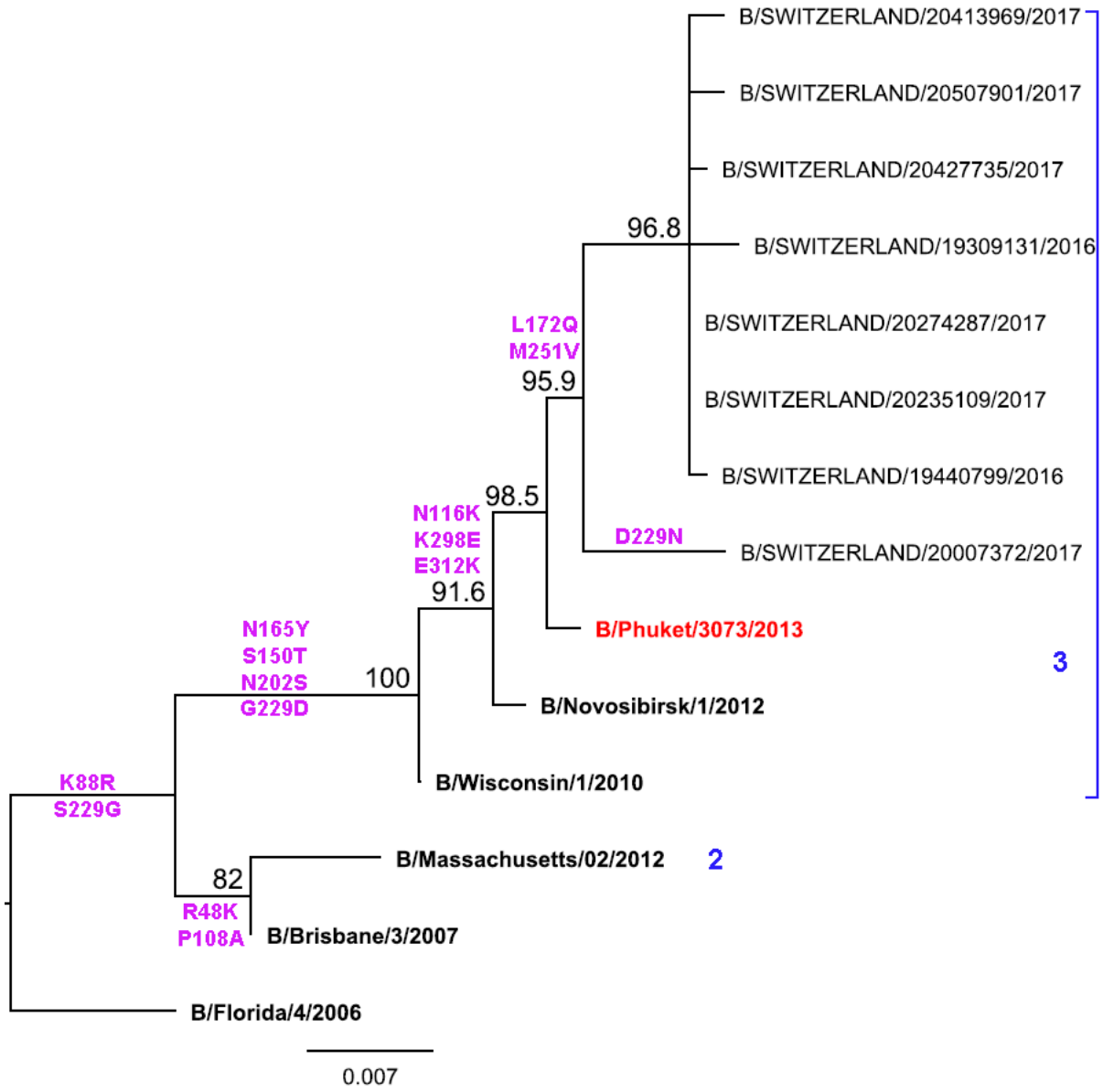


**Figure 14. Phylogenetic analysis of the HA1 gene of B Yamagata viruses.** Black: influenza virus detected in the Sentinel Network during the 2016/17 season. Red: 2016/17 vaccine strain. Blue: genetic groups (clades). Bold: selected reference sequences used by the WHO. Purple: some typical mutations described by the WIC. Sequences were aligned using Geneious 6.1.8 MAFFT alignment (v7.017) with default settings. A consensus tree was built from 1000 original trees in ML (70% support threshold) constructed using Geneious 6.1.8 PHYML default settings.

**B/Yamagata/16/88 viruses**

Yamagata lineage viruses were all antigenically close to the 2016/17 vaccine strain, B/Phuket/3073/13.

#### *4.3.3.2. B/Victoria/2/87 viruses:*

Only two B/Victoria viruses were identified at the NRCI during the 2016/17 season. B/Switzerland/*****129/2016 and B/Switzerland/*****954/2016 isolates were both recognized within two-fold of the homologous titer by the B/Johannesburg/3964/2012 antiserum but only B/Switzerland/*****129/2016 virus was closely related to the vaccine strain B/Brisbane/60/2008 (Annex 5).

The B/Switzerland/*****129/2016 virus was also successfully recovered at the WIC. B/Switzerland/19357129/2016 was recognized by the antiserum raised against the vaccine virus egg-propagated B/Brisbane/60/2008 at a titer four-fold lower than the homologous titer of the antiserum. Antisera raised against the other egg-propagated viruses, including B/Johannesburg/3964/2012, recognized B/Switzerland/*****129/2016 at lower titers relative to the homologous titers of the antisera. Concerning the low homologous titer of the antisera raised against three of the cell culture-propagated reference viruses (B/Hong Kong/514/2009, B/Ireland/3154/2016 B/Formosa/V2367/2012, and B/Nordrhein-Westfalen/1/2016) only the antiserum raised against B/Ireland/3154/2016 recognized B/Switzerland/*****129/2016 at a titer similar to the titer of the antisera for the homologous viruses. However, the antisera raised against B/Hong Kong/514/2009 and B/Nordrhein-Westfalen/1/2016 recognized B/Switzerland/*****129/2016 at a titer four-fold lower than the homologous titer. (Annex 9)

Sequence analysis of the HA (B/Switzerland/*****129/2016 and B/Switzerland/*****954/2017) and the NA (B/Switzerland/*****129/2016 recovered only) genes of B/Victoria viruses shown that both HA (Figure 15) and NA (not shown) genes fell in clade 1A, the B/Brisbane/60/2008 clade. Similar results were obtained by the WIC for the isolate B/Switzerland/*****129/2016 (Annexes 16 and 17).

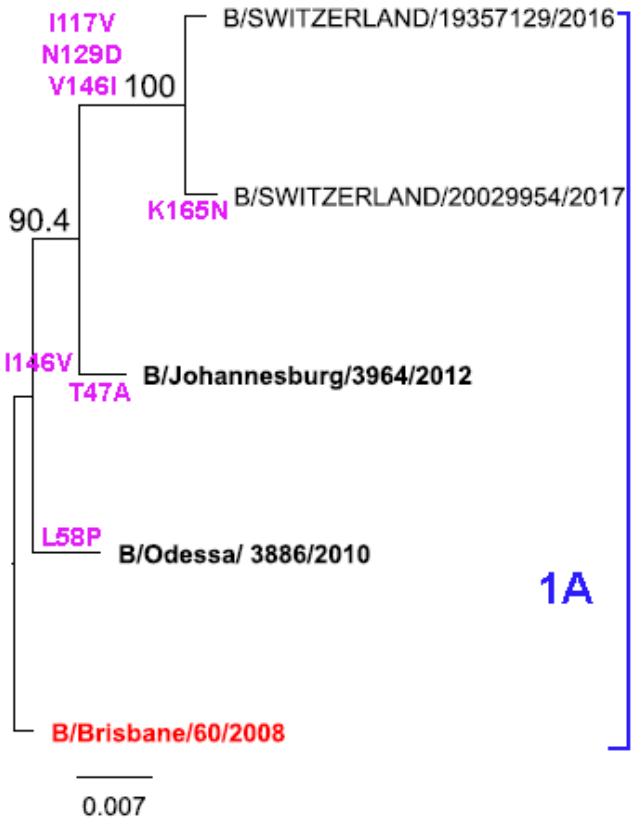


**Figure 15. Phylogenetic analysis of the HA1 gene of B Victoria-like viruses.** Black: influenza virus detected in the Sentinel Network during the 2016/17 season. Red: 2016/17 vaccine strain. Bold: selected reference sequences used by the WHO. Purple: some typical mutations described by the WIC. Blue: genetic groups (clades). Sequences were aligned using Geneious 6.1.8 MAFFT alignment (v7.017) with default settings. A consensus tree was built from 1000 original trees in ML (70% support threshold) constructed using Geneious 6.1.8 PHYML default settings.

**B/Victoria/2/87 viruses**

One of two Victoria lineage viruses was antigenically close to the 2016/17 vaccine strain, B/Brisbane/60/08.

### 4.4. Antiviral resistance

One-hundred-and-five influenza viruses were submitted to NA gene sequencing analysis to assess the antiviral resistance of circulating strains. Among the 89 A(H3N2), four A(H1N1 09), six B/Yamagata and one B/Victoria NA sequences successfully recovered, none had the common strain-specific mutations associated with resistance to NAIs. As mentioned in section 1.6.1, phenotypic antiviral resistance was performed for three isolates carrying mutation V149A and one harbouring mutation V149I. All shown normal inhibition of the NA activity in presence of both oselatmivir and zanamivir (Table 2).

**Table 2: Zanamivir and oseltamivir resistance assessment of isolates with V149A mutation**. *Nd: not done.*

| | | Inhibitor | Oseltamivir | Zanamivir |
|---|---|---|---|---|
| | **AA in NA** | **Dilution** | **IC50** | **IC50** |
| **20145462 S/1** | V149A | 14.6X | 0.297 | 0.593 |
| **19778161 MD/1** | V149A | 32.4X | 0.379 | 0.412 |
| **20164082 MD/1** | V149A | 51.3X | 0.362 | 0.389 |
| **20290645 MD/1** | V149 | 49X | 0.386 | 0.394 |
| **19289013 S/1** | V149 | 8.1X | 0.395 | nd |
| **20235010 MD/1** | V149I | 1.22X | 0.529 | 0.585 |
| **E119** | | 4.79X | 0.332 | 1.78 |
| **E119V** | | 5.03X | 44.36 | 0.402 |

Eighteen (14 A(H3N2), 2 A(H1N1 09), 1 B/Yamagata and 1 B/ Victoria) of the 20 Sentinel influenza viruses sent to the WIC had sufficient sialidase activity for the assessment of resistance to the NAIs oseltamivir and zanamivir in NA inhibition assays. All were sensitive to the two NAIs, oseltamivir and zanamivir (Annex 18).

**NAI resistance**

No NAI-resistant influenza strains were identified during the 2016/17 influenza season.

## 5. WHO recommendation for the composition of influenza virus vaccines for the 2017/18 influenza season

As most of the A(H1N1 09) viruses circulating worldwide were generally better recognized by antisera raised against A/Michigan/45/2015 isolate, the latter strain will replace the former vaccine strain for the 2017/18 influenza season. The vaccine strains recommended for the 2017/18 Northern hemisphere influenza vaccine by the WHO experts are:

| | **Vaccine strains 2017/18** |
|---|---|
| **A(H1N1 09)** | A/Michigan/45/2015 (H1N1) |
| **A(H3N2)** | A/Hong Kong/4801/2014-like virus |
| **B** | B/Brisbane/60/2008-like virus* |

**Table 3. Recommended influenza vaccine composition for the 2017/18 season**. *B/Phuket/3073/2013-like virus is advised for quadrivalent vaccines.

## 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 seem to require a close contact with infected animals for spread and do not (or at least not efficiently) sustain human-to-human transmission 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 2016/17 influenza season, two 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.

**Table 4. Pig breeders influenza rtRT-PCR results.**

| Sample ID | Result | Sample date |
|---|---|---|
| *****823 | Porcine IA | 20.12.16 |
| *****442 | NEG | 21.12.16 |

IA:influenza A, NEG: negative

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 and as of May 2017, 402 (373 A(H3N2)v, 20 A(H1N1)v and 9 A(H1N2)v) human cases of variant influenza have been reported within several States.[15]

### 6.2. Avian influenza A subtypes in humans

As of May 2017, a total of 859 laboratory-confirmed human cases of A(H5N1), including 453 deaths, have been reported to WHO (Figure 16).

| Country | 2003-2009* | | 2010-2014** | | 2015 | | 2016 | | 2017 | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | cases | deaths | cases | deaths | cases | deaths | cases | deaths | cases | deaths | cases | deaths |
| Azerbaijan | 8 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 5 |
| Bangladesh | 1 | 0 | 6 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 8 | 1 |
| Cambodia | 9 | 7 | 47 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 56 | 37 |
| Canada | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| China | 38 | 25 | 9 | 5 | 6 | 1 | 0 | 0 | 0 | 0 | 53 | 31 |
| Djibouti | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Egypt | 90 | 27 | 120 | 50 | 136 | 39 | 10 | 3 | 3 | 1 | 359 | 120 |
| Indonesia | 162 | 134 | 35 | 31 | 2 | 2 | 0 | 0 | 0 | 0 | 199 | 167 |
| Iraq | 3 | 2 | 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 | 2 | 2 |
| Myanmar | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Nigeria | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Pakistan | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 1 |
| Thailand | 25 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 25 | 17 |
| Turkey | 12 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 4 |
| Viet Nam | 112 | 57 | 15 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 127 | 64 |
| **Total** | **468** | **282** | **233** | **125** | **145** | **42** | **10** | **3** | **0** | **0** | **859** | **453** |

* 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 16 May 2017

World Health Organization

**Figure 16. Influenza A/H5N1.** Cumulative number of laboratory-confirmed H5N1 cases and deaths from 2003 to 2017.
(http://www.who.int/influenza/human_animal_interface/2017_05_16_tableH5N1.pdf).

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

Since February 2013, four “waves” of A(H7N9) infection cases were reported, and the fifth one is currently ongoing. Of major concern is the fact that the number of confirmed cases is increasing each year. As of 7 June 2017, 1542 laboratory-confirmed A(H7N9) human cases, including 582 deaths, have been reported to WHO. All cases were of Chinese origin and the majority were isolated in China (Figure 17). An increasing number of recent viruses belong to the Yangtze River Delta-lineage, but antisera raised against available candidate vaccine viruses (A/Anhui/1/2013 and A/Shanghai/2/2013) reacted poorly with viruses from this lineage. Therefore A/Hunan/2650/2016-like viruses were proposed as candidate vaccine viruses. In addition, some highly pathogenic avian influenza (HPAI) A(H7N9) viruses of the Yangtze River Delta-lineage, detected also in humans, were genetically and antigenically distinct from A/Hunan/2650/2016-like viruses. Thus A/Guangdong/17SF003/2016-like viruses were proposed as candidate vaccine viruses to cover those particular HPAI viruses.[18]

**Figure 17. 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 also not represented. [18]

Thirty-one laboratory-confirmed A(H9N2) human infections have been reported since 1999. The first A(H9N2) causality was reported in 2016 in a 57-year-old woman with underlying conditions. Until this fatal case, A(H9) viruses were considered to cause only mild diseases. No human-to-human transmission of A(H9N2) viruses has been documented so far.[19] In December 2016, a human case of H7N2 was reported in New York City, USA. This infection resulted from the direct contact of the patient with H7N2-infected cats in an animal shelter. H7N2 infections are rare in both human and cats.[20]

## 7. Avian influenza A in animals (current update)[21]

Highly and low pathogenic avian (LPAI) influenza A viruses continuously circulate within the avian population. Some of these viruses periodically cause moderate to large outbreaks in poultry and/or wild birds particularly during the winter season (Figure 18). HPAI and LPAI A(H5N1) and A(H7N9) viruses are (as of 6 June 2017) currently present in Africa and Asia, and the Americas and Asia respectively. Of note, the A(H7N9) strains circulating in America and Asia do not belong to the same lineage.

In 2016/17 significant epidemics of A(H5N8) and A(H5N6) were observed in Europe and Asia, respectively. Despite the fact that A(H5N8) outbreaks in Europe show encouraging signs of being brought under control, A(H5N8) viruses continue to circulate in Europe, Africa, Asia, and very recently in Zimbabwe, thus suggesting an

increase in their geographical distribution. Wild birds death due to A(H5N8) was reported in Switzerland in November 2016. Therefore, prevention and containment measures were issued from November 12, 2016 to March 17, 2017. Despite the high number of wild birds infected, Switzerland was one of the rare countries in Europe where no A(H5N8) infection was detected in domestic birds and poultry[22]. A(H5N6) outbreaks are still being reported in Asia. Other avian influenza strains responsible for ongoing outbreaks in poultry and wild birds are H7N3 in the Americas, H5N2 in Asia, and H5N5 in Europe. No human infection with these viruses have been reported in European countries 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.

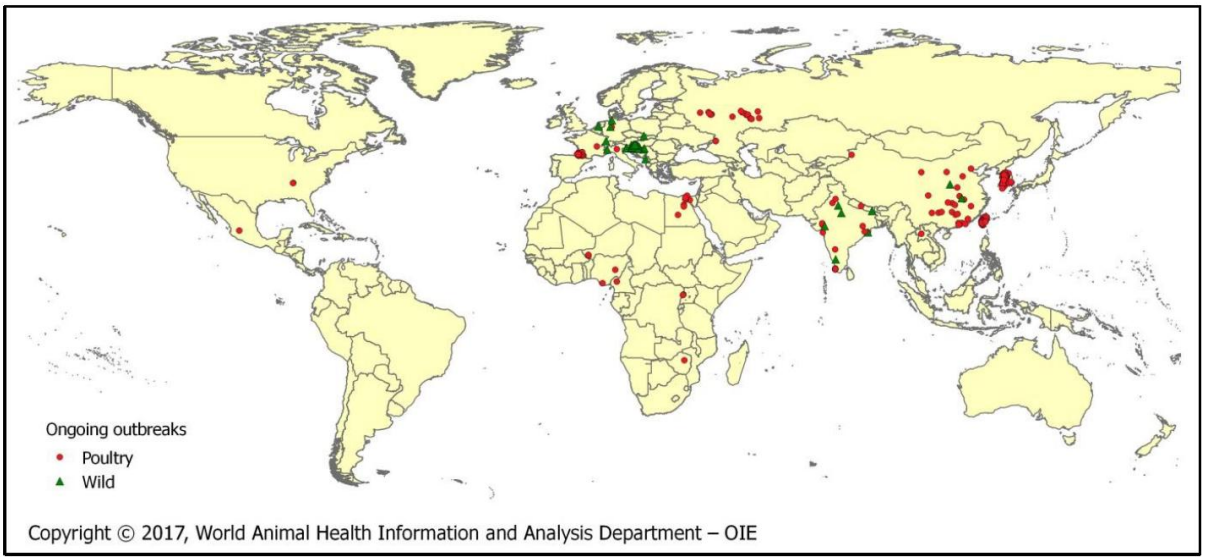


**Figure 18. Current (as of 6 June 2017) outbreaks of highly pathogenic avian influenza in poultry and wild birds worldwide.** Only outbreaks of the most common strains are shown here.[21]

## 8. Conclusion

The 2016/17 influenza season epidemics started earlier than previous seasons, in Switzerland and Europe in general, but not in the USA, with exception of the 2009/10 pandemics. Similarly, the epidemic peak also occurred earlier at week 2/2017 for Switzerland, between weeks 52/2016 and 4/2017 in Europe,[23] and 6/2017 in the USA[24]. Even if later than in Europe, the onset and activity peak of 2016/17 influenza season in the USA were earlier than in 2015/16. Despite this earlier onset, the duration (11 weeks) of the epidemic phase was similar to the 2012/13, 2014/15 and 2015/16 seasons (12 weeks). Apart from this, the 2016/17 influenza season in Switzerland was comparable to 2014/15 season in terms of and the overall rates of MC-ILI and the dominant types of circulating viruses.

Nine hundred and eighty-two samples were screened for influenza this season; 49% were positive for influenza. This season was marked by a large dominance of influenza A viruses over influenza B. Similar to Switzerland, in Europe[23] and the USA[24] influenza viruses of the A(H3N2) subtype were dominant and largely exceeded A(H1N1 09) viruses. Influenza B viruses were more abundant ,only after week 11/2017, with low detection absolute numbers.

The prevalence of influenza observed during 2016/17, was comparable to that measured during the 2015/16 season for the groups 0-4 years, 5-14 years and 30-64 years. For the second consecutive year, the positivity rate in the 0-4 years group was surprisingly low (≤35%). This observation is certainly due to multiple factors among which an insufficient compliance to the ILI definition for case selection by primary care practitioners and/or a suboptimal (or incomplete) ILI definition for the 0-4 years group. Indeed, several studies show that using age-specific ILI definitions would improve the detection of influenza positive cases.[25-27] A(H3N2) were the most abundant influenza viruses across all age groups. The ≥65 years group, and to a lower extent the 15-29 years group, showed a reverse image of positive versus negative samples from 2015/16 (40%, 51% positive and 60%, 49% negative samples, respectively) to 2016/17 (57%, 42% positive and 43%, 58% negative samples, respectively). In addition, the percentage of samples originating from elderly individuals in 2016/17 (12%) was almost two-fold higher than in 2015/16 (7%). This is concordant with the fact that the influenza A(H3N2) subtype is known to cause a more severe disease in this population, thus potentially increasing the need for a medical consultation. Therefore, it is not surprising that influenza A virus

infection cases reported by hospitals in both Europe[23] and the USA[24] mainly occurred in adults aged ≥65 years. An excess of all-cause mortality was also observed in the early 2016/17 influenza season in the ≥65 years group. Ninety-nine cases of influenza-related deaths among children were reported by the CDC during 2016/17 influenza season.[24].

In contrast to 2016/17 and 2014/15 A(H3N2) influenza seasons, A(H1N1 09) viruses predominated during the 2015/16 and the majority of intensive care unit admissions related to influenza infection concerned 15-64-year-old patients in Europe.[23] Interestingly, despite differences in disease severity among age groups, the A(H3N2) viruses attack rate was shown to be comparable across all the age groups in subtropical and tropical regions and regions where influenza is present all year.[28]

As expected for A(H3N2) viruses, a greater phylogenetic and antigenic variation was observed for A(H3N2) strains isolated during 2016/17 influenza season. Most of A(H3N2) belonged to clade 3C.2a (A/Hong Kong/4801/14-like viruses) and subclade 3C.2a1 (A/Slovenia/3188/15-like viruses). A couple of A(H3N2) viruses fell into the genetic clade 3C.3a (A/Switzerland/9715293/2013-like). A majority of influenza A(H3N2) viruses characterized this season, including 3C.2a1 viruses, were recognized well by antisera raised against A/Hong Kong/4801/14, the 2016/17 and 2017/18 Northern hemisphere vaccine strain. MN performed with post-vaccinated human sera and circulating viruses gave similar results.[29,30]

Only few influenza A(H1N1 09) viruses circulated in Europe, including Switzerland, and USA this season and all belonged to the phylogenetic clade 6B/subclade 6B.1 (A/Michigan/45/2015-like viruses). HAI assay performed on those viruses shown that they were antigenically similar to A/California/7/2009 the 2016/17 vaccine strain. Nevertheless HAI assays results with post-vaccinated human sera and some representative circulating viruses revealed a significant reduction in the geometric mean of HAI titers as compared to those obtained to A/California/7/2009. This observation lead to the replacement of the A/California/7/2009 strain by the A/Michigan/45/2015 strain in the 2017/18 Southern and Northern hemisphere vaccine.[29,30]

Even if present in higher numbers than A(H1N1 09) viruses, only few influenza B viruses of both B/Yamagata/16/88 and B/Victoria/2/87 lineages co-circulated during this season. The proportions of each lineage was country-dependent. A vast majority

of B/Yamagata viruses, including those circulating in Switzerland, belonged to genetic clade 3 and were all well recognized by antisera raised against the vaccine strain B/Phuket/3073/2013 (clade 3). This observation was also confirmed in HAI assay results using sera from humans vaccinated with the quadrivalent vaccine.

Concerning the viruses of B/Victoria viruses, all belonged to genetic clade 1A (B/Brisbane/60/2008-like viruses) and none contained the HA 162-163 deletion observed in some B/Victoria viruses circulating this year. Recent viruses submitted to HAI assays, were well recognized by antisera against the cell culture-propagated vaccine strain B/Brisbane/60/2008 viruses, but were less pronounced when compared to cell egg-propagated B/Brisbane/60/2008. Similar results were observed when post-vaccination human sera were used. Of note, for both influenza B lineages, higher HAI titers were obtained with the cell-propagated-cultivar vaccine strains, B/Phuket/3073/2013 and B/Brisbane/60/2008, than with the corresponding egg-propagated cultivars, especially when human sera were used. Nevertheless, this observation did not lead to the replacement of the influenza B vaccine strains.[29,30]

As observed for previous A(H3N2) seasons, preliminary 2016/17 influenza season vaccine effectiveness for A(H3N2) subtype was estimated to be moderate in Europe[31] (38%), Canada[32] (42%) and USA[33] (43%) for most age groups. Not surprisingly the estimated vaccine effectiveness for the ≥65 years-old age group was much lower (23.4% in Europe[31]).

During the 2016/17 influenza season, all influenza isolates tested at the NRCI for antiviral resistance were resistant to M2 protein inhibitors (adamantanes) but none was resistant to NAIs. In Europe, only few viruses (7 A(H3N2), one A(H1N1 09) and three B/Victoria viruses) showed phenotypic or genotypic signs for abnormal (reduced or highly reduced) inhibition in the presence of oseltamivir and/or zanamivir. As expected, almost all of the analyzed influenza A(H1N1) and A(H3N2) viruses harboured the S31N mutation associated with resistance to M2 inhibitors.

This season, a human infection with an avian-like swine influenza A(H1N1) was identified in Switzerland. The sample originated from a farm employee. Not surprisingly, at least one farm pig was also positive for swine influenza A(H1N1) according to Dr Anina Stahel from Vetsuisse Faculty. 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. The major increase in the number of

human influenza A(H7N9) cases observed during the ongoing epidemic wave is of major concern for public health. However, there are no noteworthy changes in the demographics, poultry exposition, cluster characteristics, or case-fatality proportion of human A(H7N9) cases when compared to previous epidemics.

In conclusion, as of 12 June 2017, the influenza season is slowly starting in the Southern hemisphere with ILI levels increasing in several temperate South American countries. In Chile and Paraguay, ILI levels have already crossed the seasonal threshold. In Southern Africa, Oceania and tropical South America and Asia, the overall influenza activity remains low and below the seasonal threshold. In countries reporting positive samples, influenza A(H3N2) subtype is dominant.[34]

**2016/17 season overview**

Influenza A(H3N2) were the dominant viruses during the 2016/17 season in European countries, including Switzerland.

No NAI-resistant influenza isolates were found in Switzerland. Few were isolated in Europe and the USA.

Various avian influenza strains continue to circulate worldwide in birds (poultry), but also to a lower extent in humans, particularly H7N9.

Geneva, 03 July 2017

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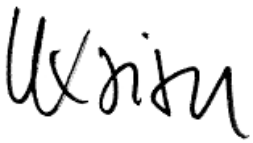

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# Annex 1: Weekly report of influenza virus detection and virus characteristics (2016/17)

| Sentinel Surveillance, Winter 2016-17 | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Weeks | Dates | | ‰ ILI | Samples received | Influenza A | | | | Influenza B | | | | Total virus (n) | % pos |
| | | | | | Undet. | A (H1N1) pdm09 | A (H3N2) seasonal | Total | Undet. | Bvic | Byam | Total | | |
| 40 | 2-Oct-16 | 8-Oct-16 | 1.7 | **8** | 0 | 0 | 0 | **0** | 0 | 0 | 0 | **0** | **0** | **0.00** |
| 41 | 9-Oct-16 | 15-Oct-16 | 3 | **7** | 0 | 0 | 1 | **1** | 0 | 0 | 0 | **0** | **1** | **14.29** |
| 42 | 16-Oct-16 | 22-Oct-16 | 1.8 | **7** | 0 | 0 | 0 | **0** | 0 | 0 | 0 | **0** | **0** | **0.00** |
| 43 | 23-Oct-16 | 29-Oct-16 | 3.1 | **12** | 0 | 0 | 0 | **0** | 0 | 0 | 0 | **0** | **0** | **0.00** |
| 44 | 30-Oct-16 | 5-Nov-16 | 1.9 | **14** | 0 | 0 | 0 | **0** | 0 | 0 | 0 | **0** | **0** | **0.00** |
| 45 | 6-Nov-16 | 12-Nov-16 | 2.6 | **15** | 0 | 0 | 0 | **0** | 0 | 0 | 0 | **0** | **0** | **0.00** |
| 46 | 13-Nov-16 | 19-Nov-16 | 2.9 | **17** | 0 | 0 | 0 | **0** | 0 | 0 | 0 | **0** | **0** | **0.00** |
| 47 | 20-Nov-16 | 26-Nov-16 | 2.2 | **13** | 0 | 0 | 1 | **1** | 0 | 0 | 0 | **0** | **1** | **7.69** |
| 48 | 27-Nov-16 | 3-Dec-16 | 5 | **29** | 0 | 0 | 7 | **7** | 0 | 0 | 0 | **0** | **7** | **24.14** |
| 49 | 4-Dec-16 | 10-Dec-16 | 6.7 | **27** | 0 | 0 | 9 | **9** | 0 | 0 | 1 | **1** | **10** | **37.04** |
| 50 | 11-Dec-16 | 17-Dec-16 | 8.2 | **43** | 0 | 1 | 14 | **15** | 0 | 1 | 0 | **1** | **16** | **37.21** |
| 51 | 18-Dec-16 | 24-Dec-16 | 15.4 | **82** | 0 | 3 | 48 | **51** | 0 | 0 | 1 | **1** | **52** | **63.41** |
| 52 | 25-Dec-16 | 31-Dec-16 | 45.2 | **76** | 0 | 1 | 43 | **44** | 0 | 0 | 0 | **0** | **44** | **57.89** |
| 1 | 1-Jan-17 | 7-Jan-17 | 56.1 | **82** | 0 | 0 | 53 | **53** | 0 | 0 | 0 | **0** | **53** | **64.63** |
| 2 | 8-Jan-17 | 14-Jan-17 | 44.4 | **92** | 0 | 0 | 56 | **56** | 0 | 0 | 0 | **0** | **56** | **60.87** |
| 3 | 15-Jan-17 | 21-Jan-17 | 36.6 | **73** | 0 | 0 | 46 | **46** | 0 | 0 | 0 | **0** | **46** | **63.01** |
| 4 | 22-Jan-17 | 28-Jan-17 | 34.6 | **71** | 0 | 0 | 47 | **47** | 0 | 0 | 0 | **0** | **47** | **66.20** |
| 5 | 29-Jan-17 | 4-Feb-17 | 34.5 | **59** | 0 | 1 | 38 | **39** | 0 | 0 | 1 | **1** | **40** | **67.80** |
| 6 | 5-Feb-17 | 11-Feb-17 | 26.2 | **70** | **0** | 0 | 44 | **44** | 1 | 0 | 1 | **2** | **46** | **65.71** |
| 7 | 12-Feb-17 | 18-Feb-17 | 16.1 | **68** | 1 | 0 | 24 | **25** | 1 | 1 | 3 | **5** | **30** | **44.12** |
| 8 | 19-Feb-17 | 25-Feb-17 | 9.5 | **29** | 0 | 1 | 8 | **9** | 0 | 0 | 2 | **2** | **11** | **37.93** |
| 9 | 26-Feb-17 | 4-Mar-17 | 7.2 | **34** | 0 | 0 | 9 | **9** | 0 | 0 | 1 | **1** | **10** | **29.41** |
| 10 | 5-Mar-17 | 11-Mar-17 | 5.2 | **20** | 0 | 1 | 3 | **4** | 0 | 0 | 3 | **3** | **7** | **35.00** |
| 11 | 12-Mar-17 | 18-Mar-17 | 2.7 | **11** | 0 | 0 | 1 | **1** | 0 | 0 | 0 | **0** | **1** | **9.09** |
| 12 | 19-Mar-17 | 25-Mar-17 | 1.8 | **8** | 0 | 0 | 0 | **0** | 0 | 0 | 1 | **1** | **1** | **12.50** |
| 13 | 26-Mar-17 | 1-Apr-17 | 1.2 | **3** | 0 | 0 | 0 | **0** | 0 | 0 | 0 | **0** | **0** | **0.00** |
| 14 | 2-Apr-17 | 8-Apr-17 | 1 | **10** | 0 | 0 | 1 | **1** | 0 | 0 | 1 | **1** | **2** | **20.00** |
| 15 | 9-Apr-17 | 15-Apr-17 | 0.2 | **1** | 0 | 0 | 0 | **0** | 0 | 0 | 0 | **0** | **0** | **0.00** |
| 16 | 16-Apr-17 | 22-Apr-17 | 0.6 | **1** | 0 | 0 | 0 | **0** | 0 | 0 | 0 | **0** | **0** | **0.00** |
| | | | | | **1** | **8** | **453** | | **2** | **2** | **15** | | | |
| | | | | **982** | **462** | | | | **19** | | | | **481** | |

## Annex 2a: 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 |
| | | A/Slovenia/3188/15 | 32 | 32 | 32 | **32** |
| **Isolates** | **HA titre** | **Typisation** | | | | |
| *****514 | 32 | A/Hong Kong 4801/14 like | 64 | 64 | 32 | 64 |
| *****032 | <2 | Non typable | x | x | x | x |
| *****865 | 64 | A/Slovenia/3188/15 like | 32 | 32 | 32 | 32 |
| *****958 | 64 | A/Slovenia/3188/15 like | 32 | 32 | 32 | 32 |
| *****213 | 256 | A/Slovenia/3188/15 like | 32 | 32 | 32 | 32 |
| *****304 | 128 | A/Slovenia/3188/15 like | 16 | 16 | 16 | 16 |
| *****442 | 32 | A/Hong Kong 4801/14 like | nd | 64 | 64 | 64 |
| *****512 | <2 | Non typable | x | x | x | x |
| *****013 | <2 | Non typable | x | x | x | x |
| *****203 | 64 | A/Switzerland/9715293/13 like | 128 | 128 | 128 | 256 |
| *****890 | 32 | A/Hong Kong 4801/14 like | 128 | 64 | 64 | 32 |
| *****073 | <2 | Non typable | x | x | x | x |
| *****103 | 128 | A/Hong Kong 4801/14 like | 64 | 64 | 64 | 64 |
| *****699 | 128 | A/Slovenia/3188/15 like | 16 | 16 | 16 | 16 |
| *****800 | 128 | A/Slovenia/3188/15 like | 32 | 32 | 32 | 32 |
| *****147 | 128 | A/Switzerland/9715293/13 like | 32 | 128 | 64 | 1024 |
| *****938 | 128 | A/Hong Kong 4801/14 like | 32 | 64 | 32 | 64 |
| *****860 | 128 | A/Slovenia/3188/15 like | 32 | 32 | 32 | 32 |

Provided HA titers were established in MDCK-SIAT cells. HAI titers should be multiplied by 8.

## Annex 2b: 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 |
| | | A/Slovenia/3188/15 | 32 | 32 | 32 | **32** |
| **Isolates** | **HA titre** | **Typisation** | | | | |
| *****960 | 128 | A/Hong Kong 4801/14 like | 64 | 64 | 128 | 256 |
| *****606 | 32 | A/Texas/50/12 like | 512 | 512 | 1024 | 4096 |
| *****629 | 128 | A/Hong Kong 4801/14 like | 64 | 128 | 32 | 64 |
| *****816 | 128 | A/Slovenia/3188/15 like | 16 | 16 | 16 | 16 |
| *****351 | 64 | A/Slovenia/3188/15 like | 16 | 16 | 16 | 16 |
| *****878 | 128 | A/Hong Kong 4801/14 like | 64 | 64 | 128 | 128 |
| *****047 | <2 | Non typable | x | x | x | x |
| *****049 | 16 | A/Slovenia/3188/15 like | 32 | 32 | 32 | 32 |
| *****069 | 16 | Non typable | x | x | x | x |
| *****269 | <2 | Non typable | x | x | x | x |
| *****626 | <2 | Non typable | x | x | x | x |
| *****506 | <2 | Non typable | x | x | x | x |
| *****619 | 16 | A/Slovenia/3188/15 like | 32 | 32 | 32 | 32 |
| *****744 | <2 | Non typable | x | x | x | x |
| *****100 | 8 | A/Hong Kong 4801/14 like | 64 | 64 | 64 | 64 |
| *****902* | 64 | A/Slovenia/3188/15 like | 32 | 32 | 32 | 32 |
| *****024* | 128 | A/Slovenia/3188/15 like | 32 | 32 | 64 | 32 |

Provided HA titers were established in MDCK-SIAT cells. HAI titers should be multiplied by 8.

## Annex 2c: 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 |
| | | A/Slovenia/3188/15 | 32 | 32 | 32 | **32** |
| **Isolates** | **HA titre** | **Typisation** | | | | |
| *****162 | 8 | A/Switzerland/9715293/13 like | 128 | 128 | 256 | 256 |
| *****578* | 128 | A/Slovenia/3188/15 like | 32 | 64 | 32 | 32 |
| *****680* | 64 | A/Slovenia/3188/15 like | 32 | 32 | 32 | 32 |
| *****853* | 64 | A/Hong Kong 4801/14 like | 32 | 32 | 128 | 64 |
| *****161* | <2 | Non typable | x | x | x | x |
| *****102 | 32 | A/Slovenia/3188/15 like | 16 | 16 | 16 | 32 |
| *****918 | 32 | A/Slovenia/3188/15 like | 32 | 32 | 32 | 32 |
| *****317 | <2 | Non typable | x | x | x | x |
| *****352 | <2 | Non typable | x | x | x | x |
| *****057 | 32 | A/Slovenia/3188/15 like | 32 | 32 | 32 | 32 |
| *****689 | <2 | Non typable | x | x | x | x |
| *****748* | <2 | Non typable | x | x | x | x |
| *****777 | 16 | A/Hong Kong 4801/14 like | 64 | 64 | 64 | 64 |
| *****805 | 32 | A/Slovenia/3188/15 like | 32 | 16 | 32 | 16 |
| *****592 | 16 | A/Hong Kong 4801/14 like | 64 | 64 | 64 | 32 |
| *****611 | 16 | A/Slovenia/3188/15 like | 64 | 32 | 32 | 64 |
| *****706 | 16 | A/Hong Kong 4801/14 like | 64 | 64 | 64 | 64 |

Provided HA titers were established in MDCK-SIAT cells. HAI titers should be multiplied by 8.

## Annex 2d: 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 |
| | | A/Slovenia/3188/15 | 32 | 32 | 32 | **32** |
| **Isolates** | **HA titre** | **Typisation** | | | | |
| *****065 | <2 | Non typable | x | x | x | x |
| *****839 | <2 | Non typable | x | x | x | x |
| *****401 | 32 | A/Slovenia/3188/15 like | 32 | 32 | 16 | 16 |
| *****634 | 16 | Non typable | x | x | x | x |
| *****682 | 32 | A/Slovenia/3188/15 like | 16 | 16 | 16 | 16 |
| *****785 | 16 | A/Slovenia/3188/15 like | 32 | 32 | 32 | 32 |
| *****324 | 16 | A/Hong Kong 4801/14 like | 64 | 64 | 32 | 64 |
| *****425 | <2 | Non typable | x | x | x | X |
| *****462 | 64 | Non typable | 8 | 8 | <8 | <8 |
| *****488 | 32 | A/Slovenia/3188/15 like | 16 | 16 | 16 | 16 |
| *****852 | <2 | Non typable | x | x | x | X |
| *****877 | <2 | Non typable | x | x | x | X |
| *****082 | <2 | Non typable | x | x | x | X |
| *****010 | 32 | Non typable | <8 | <8 | 8 | <8 |
| *****645 | <2 | Non typable | x | x | x | X |
| *****356 | 32 | A/Slovenia/3188/15 like | 32 | 32 | 32 | 32 |

Provided HA titers were established in MDCK-SIAT cells. HAI titers should be multiplied by 8.

# Annex 2e: Microneutralization assay of influenza A(H3N2) viruses

| Reference AG/AS | Characterization | A/Texas/50/12 | A/Switzerland/9715293/13 | A/Hong Kong/4801/14 | A/Slovenia/3188/15 |
|---|---|---|---|---|---|
| **A/Switzerland/9715293/13** | | **64** | **256** | **16** | **128** |
| **A/Hong Kong/4801/14** | | **16** | **32** | **128** | **128** |
| **A/Slovenia/3188/15** | | **32** | **32** | **64** | **128** |
| *****645** | **A/Switzerland/9715293/13** | 128 | 256 | 128 | 64 |
| *****082** | **A/Slovenia/3188/15** | <8 | <8 | 32 | 256 |
| *****013** | **A/Slovenia/3188/15** | <8 | <8 | <8 | 32 |
| *****626** | **A/Slovenia/3188/15** | <8 | <8 | 16 | 128 |
| *****317** | **A/Slovenia/3188/15** | <8 | <8 | 32 | 128 |
| *****010** | Non-typable | <8 | <8 | <8 | <8 |
| *****352** | Non-typable | <8 | <8 | <8 | <8 |
| *****161** | Non-typable | <8 | <8 | <8 | <8 |
| *****425** | **A/Slovenia/3188/15** | <8 | <8 | <8 | 32 |
| *****512** | **A/Slovenia/3188/15** | <8 | <8 | <8 | 32 |
| *****5462** | **A/Slovenia/3188/15** | <8 | <8 | <8 | 32 |
| *****047** | **A/Slovenia/3188/15** | <8 | <8 | <8 | 32 |
| *****744** | **A/Slovenia/3188/15** | <8 | <8 | <8 | 32 |

# Annex 3: Antigenic analyses of influenza A(H1N1 09)

| | | | Antisera | | | |
|---|---|---|---|---|---|---|
| | | **Reference viral isolates** | A/Brisbane/59/07 | A/California/07/09 | A/St Petersburg/27/11 | A/Hong Kong/3934/11 |
| | | A/Brisbane/59/07 | **1024** | <16 | <16 | <16 |
| | | A/California/07/09 | <16 | **1024** | 64 | 256 |
| | | A/St Petersburg/27/11 | <16 | 128 | **128** | 128 |
| | | A/Hong Kong/3934/11 | <16 | 256 | 128 | **512** |
| **Isolates** | **HA titre** | **Typisation** | | | | |
| *****003 | 32 | A/St. Petersburg/27/11-like | <16 | 256 | 256 | 128 |
| *****226 | 64 | A/St. Petersburg/27/11-like | 16 | 32 | 32 | 32 |
| *****892 | 16 | A/Hong Kong/3934/11-like | <16 | 1204 | 512 | 512 |
| *****972 | 8 | A/Hong Kong/3934/11-like | nd | 512 | 512 | 1024 |

Provided HA titers were established in MDCK-SIAT cells. * Corresponds to MDCK. HAI titers should be multiplied by 8.

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

| | | | Antisera | | | |
|---|---|---|---|---|---|---|
| | | **Reference viral isolates** | B/Wisconsin/01/10 | B/Novosibirsk/1/12 | B/Massachssetts/02/12 Egg | B/Phuket/3073/13 |
| | | B/Wisconsin/01/10 | **64** | 64 | 32 | 64 |
| | | B/Novosibirsk/1/12 | 256 | **256** | 64 | 16 |
| | | B/Massachssetts/02/12 Egg | 128 | 64 | **256** | 128 |
| | | B/Phuket/3073/13 | 256 | 128 | 256 | **128** |
| **Isolates** | **HA titre** | **Typisation** | | | | |
| *****131 | 128 | B/Wisconsin/01/10-like | 64 | 64 | 32 | 128 |
| *****435 | 32 | B/Novosibirsk/01/12-like | 128 | 256 | 128 | 32 |
| *****372 | 32 | B/Novosibirsk/01/12-like | 128 | 128 | 64 | 32 |
| *****422 | 32 | B/Novosibirsk/01/12-like | 128 | 128 | 64 | 32 |
| *****506 | 32 | B/Novosibirsk/01/12-like | 256 | 256 | 128 | 32 |
| *****543 | 64 | B/Novosibirsk/01/12-like | 128 | 128 | 128 | 32 |
| *****109 | 8 | B/Phuket/3073/13-like | 128 | 256 | 256 | 64 |
| *****287 | 128 | B/Novosibirsk/01/12-like | 256 | 256 | 64 | 32 |
| *****969 | 128 | B/Wisconsin/01/10-like | 64 | 64 | 16 | 16 |
| *****901 | 32 | B/Phuket/3073/13-like | 128 | 128 | 128 | 64 |

Provided HA titers were established in MDCK-SIAT cells. * Corresponds to MDCK. HAI titers should be multiplied by 8.

# Annex 5: Hemagglutination inhibition of influenza B Victoria lineage viruses

| | | | Antisera | | |
|---|---|---|---|---|---|
| | | Reference viral isolates | B/Brisbane/60/08 | B/Odessa/3886/10 | B/Johannesburg/3964/12 |
| | | B/Brisbane/60/08 | **128** | 64 | 128 |
| | | B/Odessa/3886/10 | 512 | **512** | 256 |
| | | B/Johannesburg/3964/12 | 32 | 32 | **64** |
| **Isolates** | **HA titre** | **Typisation** | | | |
| *****129 | 64 | B/Johannesburg/3964/12-like | 64 | 64 | 64 |
| *****954 | 32 | B/Johannesburg/3964/12-like | 16 | 16 | 16 |

Provided HA titers were established in MDCK-SIAT cells. * Corresponds to MDCK. HAI titers should be multiplied by 8.

# Annex 6a: Antigenic analyses of influenza A(H3N2) viruses - Plaque reduction neutralization, 01.02.2017, WIC[30]

| Viruses | Passage history / Ferret number / Genetic group | Collection Date | Passage History | Neutralisation titre[1] Post-infection ferret antisera: A/HK 4801/14 Egg NIB F53/16 3C.2a | | A/HK 7295/14 MDCK F02/15 3C.2a | | A/Cote d'Ivoire 544/16 SIAT NIB F54/16 3C.2a | | A/Oman 2585/16 SIAT NIB F50/16 3C.2a1 | | A/Norway 4436/16 SIAT F03/17 3C.2a1 | | HA1 substitutions for 3C.2a(1) viruses compared to A/Hong Kong/4801/2014: Egg adaptation HA substitutions compared to the corresponding cell isolate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 2-fold | Read | 2-fold | Read | 2-fold | Read | 2-fold | Read | 2-fold | Read | |
| ***REFERENCE VIRUSES*** | | | | | | | | | | | | | | |
| A/Hong Kong/4801/2014 | 3C.2a | 2014-02-26 | E7 | 640 | 780 | 640 | 601 | 320 | 272 | 320 | 465 | 320 | 320 | N96S, T160K (-CHO), L194P |
| A/Hong Kong/7295/2014 | 3C.2a | 2014-08-07 | MDCK3 | 80 | 61 | 640 | 570 | 160 | 144 | 320 | 311 | 320 | 251 | none |
| A/Cote d'Ivoire/544/2016 | 3C.2a | 2016-04-06 | P1/SIAT2 | 80 | 118 | 1280 | 1768 | 2560 | 2384 | 1280 | 1880 | 1280 | 993 | N121K, S144K, S198P |
| A/Oman/2585/2016 | 3C.2a1 | 2016-03-04 | SIAT2 | 160 | 203 | 320 | 386 | 640 | 508 | 2560 | 2115 | 1280 | 993 | N121K, N171K |
| A/Norway/4436/2016 | 3C.2a1 | 2016-11-03 | SIAT1 | 160 | 122 | 640 | 775 | 640 | 481 | 1280 | 1406 | 1280 | 992 | N121K, I140M, N171K |
| ***TEST VIRUSES*** | | | | | | | | | | | | | | |
| A/Spain/108862/2016 | 3C.2a | 2016-12-02 | SIAT1 | 160 | 120 | 1280 | 1021 | 1280 | 1078 | 1280 | 1180 | 1280 | 1193 | N121K, N122D (-CHO), S144K, S262N |
| A/Estonia/103732/2016 | 3C.2a | 2016-12-12 | SIAT1/SIAT1 | 80 | 78 | 1280 | 987 | 640 | 846 | 1280 | 1411 | 1280 | 1038 | N121K, S144K |
| A/Iran/68081/2016 | 3C.2a | 2016-12-14 | MDCK1/SIAT1 | 80 | 89 | 1280 | 1088 | 1280 | 1213 | 1280 | 1164 | 1280 | 1157 | A106T, N121K, S144K, I236V |
| A/Switzerland/19430223/2016 | 3C.2a | 2016-12-16 | SIAT1 | 160 | 151 | 320 | 398 | 320 | 307 | 640 | 815 | 640 | 630 | T131K, R142K, R261Q |
| A/Switzerland/19430147/2016 | 3C.2a | 2016-12-20 | SIAT1 | 160 | 217 | 1280 | 1019 | 640 | 826 | 1280 | 971 | 1280 | 1280 | N121K, N122D (-CHO), S144K, S262N |
| A/Iceland/15954/2016 | 3C.2a1 | 2016-09-14 | SIAT2 | 80 | 74 | 640 | 724 | 320 | 459 | 1280 | 1192 | 1280 | 960 | S47T, G78S, N171K, K310R |
| A/Iceland/16073/2016 | 3C.2a1 | 2016-09-15 | SIAT2 | 160 | 144 | 640 | 807 | 320 | 400 | 1280 | 1463 | 1280 | 989 | S47T, G78S, N171K, K310R |
| A/Iceland/16097/2016 | 3C.2a1 | 2016-09-15 | SIAT2 | 160 | 229 | 640 | 950 | 640 | 576 | 1280 | 1657 | 1280 | 1242 | S47T, G78S, N171K, K310R |
| A/Champagne Ardenne/3131/2016 | 3C.2a1 | 2016-12-05 | MDCK1/SIAT1 | 40 | 40 | 640 | 526 | 320 | 320 | 640 | 809 | 640 | 929 | N121E, I140M, N171K, V323I |
| A/Spain/109626/2016 | 3C.2a1 | 2016-12-05 | SIAT1 | 40 | 47 | 640 | 747 | 640 | 576 | 1280 | 1219 | 1280 | 981 | K27R, N171K |
| A/Nord Pas de Calais/3195/2016 | 3C.2a1 | 2016-12-06 | MDCK1/SIAT1 | 80 | 61 | 640 | 574 | 320 | 378 | 1280 | 1032 | 640 | 844 | D104N, N171K |
| A/Estonia/103677/2016 | 3C.2a1 | 2016-12-07 | SIAT1/SIAT1 | 40 | 46 | 640 | 501 | 320 | 282 | 640 | 776 | 640 | 704 | N121K, I140M, N171K |
| A/Paris/3199/2016 | 3C.2a1 | 2016-12-08 | MDCK1/SIAT1 | 320 | 309 | 640 | 747 | 640 | 501 | 1280 | 1779 | 640 | 731 | I34V, N171K |
| A/Estonia/103729/2016 | 3C.2a1 | 2016-12-12 | SIAT1/SIAT1 | 80 | 74 | 1280 | 960 | 640 | 533 | 1280 | 1707 | 1280 | 1055 | R142G, N171K |
| A/Estonia/103759/2016 | 3C.2a1 | 2016-12-12 | SIAT1/SIAT1 | 80 | 72 | 640 | 640 | 320 | 369 | 1280 | 1254 | 640 | 795 | R142G, N171K |
| A/Bretagne/3243/2016 | 3C.2a1 | 2016-12-12 | MDCK1/SIAT1 | 80 | 63 | 640 | 569 | 160 | 213 | 1280 | 960 | 640 | 557 | S47T, D53N, G78S, N171K |
| A/Paris/3248/2016 | 3C.2a1 | 2016-12-12 | MDCK1/SIAT1 | 40 | 50 | 640 | 576 | 320 | 320 | 1280 | 1227 | 1280 | 960 | N171K |
| A/Estonia/103796/2016 | 3C.2a1 | 2016-12-14 | SIAT1/SIAT1 | 80 | 61 | 640 | 526 | 320 | 308 | 1280 | 1008 | 640 | 683 | N121K, R142G, N171K |
| A/Iran/67534/2016 | 3C.2a1 | 2016-12-14 | MDCK1/SIAT1 | 80 | 80 | 640 | 597 | 320 | 243 | 1280 | 1138 | 640 | 618 | I3S, D53E, Q75H, K92R, N121K, N171K, S262N, H311Q |
| A/Iran/67441/2016 | 3C.2a1 | 2016-12-14 | MDCK1/SIAT1 | 80 | 102 | 640 | 853 | 640 | 486 | 1280 | 1569 | 1280 | 1222 | N121K, I140M, N171K |
| A/Iran/67702/2016 | 3C.2a1 | 2016-12-14 | MDCK1/SIAT1 | 80 | 72 | 640 | 873 | 320 | 390 | 1280 | 1166 | 1280 | 1006 | N171K |
| A/Iran/67829/2016 | 3C.2a1 | 2016-12-14 | MDCK1/SIAT1 | 80 | 60 | 640 | 626 | 320 | 250 | 1280 | 1020 | 640 | 547 | D53E, Q75H, K92R, A106T, N121K, N171K, H311Q |
| A/Iran/67362/2016 | 3C.2a1 | 2016-12-14 | MDCK1/SIAT1 | 40 | 54 | 320 | 464 | 160 | 205 | 640 | 740 | 640 | 518 | R33Q, N121K, N171K |
| A/Switzerland/19429699/2016 | 3C.2a1 | 2016-12-20 | SIAT1 | 320 | 280 | 1280 | 1029 | 1280 | 1040 | 1280 | 1463 | 1280 | 1250 | N171K |
| | | | Vaccine | | | | | | | | | | | |

# Annex 6b: Antigenic analyses of influenza A(H3N2) viruses - Plaque reduction neutralization, 06.02.2017, WIC[30]

| Viruses | Passage history / Ferret number / Genetic group | Collection Date | Passage History | Neutralisation titre[1] Post-infection ferret antisera: A/HK 4801/14 Egg NIB F53/16 3C.2a | | A/HK 7295/14 MDCK F02/15 3C.2a | | A/Cote d'Ivoire 544/16 SIAT NIB F54/16 3C.2a | | A/Oman 2585/16 SIAT NIB F50/16 3C.2a1 | | A/Norway 4436/16 SIAT F03/17 3C.2a1 | | HA1 substitutions for 3C.2a(1) viruses compared to A/Hong Kong/4801/2014: Egg adaptation HA substitutions compared to the corresponding cell isolate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 2-fold | Read | 2-fold | Read | 2-fold | Read | 2-fold | Read | 2-fold | Read | |
| *REFERENCE VIRUSES* | | | | | | | | | | | | | | |
| A/Hong Kong/4801/2014 | 3C.2a | 2014-02-26 | E7 | 640 | 780 | 640 | 601 | 320 | 272 | 320 | 465 | 320 | 320 | N96S, T160K (-CHO), L194P |
| A/Hong Kong/7295/2014 | 3C.2a | 2014-08-07 | MDCK3 | 80 | 61 | 640 | 570 | 160 | 144 | 320 | 311 | 320 | 251 | none |
| A/Cote d'Ivoire/544/2016 | 3C.2a | 2016-04-06 | P1/SIAT2 | 80 | 118 | 1280 | 1768 | 2560 | 2384 | 1280 | 1880 | 1280 | 993 | N121K, S144K, S198P |
| A/Oman/2585/2016 | 3C.2a1 | 2016-03-04 | SIAT2 | 160 | 203 | 320 | 386 | 640 | 508 | 2560 | 2115 | 1280 | 993 | N121K, N171K |
| A/Norway/4436/2016 | 3C.2a1 | 2016-11-03 | SIAT1 | 160 | 122 | 640 | 775 | 640 | 481 | 1280 | 1406 | 1280 | 992 | N121K, I140M, N171K |
| *TEST VIRUSES* | | | | | | | | | | | | | | |
| A/La Rioja/2992/2016 | 3c.2a | 2016-11-15 | SIAT1/SIAT1 | 40 | 40 | 640 | 640 | 1280 | 996 | 1280 | 1030 | 640 | 891 | D53N, N121K, S144K |
| A/Madrid/2920/2016 | 3c.2a | 2016-11-16 | SIAT1/SIAT1 | 80 | 92 | 320 | 395 | 160 | 172 | 640 | 800 | 640 | 931 | G78D, T131K, R142K, R261Q |
| A/La Rioja/2994/2016 | 3c.2a | 2016-11-17 | SIAT1/SIAT1 | 40 | 20 | 640 | 630 | 1280 | 980 | 1280 | 1203 | 640 | 867 | D53N, N121K, S144K |
| A/Asturias/3088/2016 | 3c.2a | 2016-11-17 | SIAT1/SIAT1 | 40 | 58 | 640 | 604 | 1280 | 1440 | 1280 | 1510 | 1280 | 1355 | N121K, N122D (-CHO), S144K, S262N |
| A/Switzerland/19260442/2016 | 3c.2a | 2016-12-03 | SIAT1 | 80 | 95 | 640 | 581 | 2560 | 2211 | 1280 | 1280 | 1280 | 1216 | I58V, N121K, S144K, S219Y |
| A/Switzerland/19347103/2016 | 3c.2a | 2016-12-06 | SIAT1 | 160 | 142 | 320 | 384 | 320 | 346 | 640 | 877 | 640 | 924 | T131K, R142K, R261Q |
| A/Switzerland/18821514/2016 | 3c.2a1 | 2016-10-20 | SIAT1 | 80 | 84 | 640 | 837 | 640 | 896 | 1280 | 1728 | 2560 | 1963 | D77E, N121K, R142G, N171K |
| A/La/Rioja/2995/2016 | 3c.2a1 | 2016-11-14 | SIAT1/SIAT1 | 40 | 58 | 640 | 507 | 640 | 544 | 1280 | 1152 | 640 | 832 | K92R, N121K, N171K, H311Q |
| A/Madrid/2921/2016 | 3c.2a1 | 2016-11-15 | SIAT1/SIAT1 | 80 | 76 | 640 | 640 | 640 | 775 | 2560 | 2194 | 1280 | 1177 | R33Q, N171K |
| A/La Rioja/2998/2016 | 3c.2a1 | 2016-11-16 | SIAT1/SIAT1 | 40 | 40 | 160 | 146 | 160 | 148 | 640 | 517 | 320 | 416 | K92R, N121K, N171K, H311Q |
| A/La Rioja/2997/2016 | 3c.2a1 | 2016-11-18 | SIAT1/SIAT1 | 40 | 53 | 640 | 860 | 640 | 800 | 1280 | 1440 | 1280 | 1093 | K92R, N121K, N171K, H311Q |
| A/La Rioja/3000/2016 | 3c.2a1 | 2016-11-18 | SIAT1/SIAT1 | 80 | 87 | 640 | 640 | 640 | 640 | 1280 | 1259 | 1280 | 1166 | K92R, N121K, N171K, H311Q |
| A/Andalucia/3039/2016 | 3c.2a1 | 2016-11-18 | SIAT1/SIAT1 | 80 | 80 | 640 | 940 | 640 | 597 | 2560 | 1978 | 1280 | 1829 | N171K, R261Q |
| A/La Rioja/2999/2016 | 3c.2a1 | 2016-11-22 | SIAT1/SIAT1 | 40 | 42 | 320 | 469 | 320 | 469 | 1280 | 987 | 640 | 907 | K92R, N121K, N171K, H311Q |
| A/Madrid/3014/2016 | 3c.2a1 | 2016-11-28 | SIAT1/SIAT1 | 640 | 590 | 640 | 782 | 640 | 594 | 1280 | 1600 | 1280 | 1248 | K92R, N121K, N171K, H311Q |
| | | | | Vaccine | | | | | | | | | | |

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

In phylogenetic trees

# Annex 7: Antigenic analyses of influenza A(H1N1)pdm09 viruses, 17.01.2017, WIC

| Viruses | Passage history | Collection date | Passage history | Haemagglutination inhibition titre | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Post-infection ferret antisera | | | | | | | | | | |
| | | | | A/Mich 45/15 | A/Cal 7/09 | A/Bayern 69/09 | A/Lviv N6/09 | A/Astrak 1/11 | A/St. P 27/11 | A/St. P 100/11 | A/HK 5659/12 | A/Sth Afr 3626/13 | A/Slov 2903/2015 | A/Israel Q-504/15 |
| | Passage history | | | Egg | Egg | MDCK | MDCK | MDCK | Egg | Egg | MDCK | Egg | Egg | MDCK |
| | Ferret number | | | 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 | | | | | | | 5 | 6 | 7 | 6A | 6B | 6B.1 | 6B.2 |
| *REFERENCE VIRUSES* | | | | | | | | | | | | | | |
| A/Michigan/45/2015 | | | E3/E2 | 1280 | 1280 | 640 | 320 | 1280 | 640 | 2560 | 1280 | 1280 | 2560 | 1280 |
| A/California/7/2009 Clone38-32 | | 2009-04-09 | E3/E4 | 1280 | 640 | 640 | 320 | 640 | 320 | 1280 | 1280 | 640 | 1280 | 640 |
| A/Bayern/69/2009 | | 2009-07-01 | MDCK5/MDCK1 | < | < | 160 | 80 | 40 | < | < | 40 | 40 | < | < |
| A/Lviv/N6/2009 | | 2009-10-27 | MDCK4/SIAT1/MDCK3 | 80 | 80 | 640 | 640 | 40 | 80 | 80 | 160 | 80 | 160 | 80 |
| A/Astrakhan/1/2011 | 5 | 2011-02-28 | MDCK1/MDCK5 | 640 | 640 | 320 | 160 | 640 | 320 | 1280 | 640 | 640 | 1280 | 640 |
| A/St. Petersburg/27/2011 | 6 | 2011-02-14 | E1/E4 | 1280 | 640 | 640 | 320 | 640 | 640 | 1280 | 1280 | 640 | 1280 | 640 |
| A/St. Petersburg/100/2011 | 7 | 2011-03-14 | E1/E4 | 1280 | 640 | 640 | 320 | 1280 | 640 | 1280 | 1280 | 640 | 1280 | 640 |
| A/Hong Kong/5659/2012 | 6A | 2012-05-21 | MDCK4/MDCK2 | 320 | 320 | 160 | 80 | 320 | 320 | 640 | 640 | 320 | 640 | 320 |
| A/South Africa/3626/2013 | 6B | 2013-06-06 | E1/E3 | 1280 | 320 | 320 | 320 | 320 | 320 | 640 | 640 | 640 | 640 | 640 |
| A/Slovenia/2903/2015 clone 37 | 6B.1 | 2015-10-26 | E4/E1 | 2560 | 2560 | 1280 | 640 | 1280 | 640 | 5120 | 2560 | 1280 | 2560 | 2560 |
| A/Israel/Q-504/2015 | 6B.2 | 2015-12-15 | C1/MDCK2 | 1280 | 640 | 320 | 160 | 640 | 320 | 1280 | 1280 | 640 | 1280 | 1280 |
| *TEST VIRUSES* | | | | | | | | | | | | | | |
| A/Switzerland/19464226/2016 | 6B.1 | 2016-12-21 | MDCK1 | 2560 | 640 | 640 | 320 | 1280 | 640 | 1280 | 1280 | 1280 | 1280 | 1280 |
| A/Switzerland/19374003/2016 | 6B.1 | 2016-12-12 | MDCK1 | 1280 | 640 | 640 | 320 | 640 | 320 | 1280 | 1280 | 1280 | 1280 | 1280 |

| | |
|---|---|
| Assay | HI (Turkey RBC) |
| RBC | Turkey |
| Virus | A(H1N1)pdm09 |
| Date | |
| * | Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used) |
| 1 | < = <40 |
| 2 | < = <80 |

Vaccine

# Annex 8: Antigenic analyses of influenza B viruses (Yamagata lineage), 24.01.2017, WIC

| Viruses | Passage history | Collection date | Passage history | Haemagglutination inhibition titre: 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 | Passage history | | | Egg | Egg | Egg | MDCK | MDCK | Egg | Egg | Egg | MDCK | Egg | Egg |
| Ferret number | Ferret number | | | SH614[*1,3] | F17/13[*1] | F38/14[*2] | F32/12[*2] | F05/15[*2] | F42/14[*2] | F10/13[*2] | F06/15[*2] | F35/14[*2] | F36/14[*2] | St Judes F715/14[*2,] |
| Genetic Group | 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 | 320 | 40 | 80 | 640 | 80 | 160 | 40 | 80 | 160 |
| B/Brisbane/3/2007 | 2 | 2007-09-03 | E2/E2 | 1280 | 1280 | 640 | 80 | 160 | 1280 | 160 | 160 | 20 | 160 | 320 |
| B/Estonia/55669/2011 | 2 | 2011-03-14 | MDCK2/MDCK3 | 640 | 80 | 40 | 80 | 320 | 40 | 20 | 10 | 10 | 20 | 160 |
| B/Massachusetts/02/2012 | 2 | 2012-03-13 | CK1/C2/MDCK3 | 1280 | 320 | 160 | 160 | 320 | 320 | 80 | 40 | 20 | 80 | 320 |
| B/Massachusetts/02/2012 | 2 | 2012-03-13 | E3/E3 | 1280 | 640 | 320 | 40 | 80 | 640 | 80 | 80 | 10 | 80 | 160 |
| B/Wisconsin/1/2010 | 3 | 2010-02-20 | E3/E2 | 5120 | 640 | 320 | 20 | 40 | 640 | 640 | 160 | 40 | 320 | 320 |
| B/Stockholm/12/2011 | 3 | 2011-03-28 | E4/E1 | 1280 | 160 | 80 | 10 | 20 | 80 | 40 | 80 | 10 | 40 | 80 |
| B/Phuket/3073/2013 | 3 | 2013-11-21 | MDCK2/MDCK2 | 5120 | 160 | 160 | 80 | 80 | 320 | 320 | 80 | 320 | 320 | 160 |
| B/Phuket/3073/2013 | 3 | 2013-11-21 | E4/E3 | 2560 | 320 | 160 | 10 | 40 | 160 | 160 | 80 | 40 | 160 | 160 |
| B/Hong Kong/3417/2014 | 3 | 2014-06-04 | E4/E1 | 1280 | 80 | 40 | < | 20 | 40 | 40 | 20 | < | 20 | 160 |
| *TEST VIRUSES* | | | | | | | | | | | | | | |
| B/Switzerland/19309131/2 | 3 | 2016-12-06 | MDCK1 | 1280 | 40 | 20 | 10 | 20 | 10 | 20 | 20 | 20 | 20 | 80 |

| | | |
|---|---|---|
| Assay | HI (Turkey RBC) | Vaccine NH 2015-16 NH 2016- |
| RBC | Turkey | |
| Virus | Influenza B/Yamagata-lineage | |
| Date | | |
| * | Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used) | |
| 1 | < = <40 | |
| 2 | < = <10 | |
| 3 | hyperimmune sheep serum | |
| 4 | RDE serum pre-absorbed with TRBC | |

# Annex 9: Antigenic analyses of influenza B viruses (Victoria lineage), 17.01.2017, WIC

| Viruses | | Collection date | Passage history | Haemagglutination inhibition titre | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Post-infection ferret antisera | | | | | | | | | |
| | | | | B/Bris 60/08 | B/Mal 2506/04 | B/Bris 60/08 | B/Malta 636714/11 | B/Jhb 3964/12 | B/For √2367/12 | B/Sth Aus 81/12 | B/HK 514/09 | B/Ireland 3154/16 | Nord-West 1/16 |
| Passage history | | | | Egg | Egg | Egg | Egg | Egg | MDCK | Egg | MDCK | MDCK | MDCK |
| Ferret number | | | | Sh 539,540,543,544,57 | F41/14*2 | F26/13*2 | F29/13*2 | F01/13*2 | F04/13*2 | F41/13*2 | F09/13*2 | F15/16*2 | F16/16*2 |
| Genetic group | | | | 1A | | 1A | 1A | 1A | 1A | 1A | 1B | 1A | 1A |
| *REFERENCE VIRUSES* | | | | | | | | | | | | | |
| B/Malaysia/2506/2004 | | 2004-12-06 | E3/E6 | 2560 | 320 | 80 | 80 | 160 | 40 | 160 | 20 | < | < |
| B/Brisbane/60/2008 | 1A | 2008-08-04 | E4/E4 | 2560 | 80 | 320 | 160 | 160 | 160 | 160 | 40 | 20 | 20 |
| B/Malta/636714/2011 | 1A | 2011-03-07 | E4/E1 | 2560 | 80 | 320 | 320 | 320 | 160 | 640 | 80 | 20 | 20 |
| B/Johannesburg/3964/2012 | 1A | 2012-08-03 | E1/E2 | 5120 | 640 | 1280 | 1280 | 1280 | 640 | 1280 | 320 | 80 | 160 |
| B/Formosa/V2367/2012 | 1A | 2012-08-06 | MDCK1/MDCK3 | 2560 | 20 | 640 | 320 | 160 | 160 | 640 | 80 | 40 | 20 |
| B/South Australia/81/2012 | 1A | 2012-11-28 | E4/E2 | 2562 | 80 | 320 | 320 | 320 | 160 | 640 | 80 | 40 | 20 |
| B/Hong Kong/514/2009 | 1B | 2009-10-11 | MDCK3 | 2560 | < | 40 | 40 | 40 | 20 | 80 | 80 | 40 | 20 |
| B/Ireland/3154/2016 | 1A | 2016-01-14 | MDCK1/MDCK4 | 2560 | < | 40 | 20 | 20 | 20 | 40 | 40 | 40 | 20 |
| B/Nordrhein-Westfalen/1/2016 | 1A | 2016-01-04 | C2/MDCK3 | 2560 | < | 40 | 20 | 20 | 20 | 40 | 40 | 40 | 40 |
| *TEST VIRUSES* | | | | | | | | | | | | | |
| B/Switzerland/19357129/2016 | 1A | 2016-12-12 | MDCK1 | 2561 | < | 80 | < | 20 | 10 | 10 | 20 | 40 | 10 |

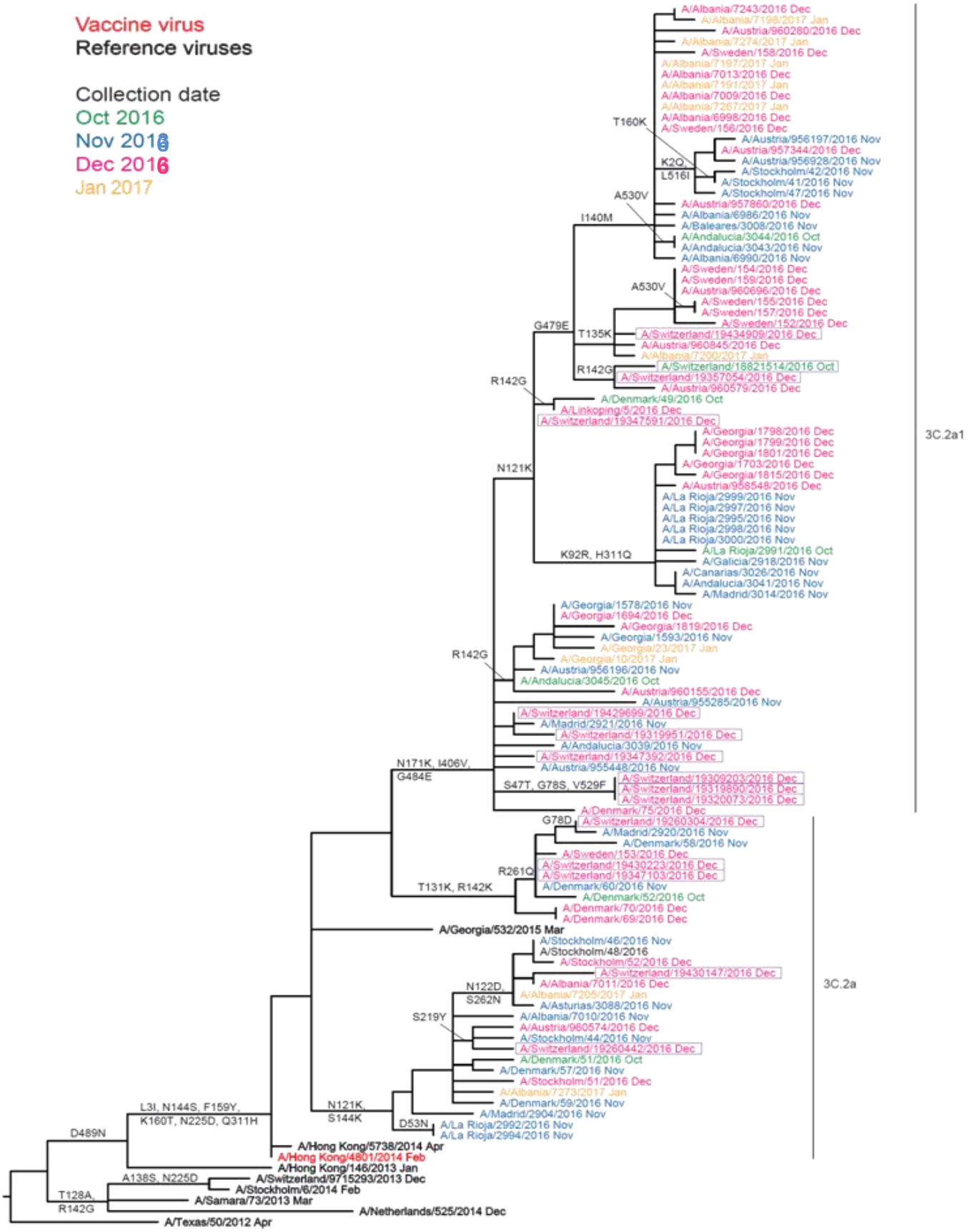
| | | |
|---|---|---|
| Assay | HI (Turkey RBC) | Vaccine NH 2015-SH 2016 |
| RBC | Turkey | |
| Virus | Influenza B/Victoria-lineage | 17 |
| Date | | |

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

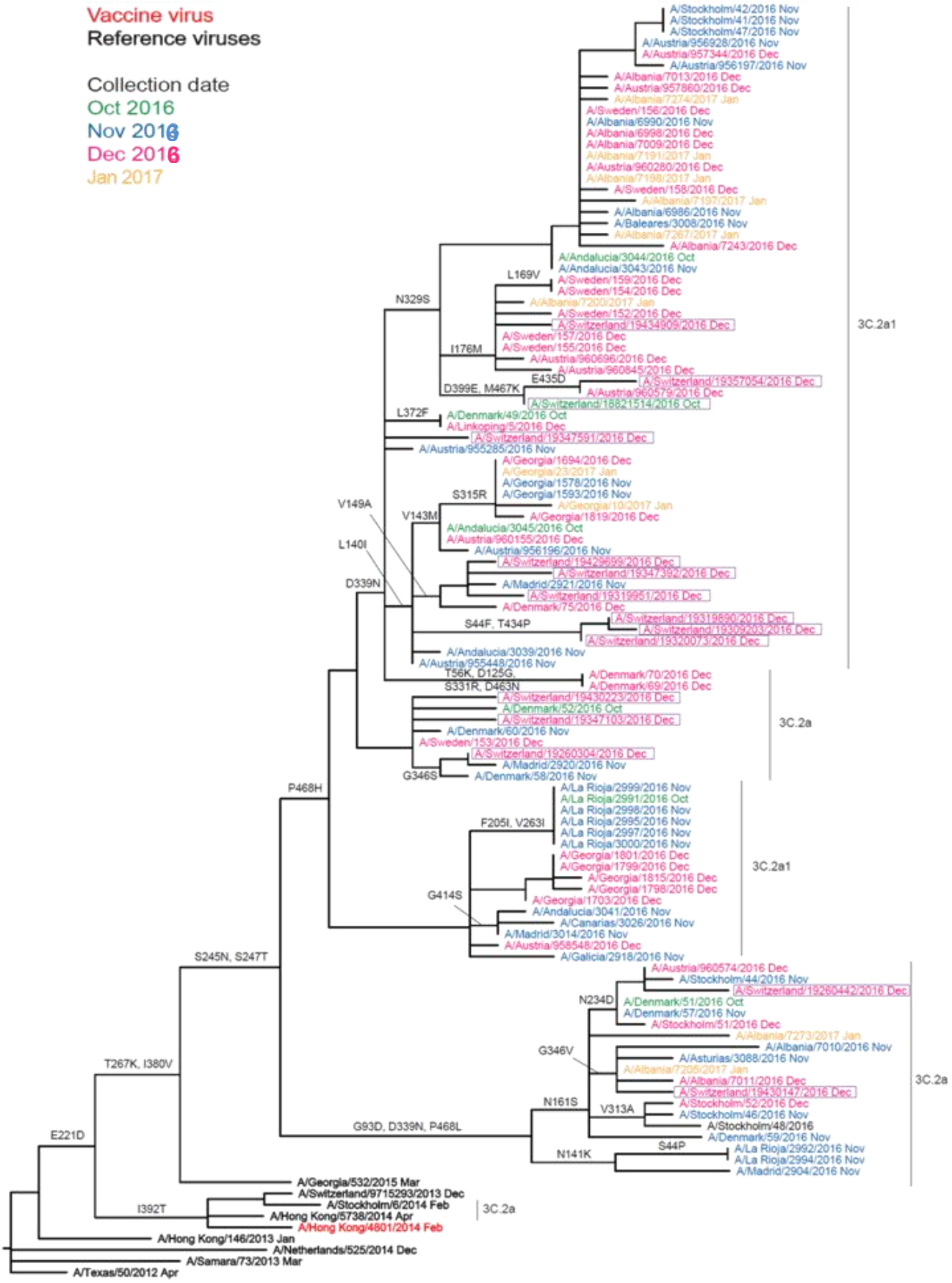
[1] < = <40

[2] < = <10

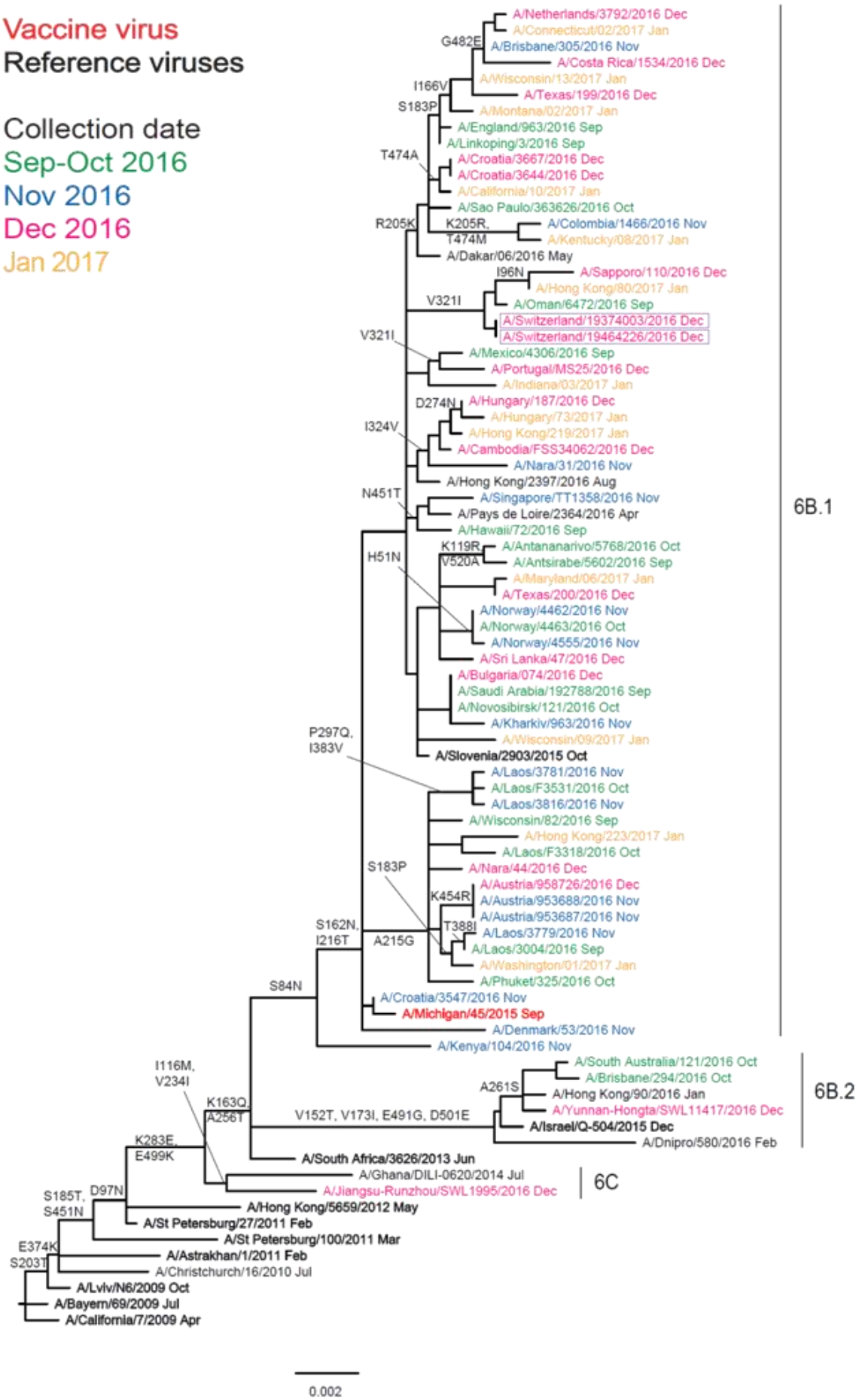
# Annex 10: Phylogenetic comparison of influenza A(H3N2), Hemagglutinin gene, WIC



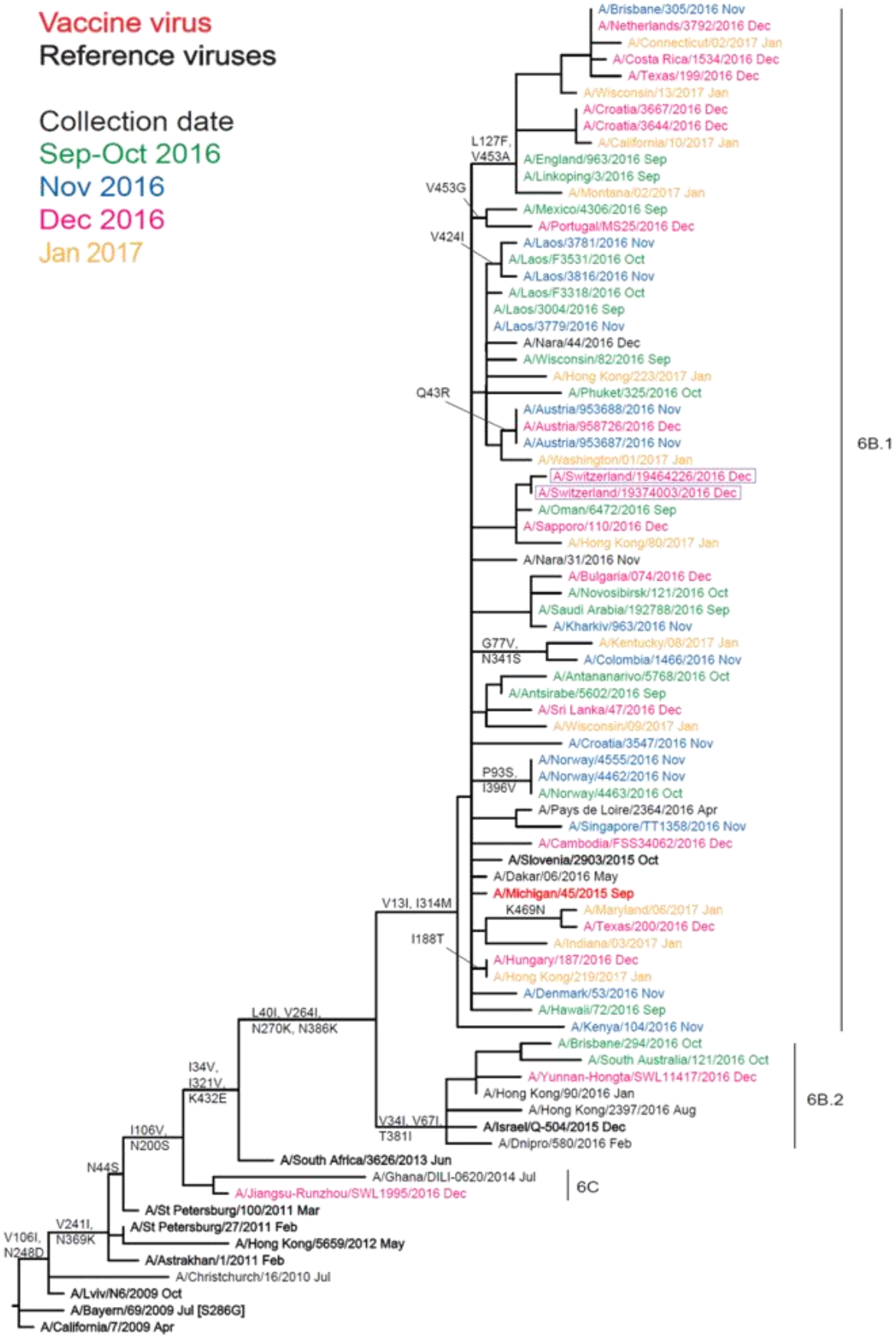
# Annex 11: Phylogenetic comparison of influenza A(H3N2), Neuraminidase gene, WIC



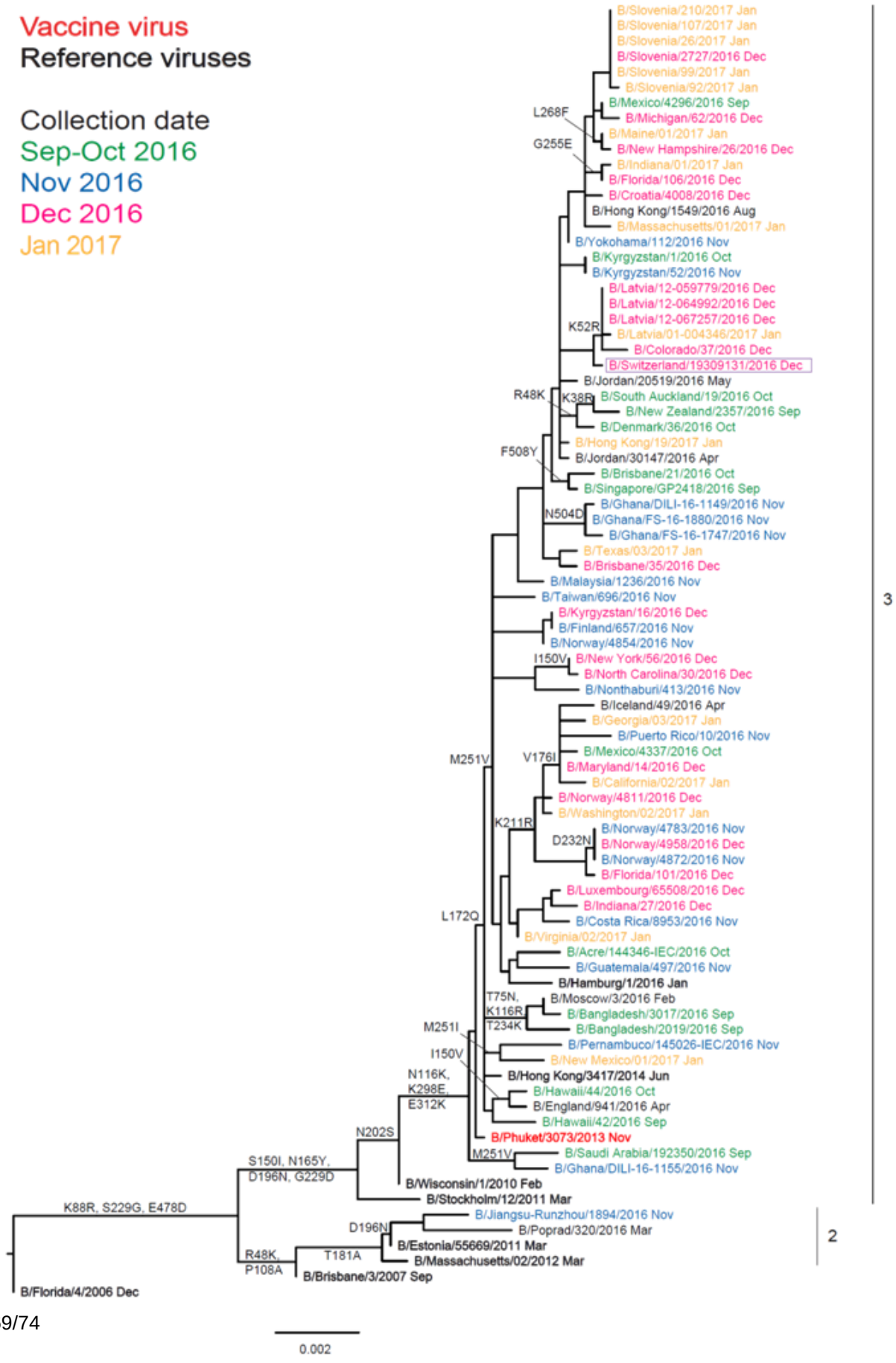
# Annex 12: Phylogenetic comparison of influenza A(H1N1 09), Hemagglutinin gene, WIC



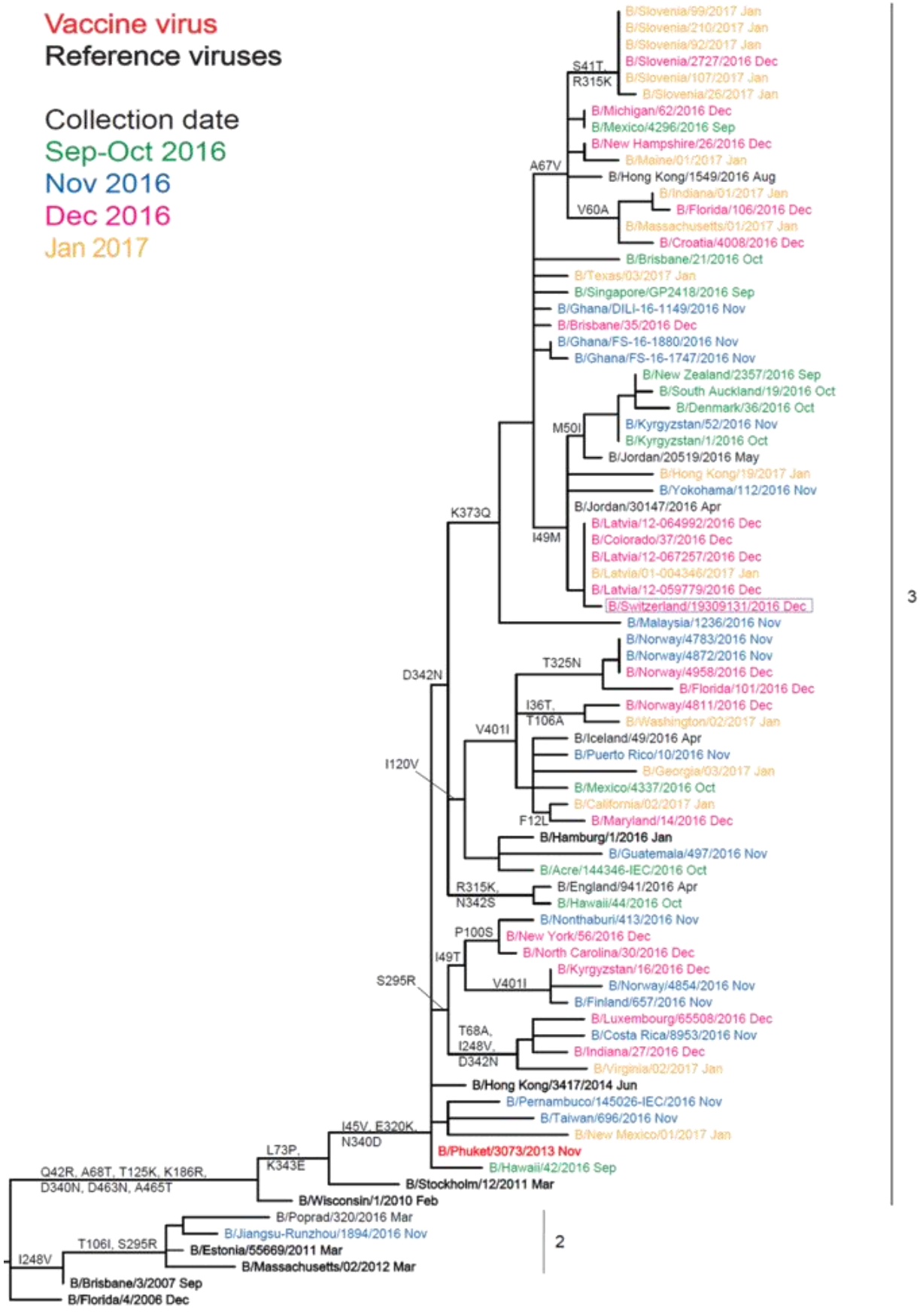
# Annex 13: Phylogenetic comparison of influenza A(H1N1 09), Neuraminisade gene, WIC



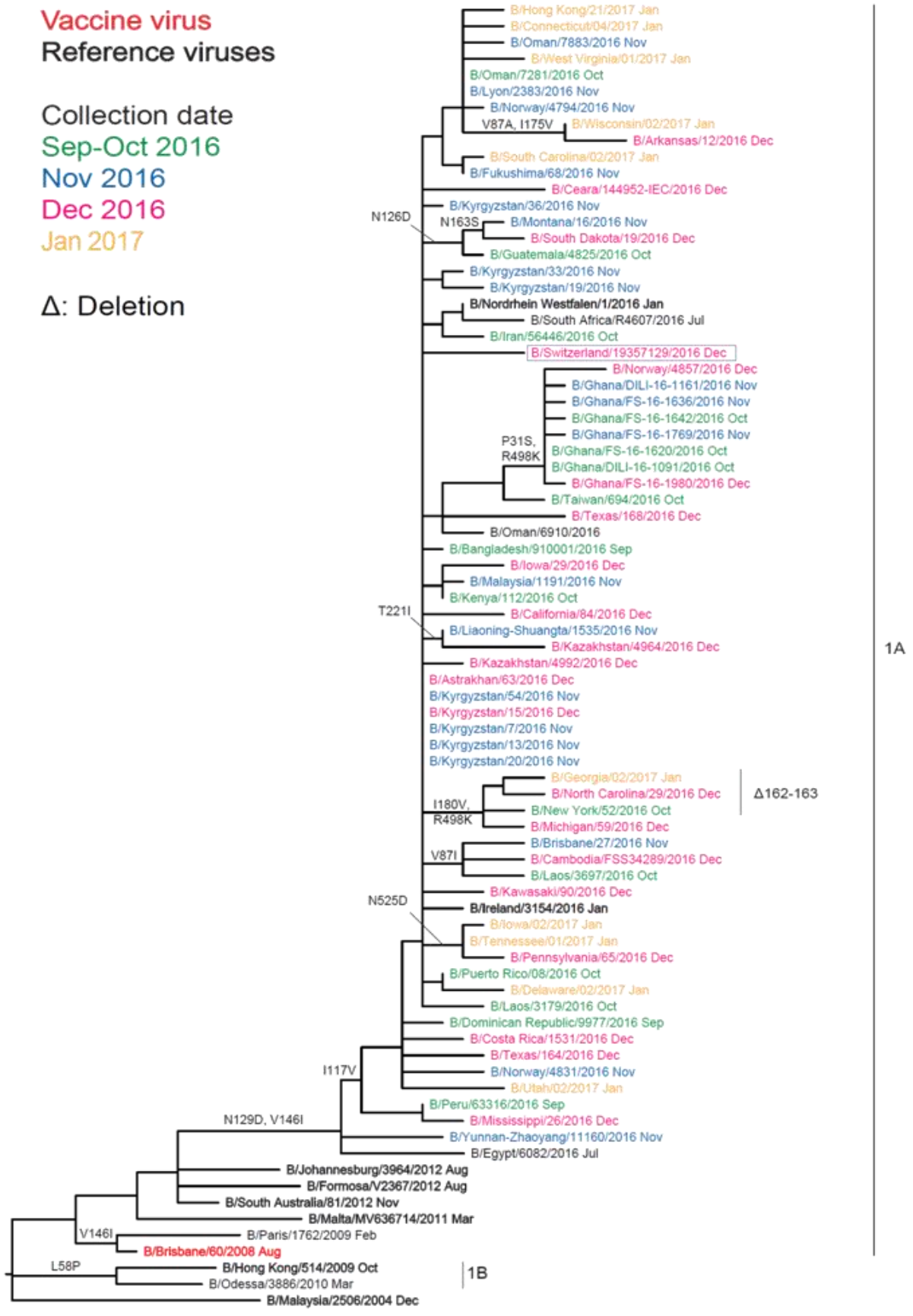
## Annex 14: Phylogenetic comparison of influenza B Yamagata, Hemagglutinin gene, WIC



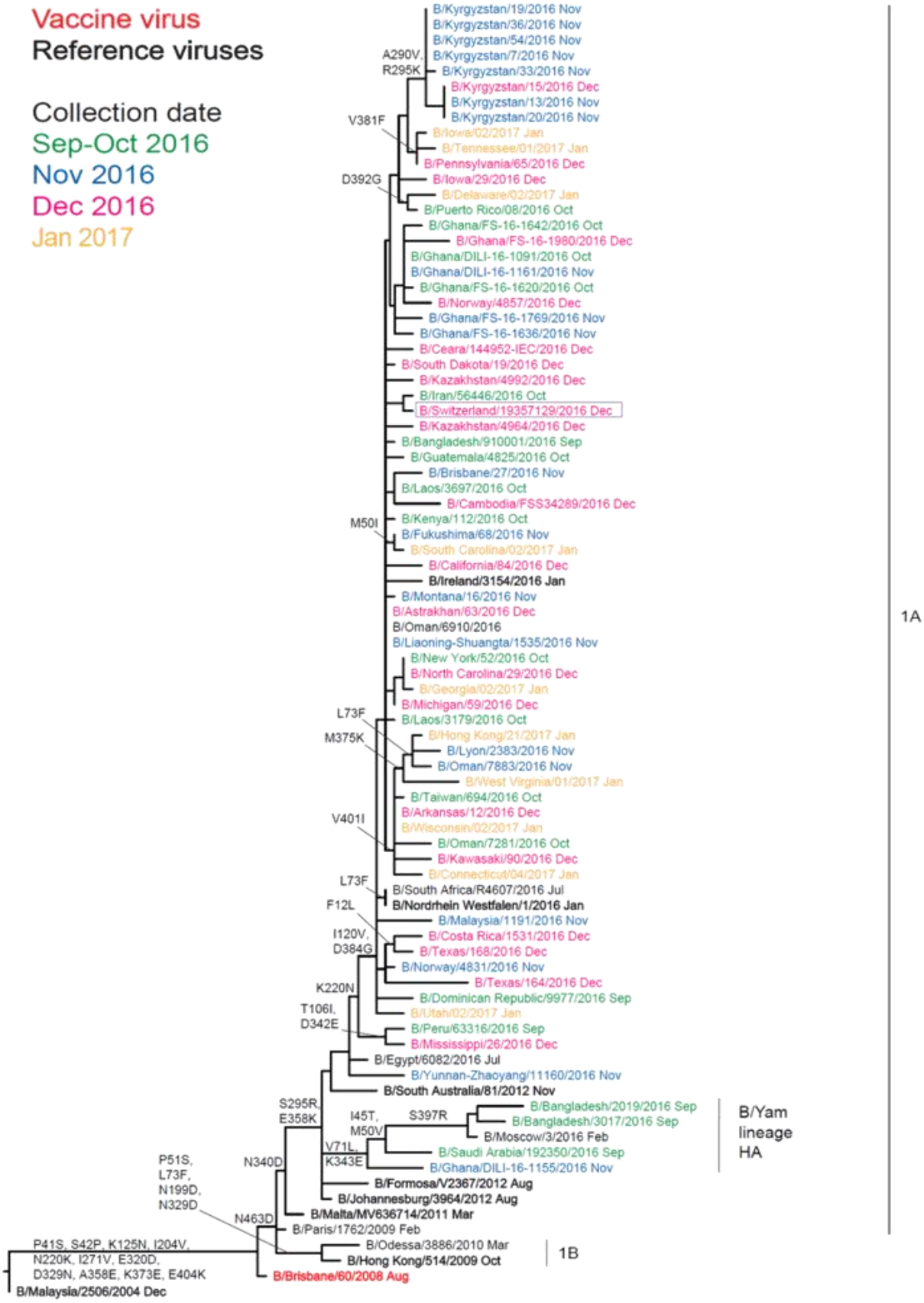
## Annex 15: Phylogenetic comparison of influenza B Yamagata, Neuraminidase gene, WIC



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



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



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

| Collection date | Virus name | Type/Subtype | OS IC50 | OS sensitivity | Zan IC50 | Zan sensitivity | HI result 1 | Centre ID | Date received |
|---|---|---|---|---|---|---|---|---|---|
| 12.12.2016 | B/Switzerland/19357129/2016 | BV | 22.14 | Normal inhibition | 3.49 | Normal inhibition | | CHE | 06.janv.17 |
| 06.12.2016 | B/Switzerland/19309131/2016 | BY | 24.24 | Normal inhibition | 1.5 | Normal inhibition | | CHE | 06.janv.17 |
| 21.12.2016 | A/Switzerland/19464226/2016 | H1pdm | 0.61 | Normal inhibition | 0.3 | Normal inhibition | | CHE | 06.janv.17 |
| 12.12.2016 | A/Switzerland/19374003/2016 | H1pdm | 1.01 | Normal inhibition | 0.44 | Normal inhibition | | CHE | 06.janv.17 |
| 12.12.2016 | A/Switzerland/19357054/2016 | H3 | 0 | Failed | 0 | Failed | Na+ Ha- | CHE | 06.janv.17 |
| 07.12.2016 | A/Switzerland/19319951/2016 | H3 | 0 | Failed | 0 | Failed | Na+ Ha- | CHE | 06.janv.17 |
| 20.12.2016 | A/Switzerland/19429699/2016 | H3 | 0.35 | Normal inhibition | 0.35 | Normal inhibition | Na+ Ha- | CHE | 06.janv.17 |
| 20.12.2016 | A/Switzerland/19430147/2016 | H3 | 0.51 | Normal inhibition | 0.45 | Normal inhibition | Na+ Ha- | CHE | 06.janv.17 |
| 16.12.2016 | A/Switzerland/19430223/2016 | H3 | 0.48 | Normal inhibition | 0.4 | Normal inhibition | Na+ Ha- | CHE | 06.janv.17 |
| 16.12.2016 | A/Switzerland/19434909/2016 | H3 | 0.65 | Normal inhibition | 0.67 | Normal inhibition | Na+ Ha- | CHE | 06.janv.17 |
| 09.12.2016 | A/Switzerland/19347392/2016 | H3 | 0.32 | Normal inhibition | 0.41 | Normal inhibition | Na+ Ha- | CHE | 06.janv.17 |
| 09.12.2016 | A/Switzerland/19347591/2016 | H3 | 0.29 | Normal inhibition | 0.38 | Normal inhibition | Na+ Ha- | CHE | 06.janv.17 |
| 07.12.2016 | A/Switzerland/19319890/2016 | H3 | 0.5 | Normal inhibition | 0.39 | Normal inhibition | Na+ Ha- | CHE | 06.janv.17 |
| 07.12.2016 | A/Switzerland/19320073/2016 | H3 | 0.6 | Normal inhibition | 0.36 | Normal inhibition | Na+ Ha- | CHE | 06.janv.17 |
| 06.12.2016 | A/Switzerland/19309203/2016 | H3 | 0.23 | Normal inhibition | 0.41 | Normal inhibition | Na+ Ha- | CHE | 06.janv.17 |
| 06.12.2016 | A/Switzerland/19347103/2016 | H3 | 0.46 | Normal inhibition | 0.46 | Normal inhibition | Na+ Ha- | CHE | 06.janv.17 |
| 03.12.2016 | A/Switzerland/19260304/2016 | H3 | 0.29 | Normal inhibition | 0.44 | Normal inhibition | Na+ Ha- | CHE | 06.janv.17 |
| 03.12.2016 | A/Switzerland/19260442/2016 | H3 | 0.38 | Normal inhibition | 0.47 | Normal inhibition | Na+ Ha- | CHE | 06.janv.17 |
| 01.12.2016 | A/Switzerland/19239213/2016 | H3 | 0.46 | Normal inhibition | 0.51 | Normal inhibition | Na+ Ha- | CHE | 06.janv.17 |
| 20.10.2016 | A/Switzerland/18821514/2016 | H3 | 0.39 | Normal inhibition | 0.43 | Normal inhibition | Na+ Ha- | CHE | 06.janv.17 |

# Annex 19: Sequencing primers used during the 2016/17 season

| Primers used for classical RT-PCR detection of influenza viruses | | | | |
|---|---|---|---|---|
| **Influenza virus** | **Target gene** | **Primer or probe** | | **Origin and reference** |
| A/H1N1pdm 2009 | Hemaggutinin (H1) | Forward | cswHAF1 | R.Daniel, MRC-NIMR Feb 2011 |
| | | Forward | cswHAF31 | |
| | | Forward | cswHAF451 | |
| | | Forward | cswHAF848 | |
| | | Reverse | cswHAR475 | |
| | | Reverse | cswHAR873 | |
| | | Reverse | cswHAR1263 | |
| | | Reverse | cswHAR1313 | |
| | Neuraminidase (N1) | Forward | cswN1F1 | R.Daniel, MRC-NIMR |
| | | Forward | cswN1F401 | |
| | | Reverse | cswN1R424 | |
| | | Forward | cswN1F1076 | |
| | | Reverse | cswN1R1099 | |
| | | Reverse | cswN1R1424 | |
| | | Reverse | cswN1R1440 | |
| | Matrix (M1) | Forward | M93c | Y. Thomas, CNRI, Geneva Aug 2009 |
| | | Reverse | MF821Y | |
| A/H3N2 seasonal | Hemagglutinin (H3) | Forward | AH3G | J. Ellis London Jan 2006 |
| | | Reverse | AH3H | |
| | | Forward | AH3B | |
| | | Reverse | AH3CII | |
| | | Reverse | AH3I | |
| | | Forward | H3HAF567 | |
| | | Reverse | H3HAR650 | |
| | Neuraminidase (N2) | Forward | H3N2F1 | V. Gregory , MRC-NIH Modified by Y. Thomas, Mar 2011 |
| | | Reverse | N2R410 | |
| | | Forward | N2F387 | |
| | | Reverse | N2R778 | |
| | | Forward | N2F1083 | |
| | | Reverse | N2R1447 | |
| | Matrix | Forward | M93c | Y.Thomas, CNRI, Geneva Feb 2007 |
| | | Reverse | MF820R | |
| B seasonal | Hemagglutinin | Forward | BHA1F1 | V.Gregory, MRC-NIMR Jan 2006 |
| | | Reverse | BHA1R1 | |
| | | Forward | BHAF | |
| | | Forward | BHA25 | |
| | | Forward | BHAF458 | |
| | | Reverse | BHAR652 | |
| | Neuraminidase | Forward | BNAF1 | V. Gregory , MRC-NIMR Modified by Y.Thomas, 2011 |
| | | Forward | BNAF336 | |
| | | Forward | BNAF725 | |
| | | Forward | BNAF1096 | |
| | | Reverse | BNAR1487 | |
| | | Reverse | BNAR1119 | |
| | | Reverse | BNAR748 | |