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Influenza virus surveillance in Switzerland
Season 2020–2021

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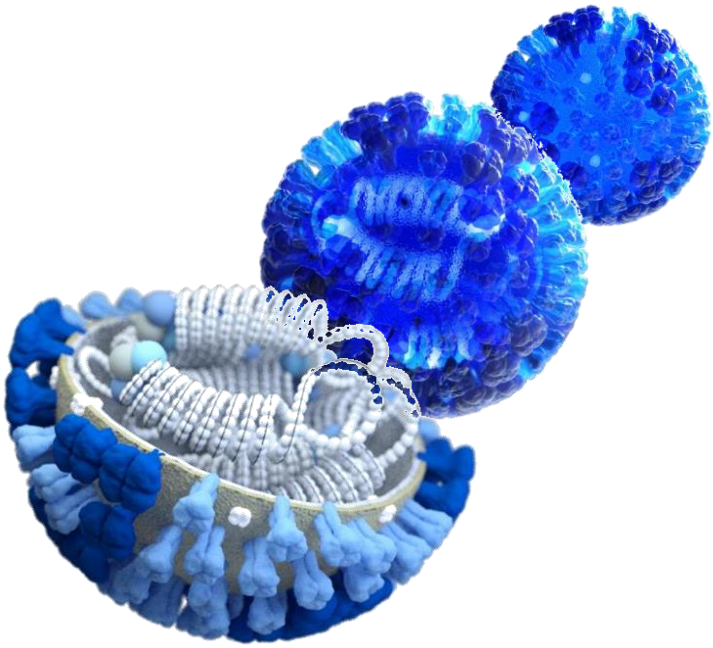

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3D computer-generated rendering of a whole influenza (flu) virus, in semi-transparent blue atop a white background. The virus' hemagglutinin (HA), and neuraminidase (NA) surface proteins, are displayed sticking out of the surface of the virus. Inside of the virus, its ribonucleoproteins (RNPs) were shown in white, with their coiled structures, and three-bulbed polymerase complex on the ends.

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Abbreviations and Acronyms

CDC	centers for disease control and prevention
COVID-19	coronavirus disease 2019
CPE	cytopathic effect
Ct	cycle threshold
ECDC	European centre for disease prevention and control
EEA	European economic area
EEIQAP	European external influenza virus quality assessment programme
EQAP	external quality assessment programme
EU	European union
FOPH	federal office of public health
HA	hemagglutinin
HAdV	human adenoviruses
HBoV	human bocavirus
HCoV	human coronavirus
HEF	hemagglutinin-esterase-fusion
H/LPAI	high/low pathogenic avian influenza
HMPV	human metapneumovirus
HPIV	human parainfluenza
ILI	influenza-like illness(es)
M	matrix
MDCK	Madin-Darby canine kidney cells
MDCK-SIAT1	sialic acid-enriched MDCK cells
NA	neuraminidase
NAI	neuraminidase inhibitor
NEP	nuclear export protein
NRCI	national reference centre of influenza
NS	non-structural
PA, PB	acidic protein, basic protein
RNA	ribonucleic acids
RNP	ribonucleoprotein
RV/EV	rhinoviruses/enteroviruses
RSV	respiratory syncytial virus
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
rRT-PCR	real-time reverse-transcription polymerase chain reaction
USA	United States of America
Vic, Yam	victoria, yamagata
WHO	world health organization
WIC	worldwide influenza centre
URTI	upper respiratory tract infection(s)

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Résumé – Zusammenfassung – Summary

Résumé de la surveillance de l'activité grippale 2020/2021

Pour la première fois au sein du réseau de surveillance Sentinella, les 1174 prélèvements nasopharyngés reçus par le NRCI, de la semaine 40/2020 à la semaine 16/2021, ont non seulement été dépistés pour la grippe mais aussi pour le SARS-CoV-2, RSV, HCoV NL63, HCoV HKU1, HCoV OC43, HCoV 229E, HPIV, HBoV, HAdV, RV/EV et HMPV. Cinq cent trente-huit des 1174 échantillons étaient positifs pour au moins un virus respiratoire. À l'exception d'un seul échantillon, aucun des frottis nasopharyngés testés n'était positif pour la grippe. De ce fait aucune donnée de caractérisation antigénique et génétique de la grippe n'est disponible cette année au CNRI. Le VRS était également presque absent jusqu'à la semaine 16/2021. Le SARS-CoV-2 et le RV/EV étaient les virus les plus fréquemment détectés cette saison.

Un dépistage rétrospectif du SARS-CoV-2, du RSV, des HCoV NL63/HKU1/OC43/229E, du HPIV, du HBoV, du HAdV, du RV/EV et du HMPV a également été exécuté sur 1220 échantillons prélevés au NRCI, des semaines 40/2019 à 39/2020. La grippe et le VRS étaient les virus majoritairement détectés. Seuls quelques échantillons étaient positifs pour le SARS-CoV-2 et seulement entre les semaines 10/2020 et 13/2020. La détection du SARS-CoV-2 a commencé à augmenter dans les échantillons de Sentinella à partir de la semaine 40/2020.

L'activité grippale chez l'Homme était en général très faible dans le monde, et seuls quelques échantillons étaient disponibles pour les réunions de sélection des futures souches vaccinales tenues en septembre 2020 et février 2021 par l'OMS. Comme d'habitude, quelques infections grippales zoonotiques ont été identifiées. Au moins deux des événements de transmission zoonotique concernaient des souches aviaires qui n'avaient jamais été détectées chez l'Homme auparavant. Aucune transmission zoonotique n'a été observée en Suisse.

Contrairement à la grippe humaine, les flambées de grippe aviaire étaient fréquentes dans plusieurs pays européens pendant 2020/2021, mais étaient absentes en Suisse.

Zusammenfassung der Grippeüberwachung 2020/2021

Zum ersten Mal wurden innerhalb des Sentinella-Surveillance-Netzwerks die 1174 Nasopharyngealproben, die das NRZI zwischen W 40/2020 und W 16/2021 erhalten hat, nicht nur routinemäßig auf Influenzaviren, sondern auch auf SARS-CoV-2, RSV, HCoV NL63, HCoV HKU1, HCoV OC43, HCoV 229E, HPIV, HBoV, HAdV, RV/EV und HMPV untersucht. Fünfhundertachtunddreißig von 1174 Proben waren für mindestens ein Atemwegsvirus positiv. Bis auf eine einzelne Probe war keine der getesteten Proben positiv für Influenza. Daher können dieses Jahr keine Daten zu antigenischen und genetischen Charakterisierung vom NRZI zur Verfügung gestellt werden. Bis W 16/2021 konnte auch fast kein RSV nachgewiesen werden. SARS-CoV-2 und RV/EV waren die am häufigsten nachgewiesenen Viren.

Außerdem ist ein retrospektives Screening auf SARS-CoV-2, RSV, HCoV NL63/HKU1/OC43/229E, HPIV, HBoV, HAdV, RV/EV und HMPV von 1220 Proben aus den Wochen 40/2019 bis 39/2020 am NRZI durchgeführt worden. Dabei wurden überwiegend Influenza und RSV nachgewiesen. Nur wenige Proben waren positiv für SARS-CoV-2 und nur von W 10/2020 bis 13/2020. Die Nachweisrate von SARS-CoV-2 in den Sentinella-Proben begann in Woche 40/2020 anzusteigen.

Die Influenza-Aktivität im Menschen war weltweit im Allgemeinen sehr gering, sodass für die im September 2020 und Februar 2021 von der WHO abgehaltenen Auswahlsitzungen für die Impfstoffstämme nur wenige Proben zur Verfügung standen. Wie üblich wurden einige zoonotische Influenza-Infektionen festgestellt. Mindestens zwei der zoonotischen Übertragungsereignisse betrafen Vogelstämme, die noch nie beim Menschen nachgewiesen wurden. In der Schweiz wurde keine zoonotische Übertragung beobachtet.

Im Gegensatz zur menschlichen Influenza kam es 2020/2021 in mehreren europäischen Ländern häufig zu Ausbrüchen von Influenza in Vögeln. In der Schweiz wurde jedoch kein Ausbruch beobachtet.

Summary of the 2020/2021 influenza surveillance

For the first time within the Sentinella surveillance network, the 1174 nasopharyngeal samples received at the NRCI, from week 40/2020 to week 16/2021, were not only routinely screened for influenza but also for SARS-CoV-2, RSV, HCoV NL63, HCoV HKU1, HCoV OC43, HCoV 229E, HPIV, HBoV, HAdV, RV/EV and HMPV. Five hundred and thirty eight out of 1174 samples were positive for at least one respiratory virus. Except a single specimen, none of the tested samples were positive for influenza. Therefore, no influenza antigenic and genetic characterization data are avaible this year at the NRCI. RSV was also almost absent until week 16/2021. SARS-CoV-2 and RV/EV were the most commonly detected viruses.

A retrospective screening for SARS-CoV-2, RSV, HCoV NL63/HKU1/OC43/229E, HPIV, HBoV, HAdV, RV/EV and HMPV of 1220 samples collected at the NRCI from week weeks 40/2019 to 39/2020 was also performed. Influenza and RSV were the viruses predominantly detected. Only few samples were positive for SARS-CoV-2 and only from week 10/2020 to 13/2020. SARS-CoV-2 detection started to increase within the Sentinella' samples at week 40/2020.

Human influenza activity was in general very low worldwide, thus only few samples were avaible for the vaccine strains candidates selection meeting held in September 2020 and February 2021 by the WHO. As usual a couple of zoonotic influenza infections were identified. At least two of the zoonotic transmission events concerned avian strains that had never been detected in humans before. No zoonotic transmission was observed in Switzerland.

In contrast to human influenza, avian influenza outbreaks were frequent in several European Countries during 2020/2021, but were absent from Switzerland.

1 Introduction

Influenza virus infections are a major clinical and economic burden worldwide.[1] In Switzerland, the sentinel surveillance system (Sentinella) is a community-based network of primary care medical practitioners who report suspected cases of influenza or influenza-like illness (ILI) to the Federal Office of Public Health (FOPH). A subgroup of sentinel practitioners collects respiratory samples from patients presenting with ILI, which are sent to the National Reference Centre of Influenza (NRCI) in Geneva for further characterization.

With a mean positivity rate of 45.1±8.7%[1], less than half of the samples from ILI cases tested at the NRCI could be attributed to an influenza infection over the last 15 years. Since week 40 of 2020, in addition to influenza, respiratory syncytial viruses (RSV), human coronavirus (HCoV) (NL63/HKU1/OC43/229E), human parainfluenza (HPIV) virus, human bocavirus (HBoV), human adenovirus (HAdV), human rhinovirus/enterovirus (RV/EV) and human metapneumovirus (HMPV) screening was included in the annual surveillance as a pilot study. Due to its recent but massive public health impact, pandemic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) detection was also implemented.

The majority of nasopharyngeal samples originally screened only for influenza, from week 40 of 2019 (week 40/2019) to week 39/2020, were retrospectively tested for other respiratory viruses as listed in the previous paragraph, SARS-CoV-2 included.

This report summarizes the demographic, epidemiological and virological data gathered from samples processed and analysed by the NRCI during 2020/2021 surveillance (week 40/2020 to week 16/2021) as well as data from the 2019/2020 retrospective screening.

2 The influenza virus

Influenza viruses are Orthomyxoviruses, a family of enveloped, negative, single-stranded ribonucleic acid (RNA) viruses (Figure 1), known to be causative agents of respiratory tract infections and referred to as influenza disease or “flu”. Influenza

[1] The mean positivity rate was calculated over a period of 15 years (2005/2006 to 2019/2020) corresponding to a surveillance period of 29 or 30 (2009 and 2015) weeks/year.

viruses are divided into four genera, A, B, C and D. They are transmitted via airborne and contact routes.[1,2]

Influenza A viruses have a wide host tropism, while influenza B viruses are mainly found in humans[3] and in harbour seals.[4] These two influenza types are responsible for the annual influenza epidemics. Influenza C viruses can be isolated from swine and humans in whom they cause mostly limited mild symptoms, but the epidemiological pattern has not been well studied. Influenza D viruses are mainly found in swine and cattle.[5] Even if the pathogenic potential of influenza D virus in humans remains unknown, specific influenza D antibodies can be found in high proportions in individuals regularly in contact with cattle.[6]

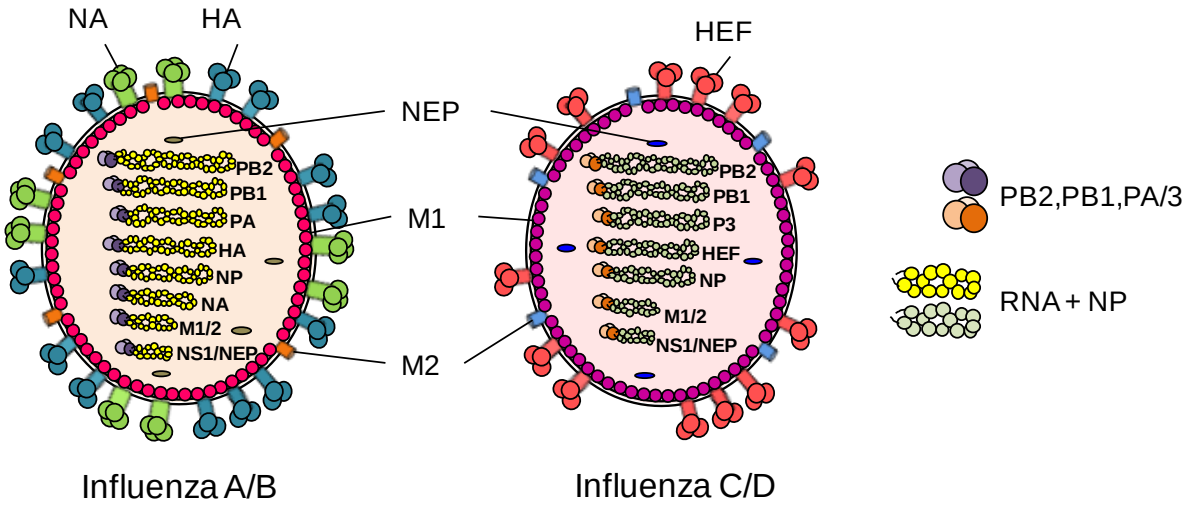

Figure 1. The structure of influenza viral particles. Basic protein 2 (PB2), 1 (PB1) and acidic protein or 3 (PA or P3) form a complex that corresponds to the RNA-dependent polymerase. The hemagglutinin (HA) and the hemagglutinin-esterase-fusion (HEF) play a role in virus attachment to sialic acids present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

Influenza viruses are known to evolve rapidly through two major mechanisms called antigenic drift and shift. The first is the consequence of the accumulation of mutations in the hemagglutinin (HA) and neuraminidase (NA) genes encoding the two major surface glycoproteins targeted by neutralizing antibodies produced against the virus. The antigenic drift drives the annual evolution of the virus and is therefore responsible for the necessity to regularly adapt the seasonal influenza vaccine strains. The

antigenic shift results from the exchange (reassortment) of the influenza A HA and NA genes from different non-human species. It drives the emergence of potential pandemic strains.[7]

Human infection with seasonal influenza A and B viruses can be asymptomatic or cause mild to severe diseases, that can be lethal. Those viruses are of major concern in vulnerable individuals, such as the elderly (≥65 years old), pregnant women, persons with underling chronic diseases and young children, in whom they represent an important health threat.

3 Other respiratory viruses pilot testing

A majority of respiratory viruses other than influenza infections are often associated with mild or moderate acute respiratory diseases. Nevertheless, they can also be linked to more severe syndromes and increased morbidity[8] in particular subpopulations.

3.1 RSV

RSV belongs to the Pneumoviridae family, genus Orthopneumovirus. This enveloped virus contains a non-segmented, single-stranded, negative sense RNA genome of ten genes coding for 11 proteins.[9]

RSV is considered as an important threat for children under age 5 years old, adults with underlying medical conditions or the immunocompromised[10], and the elderly.[11] Each year, RSV infections are estimated to be responsible for more than 3 million hospitalizations and more than 118’000 deaths globally[12], a large proportion in low income countries. Considering the high public health impact of RSV, the WHO is currently building a global RSV surveillance programme similar to the one already existing for influenza (https://www.who.int/influenza/rsv/en/).[13]

During RSV upper respiratory tract infections (URTI), clinical manifestations are generally mild with symptoms like runny nose, cough, nasal congestion, low-grade fever and low appetite. Most infants with RSV will present an URTI, whereas 20-30% will develop potentially severe lower respiratory tract infection such as bronchiolitis, pneumonia, and respiratory failure. RSV infections at early age are also suspected to be liked to asthma development. Older children mostly present URTI symptoms. In adults and elderly, RSV symptoms can be similar to those caused by influenza virus.[11]

Cases of RSV associated encephalitis[14], myocarditis[15,16], and hepatitis[17] have also been reported.

3.2 HCoVs

Coronaviruses are enveloped, single-strand, positive-sense 5′-capped and 3′-polyadenylated RNA viruses belonging to the subfamily Orthocoronavirinae of the Coronaviridae family. Their 30'000 base-pair genome codes for more than 20 proteins.[18] Orthocoronavirinae are divided in four genera : *α*-coronavirus, *β*-coronavirus, *γ*-coronavirus and *δ*-coronavirus. Alpha- and *β*-coronaviruses infect mammalian species while *γ*- and *δ*-coronaviruses are avian viruses.[19]

Before the emergence of SARS-CoV-2 in 2019, four coronaviruses were known to cause, in general, mild to moderate diseases in humans, i.e. HCoV 229E, NL63, OC43, and HKU1; and two were associated with more severe lower respiratory tract infections, i.e. severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV). Most HCoV seem to peak during winter[20] and to display biannual epidemic patterns.[21] The first cases of SARS-CoV and MERS-CoV were respectively identified in 2002 and 2012. SARS-CoV is currently not circulating in the human population, while there are sporadic laboratory-confirmed MERS-CoV infections reported to the WHO as of December 2020.[22]

HCoV 229E, NL63, OC43, and HKU1 can infect the upper and lower respiratory tracts of both adults and children; and are, as many other respiratory viruses, often associated with common colds of mild to moderate intensity depending on the viral species. Nevertheless, in vulnerable individuals, both children and adults, HCoV 229E, NL63, OC43, and HKU1 may exhibit more severe diseases as bronchiolitis and pneumonia[21]. Neurological manifestations have also been observed.[23]

SARS-CoV-2 are *β*-coronaviruses responsible for the current coronavirus disease pandemic (COVID-19) that emerged in China in December 2019.[24] Most of the first identified cases of COVID-19 were linked to a wet market in Wuhan city where live wild animals were also traded. However, the origin of the index case(s) remains unknown.

Clinical manifestations of SARS-CoV-2 range from mild to severe diseases with non-specific symptoms similar to other respiratory diseases. Asymptomatic cases have also been described.[18] As of July 27th 2021, 715'267 cases (33 956 561 in the EU/EEA,

as of July 28th 2021) and 10’392 deaths (742 847 in the EU/EEA, as of July 28th 2021) have been reported in Switzerland[25,26].

SARS-CoV-2 viruses can be transmitted from human-to-human via respiratory droplets, fomites and by aerosols.[27] SARS-CoV-2 RNA has also been detected in blood,[28] urine and faeces.[29] However, transmission assessment via those routes requires further investigation. Wild and domestic animals infections with human SARS-CoV-2 viruses have also been observed.[30-32]

As could be expected from influenza surveillance, SARS-CoV-2 viruses have evolved into several genetic clades and subclades. Some genetic lineages were specifically identified as higher public health threats and classified in three risk groups, i.e. variant of concern (VOC) , variant of interest (VOI) and variant under monitoring. VOC, VOI and variant under monitoring are defined by the ECDC as variants with “clear evidence indicating a significant impact on transmissibility, severity and/or immunity that is likely to have an impact on the epidemiological situation in the EU/EEA”; “evidence is available on genomic properties, epidemiological evidence or in-vitro evidence that could imply a significant impact on transmissibility, severity and/or immunity, realistically having an impact on the epidemiological situation in the EU/EEA”; and variants with “properties similar to those of a VOC, but the evidence is weak or has not yet been assessed”, respectively.[26]

3.3 HPIV

HPIV are enveloped, non-segmented, single-stranded, negative-sense RNA viruses belonging to the Paramyxoviridae family. Their 15’000 base-pair genome only encodes six proteins. HPIV are divided in four serotypes. Serotype 4 is further subdivided into a and b subserotypes. HPIV 1 and 3 belong to the Respirovirus genus, while HPIV 2 and 4 to the Rubulavirus genus.

HPIV can infect both the upper and lower respiratory tracts of children, often with ages <5 years old, and adults. HPIV, with RSV, infections are major causes of morbidity and mortality in young children worldwide.[33] Even if generally considered as mild in healthy individuals, HPIV infections can also result in more severe respiratory diseases in immunocompromised individuals as well as in children.[34] HPIV 1 and HPIV 2 cause croup and cold-like symptoms, while HPIV 3 often results in bronchiolitis, bronchitis

and pneumonia.[35] HPIV 4 is less well studied but seems to exhibit symptoms similar to HPIV 3 in children.[36]

3.4 HBoV

Human bocaviruses (HBoV) 1 to 4 are non-enveloped, non-segmented, single-stranded DNA viruses belonging to the family Parvoviridae, subfamily Parvovirinae. Their approximately 5'000 base-pair genome encodes at least eight proteins.[37]

With parvovirus B19, HBoV is the second parvovirus known to be pathogenic to humans. HBoV 1 is more commonly found in respiratory specimens of young children,[37] but can also be detected in adults.[38] HBoV 2, 3 and 4 are commonly identified in stools samples.[39] They are also often found as co-infections. Their clinical presentation is similar to other respiratory viruses leading to either asymptomatic or mild URTI. However more severe clinical manifestations as encephalitis, myocarditis,[40] idiopathic lung fibrosis, as well as yet to be confirmed carcinogenesis have also been associated with HBoV, particularly type 1.[41]

3.5 HAdV

Adenoviruses are non-enveloped, double-stranded DNA viruses of more than 26'000 base-pairs encoding for several non-structural and structural proteins, that infect both animals and humans. HAdV belong to the Mastadenovirus genus of the Adenoviridae family, and are further divided into species A to G and more than 50 different serotypes infect humans. HAdV B and E both infect the conjunctiva as well as the upper and lower respiratory tracts, while D and C are specific to only one of these tissues, respectively. Finally types F and G have a tropism for the gastrointestinal tract. Most HAdV infections are either asymptomatic or mild, particularly in young children. However, in vulnerable individuals (e.g. immunocompromised), the clinical manifestations are broader and more severe with possible fatal outcome.[42,43]

3.6 RV/EV

Picornaviruses can be pathogenic for both animals and humans. They are non-enveloped, single-stranded, positive-sense RNA viruses with genomes ranging from 7'200 to 8'500 bases long. RV (A-C) and EV (A-D) are species of the Enterovirus genus of the Picornaviridae family that are responsible for a high number of human infections annually.[44,45]

Due to their resistance to low pH and high temperatures (37°C), EV can survive the stomach acidic environment and infect the small intestine. In contrast, RV replicates at the pH and temperature found in the nasal mucosa. These viruses usually cause mild gastrointestinal or respiratory diseases. However, some can also case more severe manifestations as pancreatitis, hepatitis, myocarditis, encephalitis, flaccid myelitis, paralysis and even death.[44] It is notably the case of the poliovirus, the causative agent of major poliomyelitis epidemics before the initiation of the Global Polio Eradication Initiative by the WHO in 1988.

3.7 HMPV

HMPV are enveloped single-stranded, negative-sense non-segmented RNA viruses of the Metapneumovirus genus of the Pneumoviridae family, similar to RSV. Their 13’000 base-pair genome codes for eight genes encoding for nine proteins. HMPV are divided in two genotypes and two sub-genotypes.[46]

HMPV infections are prevalent in young children <5 years old; and are second in terms of association with a hospitalisation requirement after RSV infection. Reinfection throughout life is common but disease is generally milder in young adults. HMPV have a tropism for upper and lower respiratory tracts, and could lead to bronchiolitis, pneumonia, as well as acute asthma and chronic obstructive pulmonary disease exacerbations (in adults). HMPV infections, as with all the respiratory viruses described in chapter 3, can be a major threat in vulnerable individuals and the elderly, in whom they can also be fatal.[46]

4 Methodology

4.1 Clinical identification of influenza cases

Primary care practitioners, usually 150 to 200, voluntary participate in the epidemiological national influenza surveillance network on a yearly basis. They are requested to report ILI and COVID-19 cases (since September 2020) on a weekly basis.

Within the Swiss Sentinel system, ILI cases are defined as sudden high-grade fever (>38°C) onset and cough or sore throat. The presence of other symptoms, such as malaise, myalgia, joint pain and headache, as well as gastrointestinal symptoms is not required. Patients presenting with a secondary disease (pneumonia, bronchitis, otitis, etc.) consecutive to an unreported influenza are also expected to be reported.

COVID-19 cases are defined as symptoms of acute respiratory tract disease (i.e. cough, sore throat, shortness of breath, chest pain), and/or acute confusional state or deterioration of general condition with no other aetiology in the elderly, and/or fever with no other aetiology, and/or sudden onset of anosmia, and/or ageusia.

Of note, the COVID-19 pandemic has a major impact on ILI data collection within the Sentinella network as, despite the fact that different case definitions are used, COVID-19 and influenza symptoms remain often similar. Therefore, it cannot be excluded that some clinically reported ILI were in fact COVID-19 cases and vice versa.

A subgroup of sentinel practitioners collects nasopharyngeal swabs from patients fitting the ILI and COVID-19 case definitions for subsequent viral detection and further characterization, in particular for influenza.

4.2 Sentinella population

The sentinel practitioners who send samples to the NRCI are asked to complete a brief case report form. The following data are collected: Patient identity, address and phone number (necessary for mandatory reporting only); sample type; age; gender; time of symptom onset; pneumonia; hospitalization; travel within the previous 14 days upon symptoms onset; and influenza vaccination status.

4.3 Molecular detection of influenza viruses[2]

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

Since the 2017/2018 season, influenza A positive samples are subtyped using an in-house-developed quadruplex rRT-PCR targeting the HA (H1 and H3) and the NA (N1 and N2) genes in order to discriminate between influenza A(H1N1)pdm09 and A(H3N2) strains. This new assay is a mix of already validated (in-house H1 and H3 CDC) and newly-designed (N2[3]) rRT-PCR combinations, adapted from the one used in the study by Henritzi *et al*[47] (N1). The quadruplex detection limit is similar to that of the diagnostic rRT-PCR. The N1 combination was able to detect the H1N1v[4], swH1N1[5] and H5N1[6] isolates tested during the assay validation process. The H3 and N2 rRT-PCR combinations are also able to detect the A/Wisconsin/12/2010 H3N2 triple reassortant (H3N2tr),[48] although the latter virus is not known to circulate in Switzerland. Nevertheless, if needed, additional tests are available at the NRCI to discriminate seasonal H3N2 from H3N2tr viruses. Influenza B/Yamagata/16/88-like (Yam) and B/Victoria/2/87-like (Vic) lineages are determined using a duplex rRT-PCR.

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

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

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

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

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

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

SARS-CoV-2 negative samples were submitted to cell culture starting end of February 2020.

4.4 Molecular detection of SARS-CoV-2 viruses

From week 40 of 2019 to week 39 of 2020 retrospective screening, nasopharyngeal swabs were tested for SARS-CoV-2 genome using a one-step rRT-PCR targeting the E-gene adapted from the 2019-nCoV Charité/Berlin assay[49] version 2.[50] From week 40/2020 to week 16/2021, SARS-CoV-2 positive samples were diagnosed daily using the Cobas® SARS-CoV-2 or Cobas® SARS-CoV-2 & Influenza A/B detection kits (for a small amount of samples) on a Cobas® 6800 instrument.

From week 01 of 2021 onwards, and as a consequence of the identification in the United Kingdom of a SARS-CoV-2 variant virus (Alpha variant or B.1.1.7 lineage) that was considered as a VOC, all SARS-CoV-2 positive samples with cycle threshold (Ct) values <32 (E-gene) were screened by single nucleotide polymorphism (SNP) specific rRT-PCR (VirSNiP Mutation Assays, TIB Molbiol, Berlin) for the N501Y substitution in the spike protein. Later, in reaction to the emergence of other VOCs and VOIs, the presence of E484K/Q (VOCs Beta/Gamma, and VOIs Eta/Zeta/Iota) and 417N/T (VOC beta, B.1.351/gamma, respectively) spike substitutions were also monitored.

4.4.1 Genetic characterization of SARS-CoV-2 viruses

Genetic characterization of SARS-CoV-2 was done by whole genome sequencing either by Microsynth AG (Balgach, Switzerland) or the Genome Center (Campus Biotech, Geneva, Switzerland). The resulting consensus sequences were shared internationally through submission to GISAID.

4.5 Molecular detection of respiratory viruses other than influenza and SARS-CoV-2

All nasopharyngeal swabs sent to the NRCI for influenza and SARS-CoV-2 detection were also screened for common respiratory viruses using a PCR panel already used by the Geneva University Hospitals Laboratory of Virology. RSV, HCoVs NL63/HKU1/OC43/229E, HPIV, HBoV, HAdV, RV/EV and HMPV were detected using a combination of seven custom manufactured rRT-PCR mixes produced by Eurogentec. Mixes' targets are grouped as follows: 1. RSV/ canine distemper virus (CDV, our extraction efficiency control), 2. HCoV NL63/OC43, 3. HCoV 229E/HKU1,

4. HBoV/HPIV2-4 (does not distinguish between HPIV 2 and 4), 5. HMPV/HPIV1-3 (does not distinguish between HPIV 1 and 3), 6. RV/EV, and 7. HAdV/CDV.

4.5.1 Cell culture

Both influenza positive and negative samples are cultured on MDCK and MDCK-SIAT1 cells. This allows to ensure that a low positivity rate is not due to a rRT-PCR detection defect.

In brief, 0.4 ml of transport medium containing nasopharyngeal swab are incubated for seven days under 5% CO_2 at 33°C on MDCK cells and 37°C on MDCK-SIAT1. The presence of a cytopathic effect (CPE) is monitored under visible light (Nikon®, Tokyo, Japan) for a period of 7 days. If CPE is present samples are submitted to a hemagglutination test in order to determine the virus titer. The hemagglutination and hemagglutination inhibition assays are dependent on the ability of the viral HA to bind to sialic acids present on the surface of red blood cells. If CPE is absent or low after 7 days, the cells are screened for influenza viruses by immunofluorescence using monoclonal influenza A and B antibodies combined with mouse fluorescein isothiocyanate-conjugate (Merck-Millipore, Chemicon®, Schaffhausen, Switzerland).

For biosafety reasons, only SARS-CoV-2 negative samples were submitted to cell culture. However SARS-CoV-2 isolation/amplification by cell culture is perfectly possible at the NRCI but it was not requested during 2020-2021 surveillance. It is currently done by our associated research group within our Biosecurity level 3 laboratory.

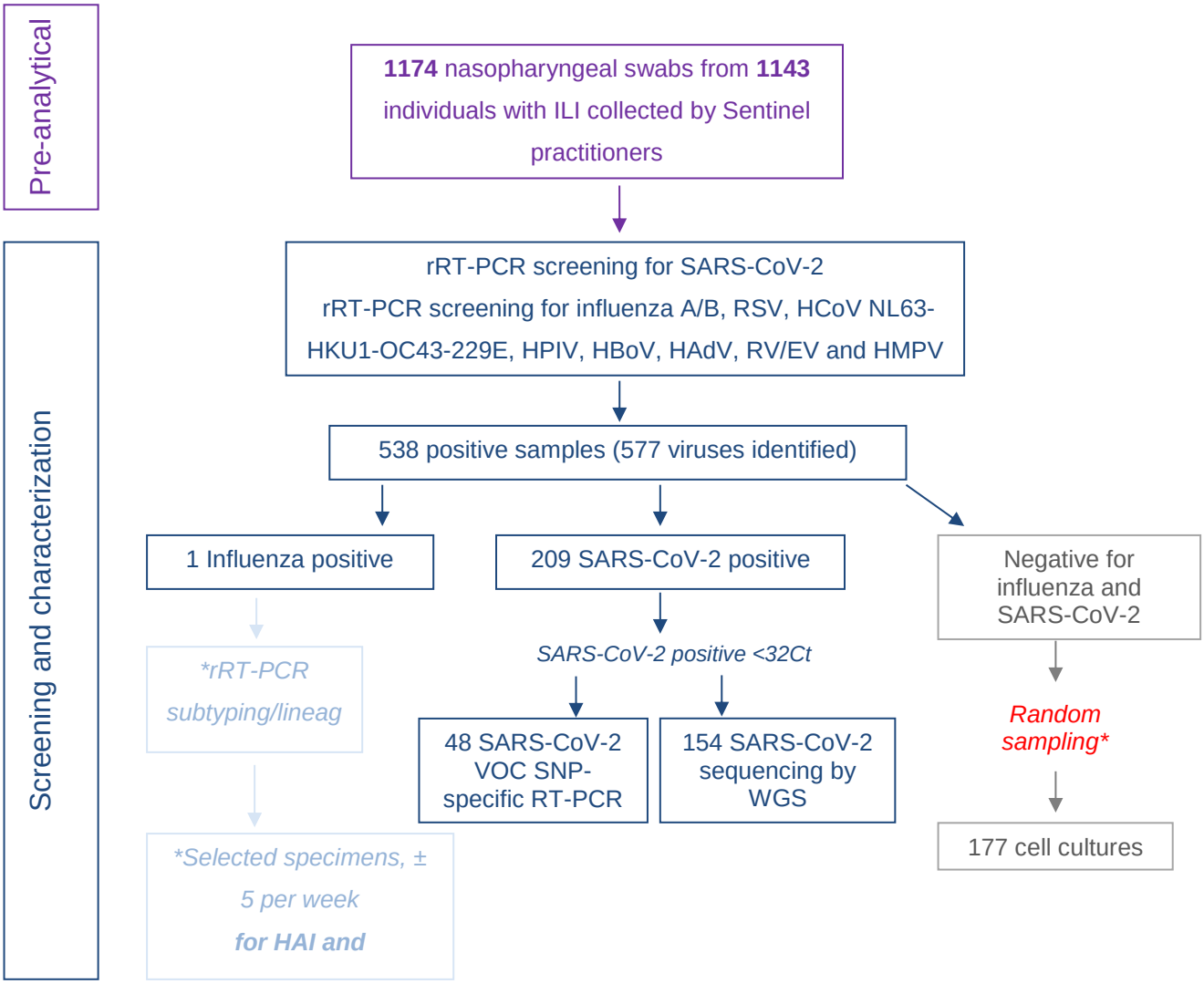


Figure 2. Flow chart of Sentinel samples collection and processing. Starting end of February 2020, only influenza positive and negative samples that were also negative for SARS-CoV-2 were submitted to cell culture. *Except for a low titer influenza B virus, no influenza was detected during the 2020-2021 surveillance period. Thus no subtyping, antigenic and genetic characterizations were performed this virus.

5 2020/2021 surveillance period

Data gathered in the present report corresponds to Sentinella samples analysed at the NRCI from September 28th 2020 to April 25th 2021, as well as to the retrospective screening for respiratory viruses from September 30th 2019 to 27th September 2020.

5.1 Population demographics

5.1.1 Annual NRCI surveillance (2020/2021)

From week 40/2020 to week 16/2021, 1143 individuals were sampled by 47 sentinel practitioners for further screening at the NRCI. Among those 583 (51%) were female and 560 (49%) were male. Seventeen males and ten females were sampled twice. One male and one female were tested 3 times during the surveillance period. (Table 1)

Except for one male, data on age was available for all individuals (median 35 years old, range [21 days to 99 years]; 95% confidence interval (CI), 33-38 years old). Median age was 33 years old for males (range [21 days to 99 years]; 95% CI, 30-37 years old) and 38 years old for females (range [25 days to 98 years]; 95% CI, 34-41 years old) (Figure 3 a and b). When further stratifying the population by age groups (i.e. 0-4, 5-14, 15-29, 30-64 and ≥65 years old), a slightly higher percentage of males in the 5-14 years old group and of females in the 30-64 group could be observed (Table 1). The age distribution within the different age groups was similar in-between male and female for all age groups (Figure 4).

Table 1: Age and sex distribution of the tested Sentinella population, from weeks 40/2020 to 16/2021

	Male	**Female**	***Totals***
Number of individuals	560	583	*1143*
Age group distribution			
0-4	61 (10.9%)	62 (10.6%)	*123 (10.8%)*
5-14	101 (18%)	71 (12.2%)	*172 (15%)*
15-29	91 (16.3%)	99 (17%)	*190 (16.6%)*
30-64	223 (39.8%)	262 (44.9%)	*485 (42.4%)*
≥65	83 (14.8%)	89 (15.3%)	*172 (15%)*
unknown	1 (0.2%)		

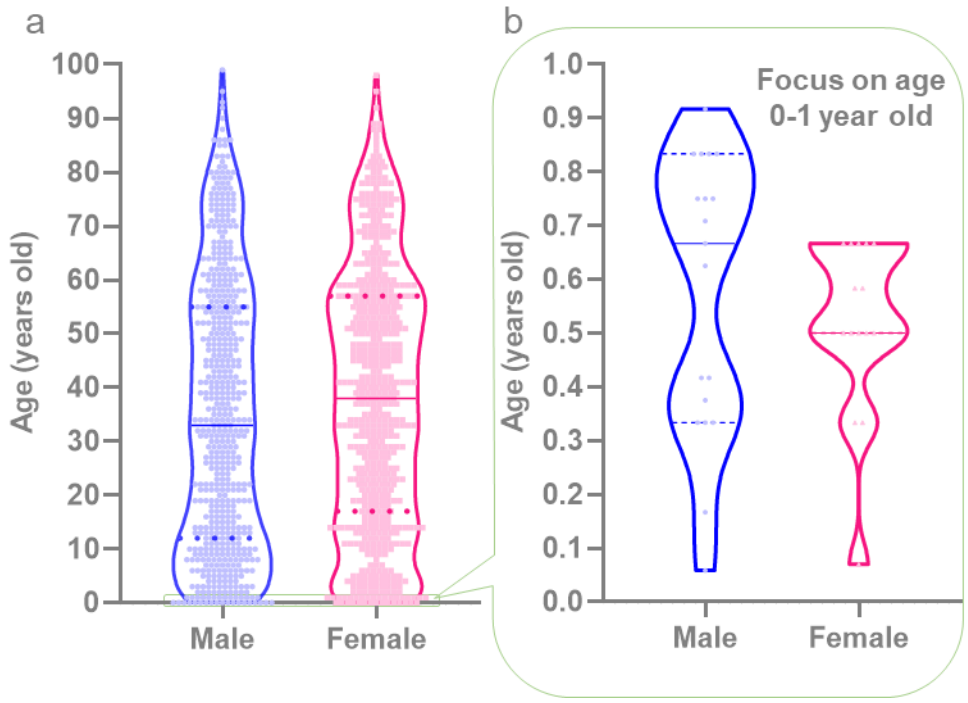


Figure 3: Age distribution by sex of the tested Sentinella population, from weeks 40/2020 to 16/2021. a. Distribution pattern for the entire population. **b.** Distribution focusing on infants less than one year. The solid lines represent median ages and dashed lines correspond to 25% and 75% quartiles.

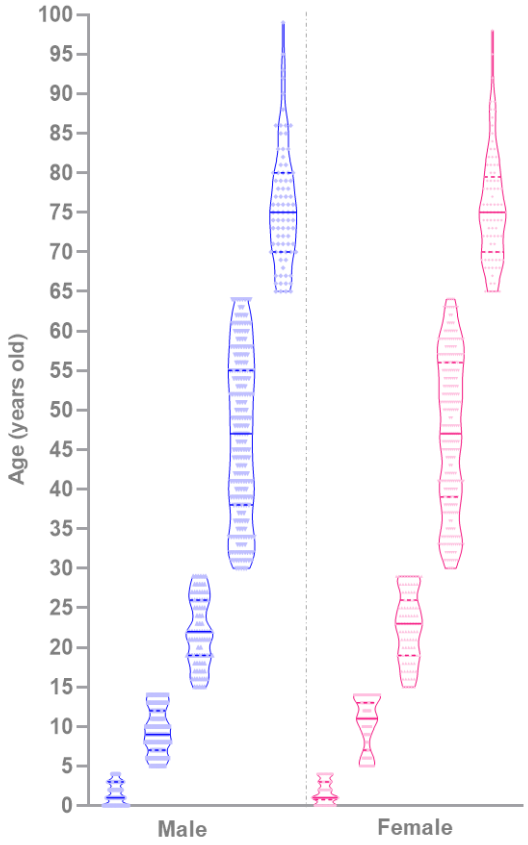


Figure 4. Age distribution within age groups for male and female. The solid lines represent median ages and dashed lines correspond to 25% and 75% quartiles. From left to right : 0-4, 5-14, 15-29, 30-64 and ≥65 years old.

The nasopharyngeal swab sampling median time after the reported onset of first symptoms was of 3 days (n=1099, 24 individuals were sampled twice) with a range from 1 to 60 days.

Influenza vaccination status was available for 1015 subjects, among whom only 113 were vaccinated against influenza during the 2020-2021 period. Interestingly, 5 individuals were reported as having received at least one dose of Comirnaty® (Pfizer Inc. and BioNTech SE) or Spikevax (Moderna).

5.1.2 Respiratory viruses retrospective screening (2019/2020)

From week 40/2019 to week 16/2020, 1118 individuals with ILI were sampled for influenza viruses diagnostic in the context of the Sentinella surveillance. This population was already described in the "Influenza virus surveillance in Switzerland, season 2019-2020" report. Of note, there was no sampled material left for 12 subjects. Of those 12, a majority were collected from weeks 40 to 46/2019 and, except two children, all were older than 31 years.

From week 17/2020 to week 39/2020, 102 persons (51 female and male) were screened for influenza and SARS-CoV-2. Median age was 35 years old for males (n= 51, range [1 to 83 years]; 95% CI, 25-45) and 41 years old for females (n=50, range [1 to 94 years]; 95% CI, 33-50) (Figure 5). All were also retrospectively tested for the "other respiratory viruses".

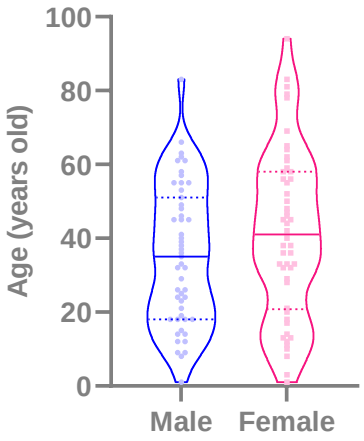


Figure 5. Age distribution per sex of the tested Sentinella population, from weeks 17/2020 to 39/2020. The solid lines represent median ages and dashed lines correspond to 25% and 75% quartiles

5.2 Detection of respiratory viruses in nasopharyngeal samples

5.2.1 Annual NRCI surveillance (2020/2021)

From week 40/2020 to week 16/2021, 1174 nasopharyngeal samples (NPS) were screened by rRT-PCR for influenza, SARS-CoV-2, RSV, HCoV NL63, HCoV HKU1, HCoV OC43, HCoV 229E, HPIV, HBoV, HAdV, RV/EV and HMPV (Annex 1).

Five hundred and thirty eight samples (46%) were positive for at least one respiratory virus and 577 viruses in total were detected. Among those, the following pathogens were detected: 36.2% of SARS-CoV-2 (209), 35% RV/EV (202), 11.3% HCoV NL63 (65), 5.7% HPIV 1/3 (33), 4.2% HAdV (24), 3.6% HCoV OC43 (21), 1.9% HBoV (11), 0.9% HPIV 2/4 (5), 0.3% RSV(2), 0.3% HMPV (2), 0.3% HCoV 229E (2) (Figure 6a) and one influenza B virus (0.2%). The latter could not be further characterized due to very low viral load. Not surprisingly, the isolation attempt by cell culture was not successful either. No HCoV HKU1 was detected from weeks 40/2020 to 16/2021.

Thirty nine co-infections (7.2%) were detected. The most common were RV/EV with SARS-CoV-2 (23.1%) and RV/EV with HAdV (17.9%). Not surprisingly SARS-CoV-2 co-infections were more common in adults (median 41 years old; 95% CI, 13-57), while other virus co-infections were mainly found in infants (median 1 year old; <1-3) (data not shown). Co-infection combinations are detailed in Table 2.

The only influenza positive sample was detected on week 06/2021. RSV was also almost absent until week 16/2021. SARS-CoV-2, RV/EV and HAdV were detected throughout the surveillance period. Even if already sporadically detected before, HCoV, in particular HCoV NL63 and OC43, and HPIV started to increase in prevalence beginning at week 4/2021 (Figure 6b).

A maximal positivity rate of 73.9% was observed during week 40/2020 (Figure 6b), the first week of surveillance, and a minimal positivity rate of 22.2% during week 3/2021 (median positivity rate : 49.2%, 95% CI [40.4, 52.5]).

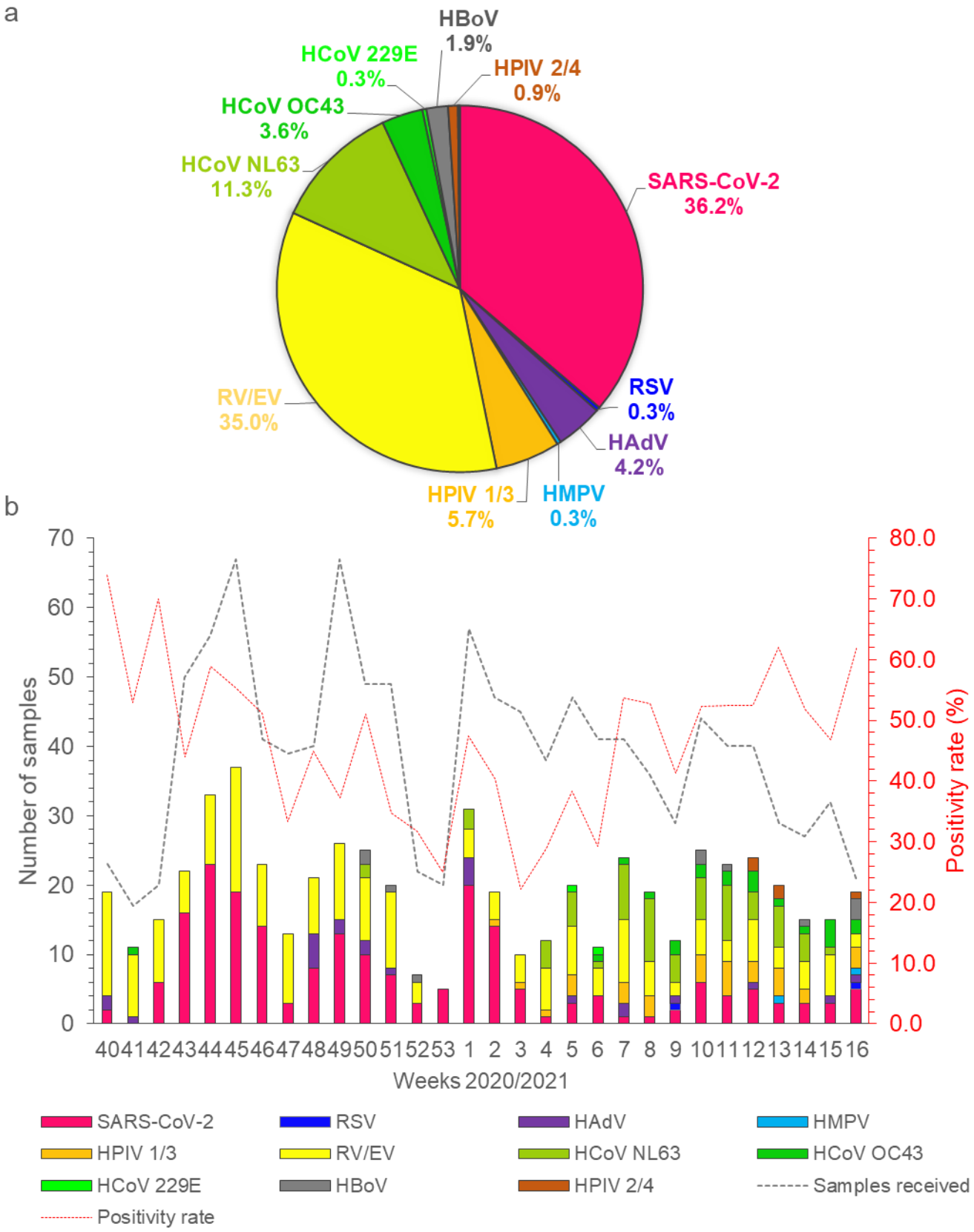


Figure 6 Percentage and temporal distribution of respiratory viruses detected in NPS collected from week 40/2020 to 16/2021. a. Percentages of the different respiratory viruses (n=577) detected in 538 NPS. **b**. Distribution of the samples tested and the detected pathogens throughout the surveillance period.
The influenza B isolated during week 6 is not shown in both panels, however it is included in the percentages and positivity rate calculation.

Table 2. Detailed description of the observed co-infections, N=39 (40/2020-16/2021)

Weeks	Co-infections						Total
40	RV/EV-HAdV	RV/EV-SARS-CoV2					2
41	RV/EV-HAdV	RV/EV-HCoV OC43					2
42	RV/EV-SARS-CoV2						1
46	RV/EV-SARS-CoV2	RV/EV-SARS-CoV2					2
48	RV/EV-HAdV	HAdV-SARS-CoV2	RV/EV-SARS-CoV2				3
49	RV/EV-HAdV						1
51	RV/EV-HAdV	RV/EV-SARS-CoV2	RV/EV-SARS-CoV2				3
1	RV/EV-HAdV	HAdV-SARS-CoV2	SARS-CoV2-HCoV NL63	RV/EV-SARS-CoV2			4
4	RV/EV-HPIV1/3						1
5	RV/EV-HCoV 229E	HAdV-SARS-CoV2					2
7	RV/EV-HPIV1/3	RV/EV-HCoV NL63					2
10	HCoV NL63-HPIV 1/3	HCoV NL63-HPIV 1/3					2
11	RV/EV-HCoV NL63	HPIV1/3-HCoV OC43					2
12	HAdV-HCoV NL63	SARS-CoV2-HCoV NL63	RV/EV-SARS-CoV2				3
13	SARS-CoV2-HPIV1/3	SARS-CoV2-HMPV					2
14	HBoV-HCoV NL63						1
16	RV/EV-HAdV	RV/EV-HBoV	HPIV1/3-HPIV2/4	SARS-CoV2-HBoV	SARS-CoV2-HBoV	SARS-CoV2-HPIV1/3	6

When stratifying positive samples by age groups, one could observe that a majority HPIV 1/3, HPIV 2/4, HAdV and HBoV were found in younger age groups (Figure 7a and b) particularly in the 0-4 year-old children were they represent 65.6%, 80%, 70.8% and 72.7%, respectively (Figure 7a). RSV and HMPV corresponded to 50% of the detected viruses in the 0-4 year-old group but only 2 samples were positive for each of those viruses. Not surprisingly, SARS-CoV2 positives samples were present in older age groups with 56.9% and 20.1% for 30-64 and ≥65 years old groups, respectively (Figure 7a). RV/EV, HCoV OC43 and HCoV NL63 positive samples were quite evenly detected throughout the age groups.

	IB	RSV	SARS-CoV2	HCoV NL63	HCoV OC43	HCoV E229	RV/EV	HPIV 1/3	HPIV 2/4	HMPV	HAdV	HBoV
≥65			42	7	3		16					
30-64	1		119	22	8	1	67	6		1		1
15-29			28	10	3		30				3	2
5-14		1	13	18	3	1	44	5	1		4	
0-4		1	7	8	4		45	21	4	1	17	8

Figure 7. Figure 7. Respiratory viruses distribution per age groups in percent (a) and in absolute numbers of positive samples (b).

All 177 influenza negative samples that were submitted to cell culture on MDCK and MDCK-SIAT cells were negative for influenza. Culture was performed under biosecurity level 2. Therefore, only samples that were also negative for SARS-CoV-2 were chosen. Of note, SARS-CoV-2 was shown not to replicate in MDCK and MDCK-SIAT cells[51,52].

5.2.2 Respiratory viruses retrospective screening (2019/2020)

From week 40/2019 to week 16/2020, 1130 NPS were received at the NRCI and, from those, 1118 were retrospectively tested for SARS-CoV-2, RSV, HCoV NL63-HKU1-OC43-229E, HPIV, HBoV, HAdV, RV/EV and HMPV. One hundred and two NPS collected from week 17/2020 to week 39/2020, all influenza and SARS-CoV-2 negative, were also retrospectively screened for the respiratory viruses listed above.

Among the 1220 samples tested from weeks 40/2019 to 39/2020, 819 were positive for at least one respiratory virus including influenza and SARS-CoV-2 (Figure 8). Seven hundred and eighty positive samples (69.8% positivity rate) were detected from week 40/2019 to week 16/2020, and only 39 (38.2% positivity rate) until week 39/2020.

Nine hundred and eight viruses were identified in total, a majority from week 49/2019 to week 12/2020 (Figure 8), which coincides with the start of a COVID-19 associated confinement period in Switzerland. The first confinement relief phase was initiated during week 18/2020, followed by the second and the third release phases during weeks 20/2020 and 24/2020, respectively. As a consequence, the number of samples received from weeks 12/2020 to 24/2020 and the following spring/summer period was very low, less than ten per week.

SARS-CoV-2 positive samples were identified at the NRCI from week 10/2020 to week 13/2020, with a peak during week 12/2020, and no additional positive samples until week 40/2020. Of note, the first SARS-CoV-2 positive sample identified in Switzerland, before the start of the COVID-19 epidemic in the country, was on February 25 2020 (week 09/2020). This was only one week before the identification of positive samples within the Sentinella network.

Influenza A and B viruses represented 55.8% of the total detected viruses (n=869), from week 40/2019 to week 16/2020, followed by RV/EV (13.1%), RSV (6.3%), HMPV (6.1%), HCoV HKU1 (4.9%), HAdV (4.7%), HCoV NL63 (2.9%), SARS-CoV-2 (2%), HCoV OC43 (1.4%) and HPIV 2/4 (1.2%) (Figure 9a). HCoV 229E, HBoV and HPIV 1/3 represented less than 1% of detected respiratory virus. An illustration excluding influenza A and B viruses is provided to offer an easier comparison between the 2019/2020 and the 2020/2021 surveillance periods (Figure 9b).

RV/EV positive samples were detected regularly starting week 40/2019 until week 12/2020. Then from weeks 23 to 27 and 32 onwards (Figure 8 and annex 1). Of note,

all positive samples collected from week 23/2020 to 39/2020 were RV/EV positive (Figure 8).

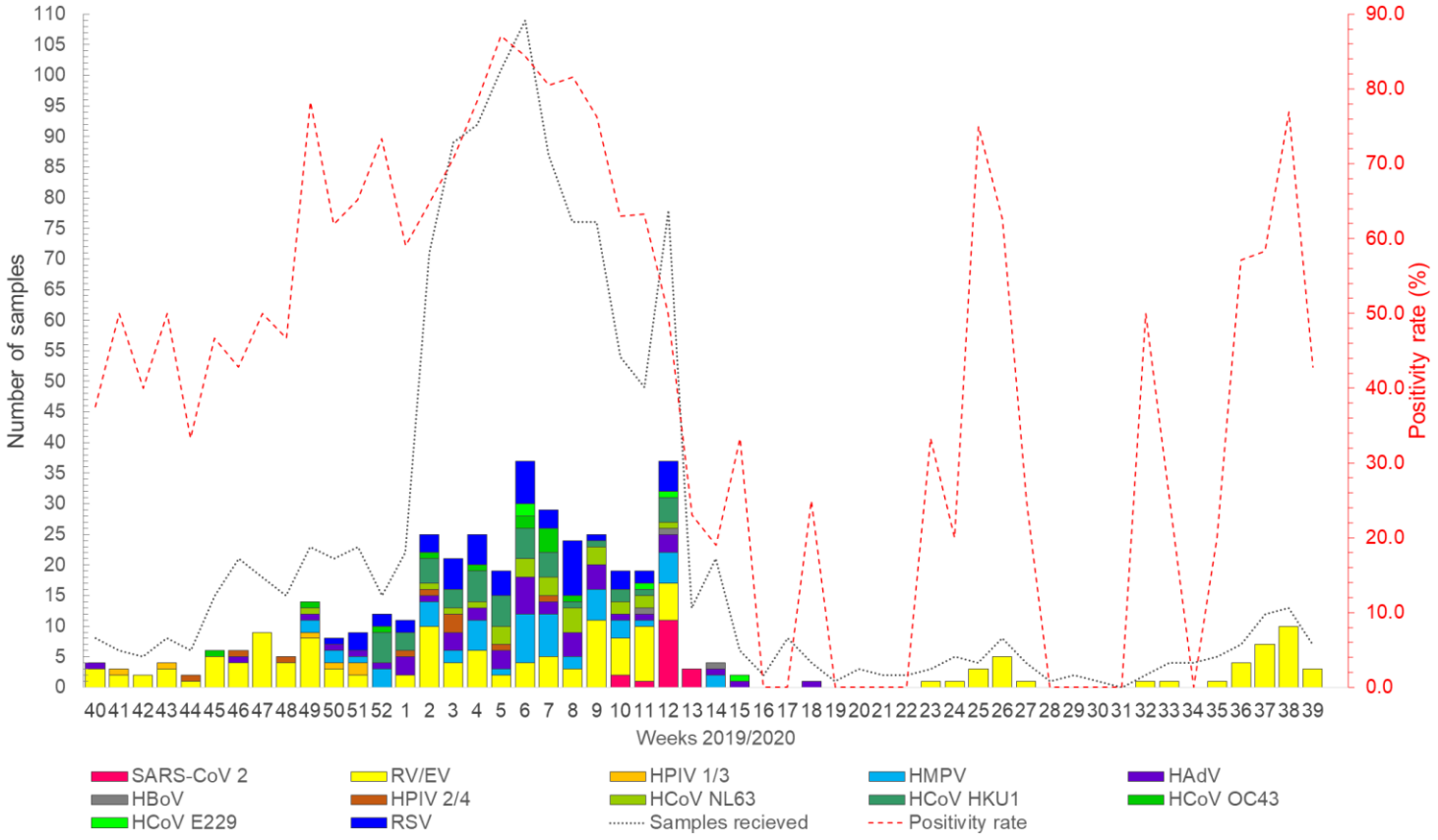


Figure 8. Temporal distribution of respiratory viruses detected in NPS collected from week 40/2019 to 39/2020. For best readability, data for influenza A and B is not plotted in this graph. The complete overview of viruses detected from week 40/2019 to week 16/2021 is shown in annex 1.

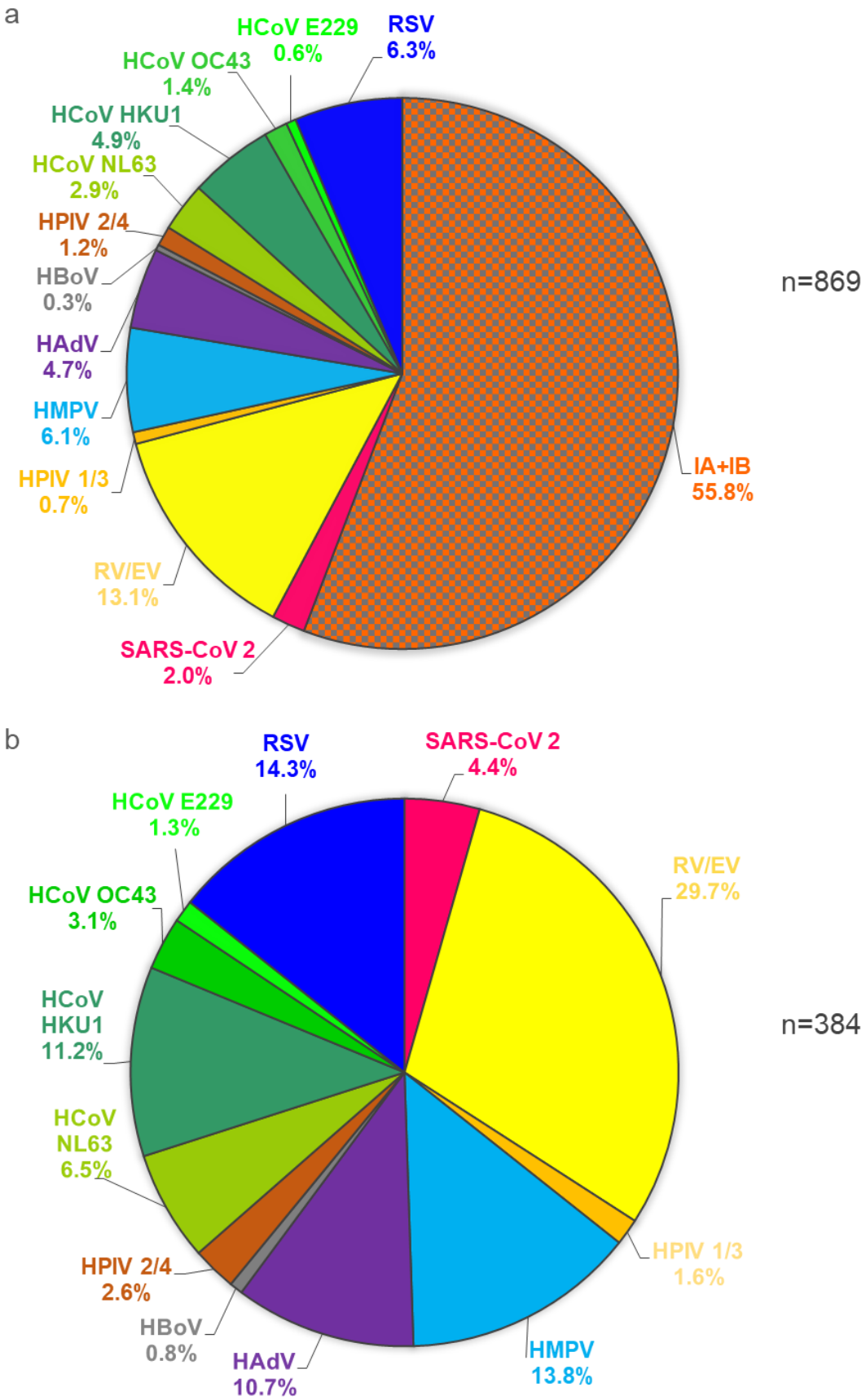


Figure 9. Proportion viruses detected from week 40/2019 to 16/2020. a. Including influenza A (IA) and B (IB). **b.** Excluding influenza A and B. This graph does not include the RV/EV viruses detected from week 23/2020 to week 39/2020 as they represented 100% of the viruses identified.

RSV, HMPV, HCoV, HKU1, HCoV NL63, HCoV OC43 and HAdV were consistently detected from week 49/2019 to week 12/2020.

Seventy nine (6.5%) co-infections were observed, all detected before week 15/2020 (Table 3). HAdV, H1N1pdm09, RV/EV and influenza B/Victoria/2/87-lineage (VIC) could be found in 35.4%, 34.2%, 25.3% and 22.8% of the total co-infections, respectively. Of the 17 SARS-CoV-2 viruses detected, two (11.8%) were associated with RV/EV or HMPV. Except for HBoV co-inflections that were all only found in infants and SARS-CoV-2 co-infections that were only found in adults, 30 and 32 years old, there was no significant difference in age-group distribution for the other co-infections. However there was a trend towards younger age groups. When present in co-infections, HAdV had often the highest Ct values, i.e. the lowest viral loads (>26 Ct, median of 34 Ct).

The median positivity rate for respiratory viruses, from week 40/2019 to week 16/2020, was 61.9% (95% [50,70.8]), with a peak (87.1%) during week 05/2020. Only two samples were received at week 16/2020 and both were negative. At week 6/2020, which corresponds to the peak of ILI consultations for 2019/2020 in Switzerland, the positivity rate was 84.4%, the second highest for this period (Figure 8). Highly variable positivity rates could be observed from week 16/2020 to 39/2020 mainly due to the very low number of samples received per week during this period.

Table 3 Detailed description of the observed co-infections, n=79 (40/2019-16/2020)

Weeks	Co-infections												Total
40	RV/EV-HAdV												1
41													
42													
43													
44													
45	H1N1-HCoV OC43												1
46													
47													
48													
49	RV/EV-HAdV												1
50													
51	VIC-RSV												1
52	HMPV-HCoV HKU1	HMPV-HCoV HKU1	HAdV-RSV	HCoV HKU1-HCoV OC43									4
1	VIC-HAdV	HAdV-RSV	HPIV 2/4-RSV										3
2	H3N2-RV/EV	H1N1-EV/RV-HMPV	VIC-HCoV HKU1										3
3	H1N1-HAdV-HCoV HKU1	H3N2-HAdV	H3N2-HCoV HKU1	H1N1-RSV	H1N1-RSV	RV/EV-HAdV-HCoV HKU1	RV/EV-RSV						7
4	VIC-HMPV	H1N1-HAdV-RSV	VIC-HCoV HKU1	RV/EV-HAdV	HMPV-HCoV HKU1								5
5	VIC-RV/EV	VIC-HAdV	H1N1-HAdV-RSV	H1N1-HAdV	H1N1-HPIV2/4	H1N1-HCoV NL63	H1N1-HCoV HKU1	H1N1-HCoV HKU1	H3N2-RSV				9
6	H1N1-RV/EV	VIC-HMPV-RSV	H1N1-HAdV	H1N1-HAdV	H1N1-HAdV	H3N2-HAdV	H1N1-HCoV NL63	H1N1-RSV	VIC-HCoV 229E	H1N1-HCoV229E	HMPV-HCoV HKU1-RSV	HCoV NL63-HCoV HKU1	12
7	H1N1-RV/EV-HCoV HKU1	H1N1-HMPV	H1N1-HAdV	VIC-HPIV2/4	H1N1-HCoV NL63	H1N1-HCoV NL63	H1N1-HCoV OC43	VIC-HCoV OC43-RSV	RV/EV-HCoV HKU1	HMPV-RSV			10
8	H3N2-RV/EV	VIC-HMPV	VIC-HAdV	VIC-HAdV	H1N1-RSV	HAdV-RSV	HAdV-RSV						7
9	VIC-RV/EV	VIC-RV/EV	VIC-RV/EV	H1N1-HAdV	RV/EV-HMPV	HCoV NL63-RSV	VIC-HAdV						7
10	SARS-CoV2-RV/EV	SARS-CoV2-HMPV	RV/EV-HAdV										3
11	RV/EV-HBoV	HAdV-HCoV NL63-HCoV 229E											2
12	RV/EV-HCoV HKU1	HAdV-HBoV											2
13													
14	HAdV-HBoV												1
15													
16													

Yellow boxes corresponds to triple infections

When stratifying positive samples by age groups, one could observe that, as expected, a majority of SARS-CoV-2-positive samples were found in adults. HBoV, HAdV, RSV were more frequent in children and adolescents, while RV/EV were similarly distributed among all age groups (Figure 10).

	IA/IB	RSV	SARS-CoV 2	HCoV NL63	HCoV HKU1	HCoV OC43	HCoV E229	RV/EV	HPIV 1/3	HPIV 2/4	HMPV	HAdV	HBoV
≥65	32	6	2	3	7			7	1	2	5		
30-64	184	12	13	11	20	9	2	46	2	5	20	7	
15-29	80	4	1	4	8	2	2	30		2	9	4	
5-14	138	12		4	6	1	1	12	1		13	13	
0-4	48	21	1	3	2			19	2	1	6	17	3

	IA/IB	RSV	SARS-CoV 2	HCoV NL63	HCoV HKU1	HCoV OC43	HCoV E229	RV/EV	HPIV 1/3	HPIV 2/4	HMPV	HAdV	HBoV
≥65								3					
30-64								16					
15-29								9					
5-14								8					
0-4								1				1	

Figure 10 Respiratory viruses distribution per age groups in percent. a. Samples collected from week 40/2019 to week 16/2020. **b.** Samples collected from week 17/2020 to week39/2020.

Detailed demographics, viral typing, antigenic and genetic characterization data on influenza A and B positive samples, analysed from week 40/2019 to week 16/2020, were already extensively discussed in the “Influenza virus surveillance in Switzerland, season 2019-2020” report. It was therefore not included in this report.

5.3 SARS-CoV-2 and influenza viruses characterisation (40/2020 to 16/2021)

5.3.1 SARS-CoV-2 variants identification and genetic analysis

SARS-CoV-2 positive samples identified from week 40/2020 to week 16/2021, with Ct values lower than 32, were further characterized by sequencing. The 154 sequenced samples, which were all submitted to GISAID except for three, fell into 34 distinct Pangolin[53] lineages (Annex 2). The most frequent, with more than ten isolates, were VOC Alpha (B1.1.7), B.1.177.44, B.1.177, B.1.160 and B.1.160.16 (Table 4). They represented, respectively, 17.5%, 12.9%, 9.1%, 8.4% and 7.8% of the of the sequenced samples.

Table 4. List of the different SARS-CoV-2 Pangolin lineages within which Sentinella isolates were distributed

Pangolin[7] lineages (VOC)	Number of isolates (n=164)	Pangolin lineages (VOC)	Number of isolates (n=164)
B.1.1.7 (Alpha)	27	B.1.160.32	1
B.1	3	B.1.160.9	2
B.1.1	1	B.1.177	14
B.1.1.232	2	B.1.177.23	6
B.1.1.317	1	B.1.177.43	4
B.1.1.39	6	B.1.177.44	20
B.1.1.519	2	B.1.177.51	1
B.1.1.70	2	B.1.177.81	1
B.1.149	1	B.1.177.86	1
B.1.160	13	B.1.221	8
B.1.160.10	1	B.1.258	5
B.1.160.12	1	B.1.258.17	3
B.1.160.14	8	B.1.367	1
B.1.160.15	1	B.1.406	1
B.1.160.16	12	B.1.416.1	1
B.1.160.22	1	B.1.474	1
B.1.160.26	1	C.36	1

The first two cases of VOC Alpha detected in Switzerland originated from the United Kingdom and were diagnosed short before Christmas 2020. As the sequencing time-to-results was over one week, and our national contact tracing strategy required variants' identification within 24 hours upon SARS-CoV-2 laboratory confirmation, all

[7] Web-based lineage assessment: https://github.com/cov-lineages/pangolin

SARS-CoV-2 positive cases with Ct value <32 were also screened using an rRT-PCR targeting the single nucleotide substitution N501Y (in Alpha, Beta and Gamma variants) in the spike gene (from week 01 to week 15/2021). The first Sentinella VOC Alpha case was detected in a sample on the 7th January 2021, and this variant became the most prevalent within the Sentinella surveillance starting February 2021. Forty eight samples in total were screened for the N501Y mutation and 21 were positive (48.5%).

A rRT-PCR targeting the spike single nucleotide substitution E484K (in beta, gamma, eta, iota and zeta variants) was also implemented starting week 05/2021. None of the 31 tested samples were E484K positive. The only case of VOC gamma identified at the NRCI so far was after week 16/2021.

5.3.2 Influenza circulation Worldwide

Despite the fact that in general few influenza positive samples were detected worldwide, some countries had influenza activity and could provide samples for characterisation to the WHO collaborating centres. Interestingly, both A(H1N1)pdm09 and A(H3N2) subtypes as well as B/Victoria/2/87 and B/Yamagata/16/88 lineages could be detected at variable rates. Data below originate from the February 2021 report from the London WHO Collaborating Centre at Francis Crick Worldwide Influenza Centre (WIC).

All A(H1N1)pdm09 viruses characterized by the WIC fell within subclade 6B.1A encoding the HA1 amino acid substitutions S74R, S164T, I295V and S183P in a majority of viruses, and predominantly in the genetic group 6B.1A5A. A high percentage (89%) of viruses examined using panels of post-infection ferret antisera remained antigenically similar to A/Guangdong-Maonan/SWL1536/2019, the 2020/2021 recommended vaccine virus. However, some viruses with the substitutions K130N, N156K, L161I, V250A in HA1 and E179D in HA2 were poorly recognised by the antiserum raised against A/Guangdong-Maonan/SWL1536/2019, but were recognised well by an antiserum raised against the 2021/2022 Northern hemisphere recommended vaccine strain A/Victoria/2570/2019.

The A(H3N2) viruses analysed by the WIC belonged to two main genetic subgroups, 3C.2a1b+T131K-A and 3C.2a1b+T135K-A, of subclade 3C.2a1b. They were generally poorly recognised by antisera raised against clade 3C.3a viruses. The two 3C.2a1b subgroups reacted best with respective homologous post-infection ferret antisera. Of

note, some viruses originating from Bangladesh and one from Norway reacted poorly with the most antisera used. Therefore the recommended 2021/2022 northern hemisphere influenza vaccine strain was updated to an A/Cambodia/e0826360/2020-like virus.

B/Victoria/2/87-lineage viruses predominated over B/Yamagata/16/88-lineage and all belonged to the clade 1A, subclade 1A(Δ3)B, characterized by HA1 deletion Δ162-164 and amino acid substitution K136E. A majority of the few viruses collected after August 2020, belonged to a subgroup named 1A.3-150K, itself subdivided in two subgroups with specific HA mutations. Antisera raised against the cell culture-propagated and egg-propagated cultivars of the vaccine strain B/Washington/02/2019 reacted only poorly with those “recent” viruses. However, a good reaction could be observed for 1A.3 HA 150K group viruses with post-vaccination human sera.

The few B/Yamagata/16/88-lineage viruses that could be analysed during 2020 fell within genetic clade 3, corresponding to the vaccine strain the B/Phuket/3073/2013 clade. Those samples also reacted well with antisera raised against clade 3 viruses.

None of the tested viruses shown a decreased susceptibility to oseltamivir, zanamivir and baloxavir marboxil. However very few samples were tested/analysed.

Currently in the Southern Hemisphere, notably in Australia[54], influenza activity remains at a historically low level. Influenza A accounted for 2/3 of the reports. Few virus were subtyped or attributed to a lineage in case of influenza B and even fewer were characterized antigenically. However when looking globally, influenza B viruses largely outnumber influenza A viruses, even if the number of detections remains quite low.[55]

6 WHO recommendation for the composition of influenza virus vaccines for the 2020/2021 influenza season

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

The vaccine strains recommended for the 2021/2022 northern hemisphere influenza vaccine by the WHO experts are:

Table 5. Recommended influenza vaccine composition for the 2021/2022 influenza season.
a: for egg-based vaccines. b: for cell-based vaccines

a	Virus strains
A(H1N1)pdm09	A/Victoria/2570/2019-like
A(H3N2)	A/Cambodia/e0826360/2020-like
B/Yamagata/16/1988 lineage	B/Phuket/3073/2013-like
B/Victoria/2/1987 lineage	B/Washington/02/2019 (B/Victoria lineage)-like*

**Only B strain included in the trivalent vaccine*

b	Virus strains
A(H1N1)pdm09	A/Wisconsin/588/2019-like
A(H3N2)	A/Cambodia/e0826360/2020-like
B/Yamagata/16/1988 lineage	B/Phuket/3073/2013-like
B/Victoria/2/1987 lineage	B/Washington/02/2019 (B/Victoria lineage)-like*

**Only B strain included in the trivalent vaccine*

7 Human infection with influenza viruses of zoonotic origin

Transmission of zoonotic influenza viruses to humans often leads to single individual or/and close contact limited infections. However widespread outbreaks and pandemics are also possible in the case of efficient human-to-human transmission. Recombination events between porcine/avian and human viruses due to concomitant circulation can drive human adaptation of zoonotic strains. In order to allow for 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 such as poultry, wild birds and farm pigs for the presence of the respective influenza strains.

7.1.1 Swine-to-human influenza virus transmission

Human infections with influenza A viruses of porcine origin are identified as “variant” viruses and denoted with a letter “v”, such as A(H1N2)v, A(H3N2)v and A(H1N1)v.

7.1.1.1 In Switzerland

In 2001, the Federal Food Safety and Veterinary Office initiated a collaborative project with the Federal Office of Public Health project, the Institute of Virology of the Vetsuisse Faculty of the University of Zurich and the Pig Health Service (SSP) of SUISAG, which aimed to monitor the swine flu circulation in Switzerland. The project is named “Surveillance of swine influenza in pigs and humans”. In this context, specimens from farm pigs with respiratory symptoms are sent to, and analysed by, the National Veterinarian Institute (Vetvir, Zurich). In parallel, samples from pig breeders (or their employees), who have been in contact with influenza-infected animals and present with ILI symptoms, are sent to the NRCI. The latter are analysed using a rRT-PCR with the capacity to distinguish influenza A viruses of human and animal origin, both avian and porcine. Positive samples are confirmed by sequencing.

No samples were sent to the NRCI during the 2020/2021 season for suspicion of zoonotic transmission.

7.1.1.2 Worldwide

Since 2010, 472 [432 A(H3N2)v, 13 A(H1N1)v and 26 A(H1N2)v] human cases of variant influenza have been reported in several states in the USA. These cases were often mild with no evidence of further human-to human transmission. Three cases of A(H1N1)v, one case of A(H1N2)v and one case of H3N2v were reported in 2020/2021. (https://gis.cdc.gov/grasp/fluview/Novel_Influenza.html)

In 2020/2021, A(H1N1)v infections were also reported from China, Netherlands and Denmark. The latter had an HA belonging to the 1A.3.3.2 H1N1pdm09-like lineage. This virus was antigenically distinct from the A/H1N1pdm09-like viruses typically found in humans.

On October 2020, Canada reported an A(H1N2)v infection in a child, whom had indirect contact with pigs.

7.1.2 Avian influenza A subtypes in humans[56]

As for porcine influenza, human cases of infection with various avian influenza viruses are sporadically reported. As of June 2021 and since 2003, a total of 862 laboratory-confirmed human cases of A(H5N1), including 455 deaths, have been reported from 17 countries. The last confirmed case was reported in October 2020 by the Lao People's Democratic Republic in a one year-old female who recovered. Five cases were reported by the Nigerian CDC in April 2021, but final confirmation is pending.

Since February 2014, 32 cases of highly pathogenic avian influenza (HPAI) A(H5N6), including 16 deaths, have been reported. The last case dates back to May 2021 and was reported by China.

Since February 2013, 1568 human cases and 616 deaths of A(H7N9) infections have been reported. All cases were of Chinese origin and most were isolated in China. Since 2017, HPAI A(H7N9) viruses were detected in 32 human cases as well as in several poultry and environmental samples. No human cases were reported since March 2019.

Since 1998, 88 laboratory-confirmed human cases of A(H9N2) infections, and one death, have been reported, mainly in mainland China. A couple of cases were found in Senegal, Pakistan, Oman, India, Cambodia, Bangladesh and Egypt. This influenza subtype is mainly found in young children, in whom it causes mild disease.

In February 2021, the Russian Federation reported the first seven human infections caused by A(H5N8). All cases were farm workers participating in a response operation to contain an avian influenza outbreak. The infected individuals remained asymptomatic and no sustained human-to-human transmission was observed.

On May 2021, the People's Republic of China reported the first A(H10N3) human infection ever detected for this subtype. Interestingly, but not surprisingly, the virus had internal genes that were closely related to influenza A(H9N2) viruses circulating in poultry in China during recent years.

8 Avian influenza A in animals[57]

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

In strong contrast with its human counterpart, the 2020/2021 avian influenza epidemic was of particular concern, as stated by the ECDC “with a total of 3,555 reported HPAI detections and around 22,400,000 affected poultry birds in 28 European Countries, 2020/2021, appears to be one of the largest and most devastating HPAI epidemics ever occurred in Europe”. Infections were detected both in poultry and wild birds. Both LPAI (A(H7N3, A(H5N3)) and HPAI A(H5Nx, one A(H7N7)) strains were observed. Not surprisingly, as influenza is known for its high reassortment potential, the different outbreaks were due to more than 15 different genotypes.

9 Discussion

A similar amount of samples were received at the NRCI during 2020/2021 and 2019/2020. This was somehow surprising as we were expecting a high increase in the number of swabs in response to the SARS-CoV-2 circulation and to the introduction of its diagnostic within the Sentinella' screening process. One of the reasons for this phenomenon may have been the decline in the participating practitioners agreeing to collect and send samples to the NRCI during 2020/2021. Then the difficulty faced by some doctors to receive the SARS-CoV-2 results in a timely manner may also have had a major impact on the choice of whether to send their samples to the NRCI or to a closer private laboratory. Indeed, even if, once in the laboratory, the NPS were processed within 6 hours on average, some parcels took more than 48 hours to arrive at the laboratory, making participation in the national surveillance less attractive in the context of the COVID-19 pandemic. It is difficult to explain such unexpected delay in the national mail delivery, which was not correlated with the distance to be covered.

The sex and age distribution, the sampling time after first symptoms onset and influenza vaccination rate of the tested individuals were comparable to previous years.

Week 40/2020 to week 16/2021 median positivity rate was lower (49.2%) than during 2019/2020 (61.9%). We would have expected it to be higher if influenza and RSV had been present as usual.

For the first time since the beginning of the influenza monitoring in Switzerland, in 1986, there were only a few sporadic influenza detections within the Swiss mandatory reporting system and only one influenza B virus was identified in samples analysed in the context of the Sentinella surveillance. This lack of influenza circulation was a global phenomenon already observed during the 2020 Southern Hemisphere influenza surveillance.[58] The remarkable decrease in influenza activity, that is still being observed at week 26/2021 in the Southern Hemisphere, is most likely multifactorial. Possible explanations may be reduced testing for influenza due to reagents shortages and/or screening procedures prioritizing SARS-CoV-2 screening, introduction of personal protection measures, stringent hand hygiene, social distancing, as well as movement and travel limitations/bans instituted in several countries worldwide.[59] SARS-CoV-2 interference with influenza can also be hypothesized as a cause for reduced influenza circulation.[59,60] Of note, as mentioned previously, the number of swabs tested within the sentinel surveillance during 2020/2021 was akin to previous

seasons and all samples tested for SARS-CoV-2 were also tested for influenza. Thus reduced testing likely does not explain the lack of influenza activity in Switzerland. Consistently, the influenza testing within the GISRS was also comparable to previous years.

Influenza scarcity during the COVID-19 pandemic was a relief, as concomitant circulation of two viruses, which have a major public health impact, may have worsened the already substantial hospitalization and death burden. Nevertheless, this beneficial situation raises the concern that the proportion of influenza susceptible individuals may increase in the population presaging a potential “higher than normal” number of cases for 2021/2022 influenza season. Indeed, people that would have had their immunity boosted by natural infection were not exposed to the virus. In addition, even if vaccination rates against influenza may have been higher than usual in adults in some countries[61] during 2020/2021, they are overall far from being good.

As already mentioned, rRT-PCR detection data on respiratory viruses other than influenza is only available since 2019/2020 within the Sentinella network, and the emergence of SARS-CoV-2 was shown to significantly impact the circulation of other respiratory viruses.[62] Therefore caution has to be taken when comparing 2019/2020 and 2020/2021 data.

As for influenza, RSV circulation was unusual during 2020/2021. Indeed, when compared to 2019/2020, during which the virus was present from weeks 50/2019 to 12/2020, RSV detection was delayed by around 20 weeks; starting to increase after week 16/2021. Indeed, from week 17/2021 up to week 26/2021, 172 samples were tested at the NRCI, 9.9% (n=17) being RSV positive. Reduced and delayed RSV circulation was also observed in other countries.[63-66]. Before COVID-19 emergence, RSV was shown to alternate between low prevalence directly followed by high prevalence annual epidemics in Switzerland[67].

Lower than usual RSV circulation means reduced and/or delayed exposure to the virus for infants that would normally have been infected. We may therefore expect an increased RSV attack rate during the next sustained circulation period. We can also hypothesize that a higher proportion of older infants will be infected.

As for RSV, HMPV detections were rare from week 40/2020 to week 16/2021, while this virus accounted for 6.1% of the positive samples in 201/2020. Its positivity rate

slowly increased from week 17/2021 onwards to reach 4.1% (n=7) at week 26/2021. We cannot give an explanation for delayed RSV and HMPV circulation. However it is interesting to stress that both belong to de same family. To be mentioned, HMPV was shown, in some studies[68,69], to have biannual circulation including in Switzerland.

SARS-CoV-2 viruses were regularly detected from week 40/2020 to week 16/2021 and represented, with RV/EV, the most prevalent virus until week 3/2021. Then other respiratory viruses became more abundant. Only a few cases (17) of SARS-CoV-2 were detected within the sentinella network until week 40/2020. In general, an increase in detection at the NRCI fit well with an increase in the number of cases identified in the Swiss population (annex 1). Consistently, the detection rate of the SARS-CoV-2 Alpha variant, that became predominant end February 2021 in the Swiss population, matched that of the sentinel population as shown by the data of the national SARS-CoV-2 genomic surveillance.[70] A continuous update of SARS-CoV-2 variants evolution in Switzerland can be found on CoV-Spectrum[71] (https://cov-spectrum.ethz.ch/explore/Switzerland/AllSamples/AllTimes). The current increased detection of the SARS-CoV-2 Delta variant coincides with a decrease in the proportion of the Alpha variant in Switzerland. The Delta variant is known to be more transmissible[72] than Alpha, which it already replaced in several European countries within only weeks after its first detection. While in vitro studies show a reduced neutralization capacity from vaccinated sera[73], recent real-life data indicate that SARS-CoV-2 mRNA vaccines available in Switzerland keep a good protection against symptomatic and severe disease caused by the Delta variant, with a slight diminution of protection against infection[74]. Knowing the actual epidemiological situation in Switzerland and that only about 44.8% of the population is fully vaccinated, a new wave of SARS-CoV-2 infections associated with the Delta variant is plausible.

RV/EV were the second most commonly detected viruses after influenza and SARS-CoV-2 during 2019/2020 and 2020/2021 respectively. This is consistent with data already published for in- and out- patients in Geneva.[75] As expected RV/EV detection was regular throughout the year with some gaps between week 12/2020 and week 31/2020, but the number of samples received during this period was also very low. From week 17/2021 to week 26/2021, 38 (22.1%) out of 172 samples tested positive for RV/EV. This corresponds to all the RV/EV detected from week 17/2020 to week 39/2020. Of note, there was about 2.5-fold increase in RV/EV positive samples in

2020/2021 compared to 2019/2020, perhaps due to the absence of influenza interference[76].

HCoVs HKU1/NL63/OC43 and HCoVs NL63/OC43 were present during both 2019/2020 and 2020/2021 respectively. Of note, only one HCoV, HKU1, was detected as late as week 20/2021, while it represented 4.9% of the virus identified during 2019/2020. HCoV 229E circulated at very low numbers during both surveillance periods. Despite the fact that HCoVs tend to exhibit seasonal circulation, with peaks mostly in winter in Northern Hemisphere, the prevalence pattern of each HCoV strains varies in-between countries and from year to year[77,78].

While they were only sporadically detected from week 40/2019 to week 51/2019, HPIV 1/3 positive samples were regularly found from week 2/2021 to week 26/2021 with a peak in March 2021. As our rRT-PCR does not differentiate type 1 and 3, the discrepancy in prevalence may potentially be due to alternate circulation of different viruses types as it has been shown in Autralia[79] and USA.[80] HPIV 2/4 were not commonly detected in general for both 2019/2020 and 2020/2021 periods. Of note, we observed a reduced sensitivity, specific to the HPIV 4 target, for one of the HBoV-HPIV 2/4 mix lot we used at the beginning of the 2020/2021 monitoring period. However, we estimate that this event had only a moderate impact on the HPIV 4 detection rate. HBoV and HPIV 2, which were detected using the same mix, were not impacted at all.

HBoV did not have a high prevalence during both study periods. As could be expected, they were mainly found in children.[81]

Co-infections represented 7.2% and 6.5% of the detections in 2020/2021 and 2019/2020 respectively. This observation is consistent with previous publications.[82,83] The rRT-PCR respiratory panel used at the NRCI does not target bacterial or fungal pathogens, however the latter are also often detected along with respiratory viruses, particularly in hospitalized patients.[83] The most common virus found in co-infections for both surveillance periods was RV/EV, SARS-CoV-2 was second during 2020/2021, while and influenza A and HAdV were second duringand 2019/2020. This finding was also very similar to what Lansbury et al[83] found, except that we did not detect SARS-CoV-2 co-infections with other HCoVs as much. A high SARS-CoV-2 co-infection rate was also observed in France[84] in 2020.

Taking into account the low number of influenza isolates that could be recovered and successfully characterized, 2020/2021 was a difficult period for the selection of future influenza vaccine candidate strains. Nevertheless, the A(H1N1)pdm09 and the A(H3N2) vaccine components were updated for the Northern Hemisphere 2021/2022 formula.

Even if few zoonotic influenza transmissions were observed, first-time in human infections with avian A(H5N8) and A(H10N3) subtypes were detected. Both viruses were ranked “at low pandemic risk” by the WHO. However such events justify the extensive and continuous surveillance of zoonotic influenza infections.

As expected, and in strong contrast with the situation observed in humans, influenza outbreaks in domestic and wild birds remained frequent during 2020/2021. They are a major economic burden for poultry farming and an increased risk for zoonotic transmissions.

It is currently impossible to draw a “most plausible” scenario for 2021/2022 influenza season. However, if COVID-19 pandemic associated protective measures are lowered, one may expect that influenza viruses will start to re-circulate. They may also not “come back” but this is very unlikely. The worst case scenario would be if a new A subtype, against which the human population lacks immunity, takes the almost vacant spot left by seasonal strains. We would potentially face a new pandemic concomitantly with SARS-CoV-2 VOC infections.

10 Other activities of the NRCI

10.1 Validation and/or evaluation of assays

10.1.1 Validation of the respiratory panel (NCRI version) on the Quantstudio 7 Pro instrument

In order to rationalize the batch testing of samples received at the NRCI for the respiratory viruses panel testing that is part of our current weekly surveillance, as well as to be able to rapidly upscale our testing capacity in case of an influenza pandemic, we acquired the only thermocycler in our laboratory that is able to perform both conventional 96 wells but and 384 wells rRT-PCR. It is routinely working in 384-well mode.

The respiratory panel we used during the 2020/2021 was previously validated by the hospital's laboratory of virology for the routine screening of hospitalised patients. However, it was intended to be performed on a Quantstudio 5 (96 wells) instrument (Thermofisher). Therefore we had to ensure that the performances of the respiratory panel were at least equivalent to the routine ones when run on the Quanstudio 7 Pro instrument (Thermofisher).

We used 56 well characterised samples for the performance testing. Nineteen negative samples for RSV, RV/EV, HAdV, HMPV, HCoV HKU1-E229-NL63-OC43, HBoV, HPIV1 and influenza A and B. Twenty nine positive samples for at least one of the panel targets and 8 positive samples that were 10-fold serially diluted (cycle threshold 17-complete detection lose).

All the samples run on the Quanstudio 7 Pro instrument exhibited the expected results. The assay performances were comparable to that of the routine method. As for its Quantstudio 5 counterpart, the detection limit for a specific positive result on the Quanstudio 7 Pro was set at <37 Ct for all components of the panel. The detailed validation plan and report are available upon request (in French).

Of note, the canine distemper virus (CDV) is used as an internal control. It is part of the RVS and HAdV duplex detection mixes. A defined volume of CDV (full virus) is spiked in all test samples prior RNA extraction and serves it serves as an extraction and rRT-PCR inhibition control.

In our current screening configuration, we could test 46 individuals for 7 to 8 different mixes and one negative and one positive rRT-PCR per mix. Results are automatically

transferred to the UNILAB2 database and final validation is performed by the NRCI technician upon double check by the NRCI technical manager.

For cost/effectiveness reasons, the Quanstudio 7 Pro/384 wells version of the respiratory panel is run when more than 10 samples need to be tested. Otherwise the Quanstudio 5/96 wells version is preferred.

Even if not used for the moment, the possibility of loading commercial or custom card array assays on the Quanstudio 7 Pro instrument offers room for future developments.

10.1.2 Qualification of Microlab Star^{let} automatic pipetting system from Hamilton

In line with the batch testing of samples and the analytic upscaling of our testing capacity mentioned previously, the laboratory of virology, together with the NRCI, acquired a high-throughput automatic pipetting system in order to replace manual pipetting of 384 well plates and provide assistance when multiple 96-well plates were to be prepared. Upon evaluation of the specifications, consumables accessibility and order-to-supply time, as well as the machine availability, the Microlab Star^{let} from Hamilton was chosen. This machine can be fully customized to accommodate the user needs. Several different programs can be set up. However, new runs can only be programed by Hamilton technicians.

Due to COVID-19 linked constraints, two programs were prioritized and a qualification was performed for each of those. One program allowed the automatic pipetting of the 7-8 mixes of the respiratory panel and extracted up to 46 RNA samples from 1.5 ml microtubes to a 384-well rRT-PCR plate. The second program was meant to transfer 96 Nimbus deepwells, consisting of 94 RNA extracted samples as well as one positive and one negative control, into a 96-well rRT-PCR plate. For the latter program, one single mix was assigned for the whole plate. For both assays the RNA and mixes input volumes corresponded to 5µl and 15µl, respectively.

For each program, the pipetting precision was assessed first by using coloured water (Figure 11). Then the performance of each automatic pipetting process was compared to the manual pipetting equivalent using well characterized samples. Some high Ct values samples were chosen in order to address cross-well contamination during the pipetting process. The assay chosen for the qualification of the “tube-to-384 wells rRT-PCR plates” pipetting was the Quantstudio 7 Pro/384-well version of the respiratory

panel using 30 negative and positive samples. While the detection of the SARS-CoV-2 501Y single nucleotide polymorphism was chosen for the qualification of the “96 deepwells-to-96 wells rRT-PCR plates” pipetting. Ninety five samples Ct value <32 Ct were used.

	Sample	1	2	3	4
A	1	a	c	e	g
B		b	d	f	vide
C	2	a	c	e	g
D		b	d	f	vide
E	3	a	c	e	g
F		b	d	f	vide
G	4	a	c	e	g
H		b	d	f	vide
I	5	a	c	e	g
J		b	d	f	vide
L	6	a	c	e	g
L		b	d	f	vide
M	7	a	c	e	g
N		b	d	f	vide
O	8	a	c	e	g
P		b	d	f	vide

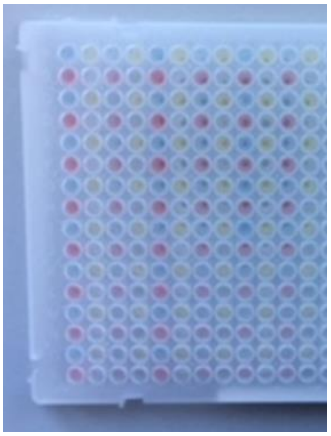


Figure 11. The programmed layout is shown on the left and the Microlab Star^{let} pipetting on the right.

Both automatic pipetting programs gave equivalent performances when compared to manual pipetting. The first program is used routinely at the NRCI in the context of the respiratory viruses panel screening. The detailed qualification reports are available upon request (in French).

Due to the good efficiency of this machine, further programs will be set up in a near future.

10.1.3 Qualification of Varioskan^{TM}

For patients and personal data protection, the University Hospital of Geneva follows strict IT rules and is constantly maintaining and updating the network. Inexorably, some IT updates result in a total and permanent incompatibility of some, often old, machines with the new IT strategy. This was the case for two microplate readers used by the NRCI, the Fluoroskan Ascent® FL (Thermofisher) and the Opsis MR^{TM} (Dynex). It was therefore decided to look for a single instrument that will be equipped with both reading modes. The Varioskan^{TM} LUX multimode microplate reader (Thermofisher) was the chosen option. Both reading modes were qualified by comparison to the already validated machines. Of note the same plate was read on both the new and the already qualified instrument.

Influenza A(H3N2) microtitration/miconeutralisation assays used to be performed using the the Opsis MR™ as plate reader. Therefore a panel of influenza A and B reference strains was used for the qualification of the absorbance on the Varioskan™ LUX. The results obtained with both instruments were similar and corresponded to the expected reference results.

The Fluoroskan Ascent® FL was used in the context of the phenotypic assessment of neuraminidase inhibitors reduced susceptibility (NA-Fluor™ Influenza Neuraminidase Assay kit, Invitrogen™). Therefore its qualification was performed using influenza A and B strains of the CDC Neuraminidase Susceptibility Reference Virus Panel (version 2) in the presence of either oseltamivir and zanamivir. The results obtained with both instruments were similar and corresponded to the expected reference results. The detailed qualification report is available upon request (in French).

10.1.4 Whole genome sequencing implementation at the NRCI

In 2020, the National Influenza Centre of Influenza collaborated with Microsynth AG in order to set up whole genome sequencing (WGS) protocols for Influenza A and B viruses, as well as for SARS-CoV-2. The latter was prioritized for obvious public health reasons and is now fully functional. Meanwhile a collaborative sequencing project was initiated by the laboratory of virology of the University Hospitals of Geneva (HUG) and the NRCI with the Genome Center in Geneva. This collaboration offers an additional whole genome sequencing platform for SARS-CoV-2, which also is easy-to-use and has a time-to-results shorter than using Microsynth AG's service. A third sequencing platform located at the HUG can also be used by the NRCI but is focused on unbiased sequencing for diagnostic purposes (e.g. a case of zoonotic influenza).

10.1.4.1 Whole genome sequencing for influenza A and B[8]

Sequential protocol optimisations were tried by Microsynth AG in order to implement whole genome sequencing (WGS) for influenza. As for SARS-CoV-2, a biased approach with a specific pre-amplification of influenza genes was chosen. Wüthrich et al[85] had tested two protocols for influenza A (IA) sequencing and came to the

[8]The 10.1.4.1 section (amplification and sequencing steps) was written using to the information and figures 12,13 and 14 kindly provided by Dr Christoph Grünig from Microsynth.

conclusion that the protocol of Zhou et al[86] works the best. This protocol uses a mix of two forward primers and a single reverse primer located in the conserved regions of the viral genome segments. For influenza B (IB) the protocol described in Zhou et al[87], including a primer cocktail of 13 different forward and reverse primers, was used. Libraries were prepared using Illumina Nextera kits and were then run on the Illumina platform MiSeq using ≥2*150 reads. Data was quality-filtered and de-multiplexed by Microsynth AG before being sent to the NRCI for sequence analysis.

For the very first trial, 16 influenza A and B RNA samples (Table 6), all extracted using the eMAG® (Biomerieux) method, were chosen. The results showed that samples with Ct values >25 only weakly amplified, or no amplification products were observed (Table 6, figure 12). Of note, the segments 4 (HA) and 6 (NA) of sample 7827 (27.2 Ct), the only ones we usually sequence in Sanger for epidemiological purposes, are complete and of good quality (data not shown). In order to try to improve the sequencing sensitivity, additional tests were run by increasing the number of one-step RT-PCR cycles from 35 to 50 for IA, and 45 to 50 for IB. Moreover, a PCR re-amplification of the one-step RT-PCR was tested. However, no visible improvements in the amplification were observed for samples with Ct values >25. This finding was in accordance with the results of Wüthrich et al.[85], who also showed that samples with low viral load perform poorly. Therefore, the PCR cycle numbers were fixed at 35 for IA and 45 cycles for IB, respectively.

Table 6. Influenza samples used for the first WGS trial. Green: full sequences for all segments, yellow: partial sequences for at least one segment, red: all/most segments 'sequencing failed

Sample ID	Ct	Type	Seg1	Seg2	Seg3	Seg4	Seg5	Seg6	Seg7	Seg8
6176	13.2	H1N1 2009	✓	✓	✓	✓	✓	✓	✓	✓
4512	16.2	H3N2	✓	✓	✓	✓	✓	✓	✓	✓
6915	22	H1N1 2009	✓	✓	✓	✓	✓	✓	✓	✓
1248	25.2	H3N2	✓	✓	✓	✓	✓	✓	✓	✓
7949	28.1	H1N1 2009	x	x	x	x	x	x	x	x
1202	31.5	H3N2	x	x	x	/	x	x	/	x
6689	33.8	H1N1 2009	x	x	x	x	x	x	x	x
5769	34.5	H3N2	x	x	x	x	x	x	x	x
2048	16.2	YAM	✓	✓	✓	✓	✓	✓	✓	✓
5774	19.2	VIC	✓	✓	✓	✓	✓	✓	✓	✓
226	25.5	YAM	✓	✓	✓	✓	✓	✓	✓	✓
8585	25	VIC	✓	✓	✓	✓	/	✓	/	✓
7827	27.2	YAM	/	/	/	✓	/	✓	✓	/
7357	30.3	VIC	/	/	x	/	x	/	x	/
8489	33.4	YAM	x	x	x	x	x	x	x	x
3615	35	VIC	x	x	x	x	x	x	x	x

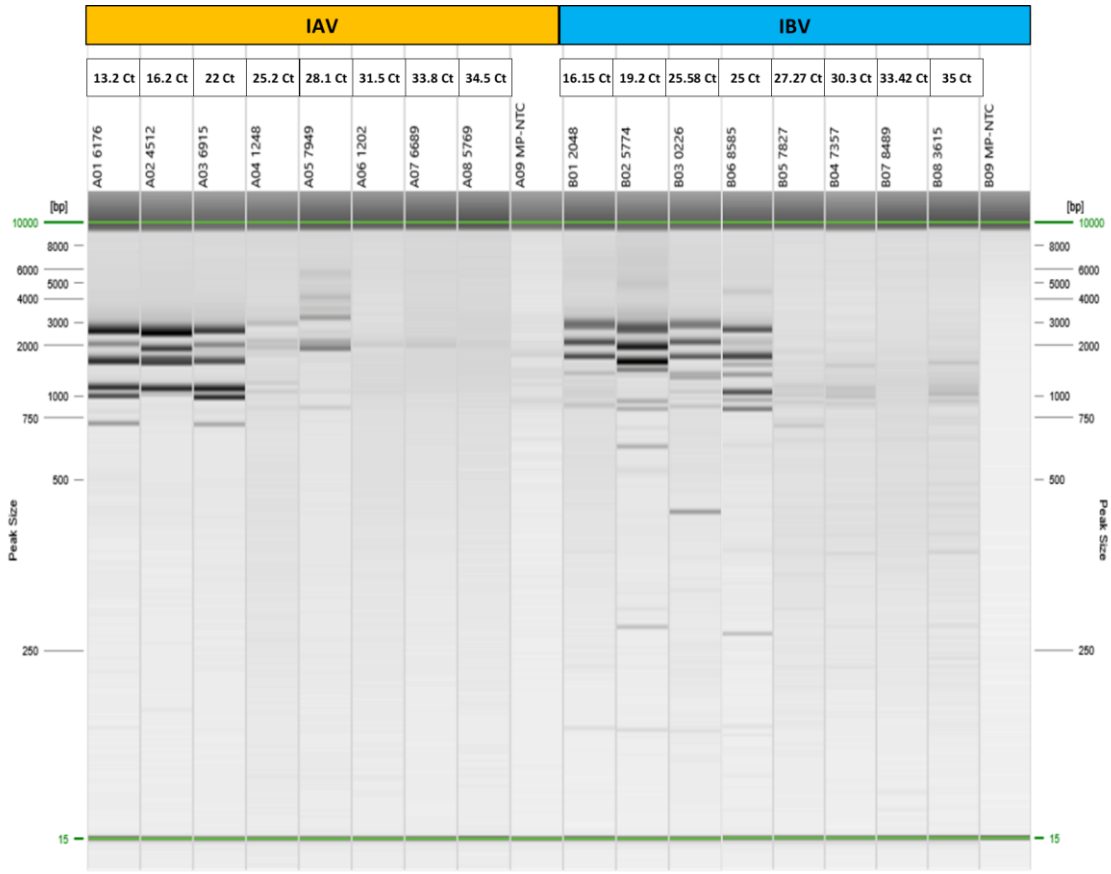


Figure 12. QIAxcel analysis of influenza A and B included in the first WGS trial.

The chosen protocols were then applied to 29 IB (both lineages) and 52 IA (both subtypes) samples with Ct values <25.5 (Table 7). Whereas the majority of samples resulted in complete or near complete sequences, some showed fragmented genomic sequences despite high viral load. Most of the samples that showed fragmented genomic information did not show a visible PCR amplification (Figure 13). Interestingly, in IB it was mostly the shortest PCR products that were absent or not amplified whereas for IA it was mostly the longest PCR products that failed to amplify. As the RNA quality can affect the PCR amplification of longer RNA fragments, some of the RNA samples used were subjected to Bioanalyzer analysis (Figure 14). However, no obvious pattern was observed that could explain the results of the sequencing. Therefore, it is most probable that a combination of inhibition and RNA quality results in the incomplete sequence information.

Table 7. List of samples used for the second WGS run. 81 influenza A and B samples were batched. Failed sequences are in red and incomplete in yellow.

ID	Type	Ct	A-SEG-1	A-SEG-2	A-SEG-3	A-SEG-4	A-SEG-5	A-SEG-6	A-SEG-7	A-SEG-8	B-SEG-1	B-SEG-2	B-SEG-3	B-SEG-4	B-SEG-5	B-SEG-6	B-SEG-7	B-SEG-8
5139	H1N1	20.4	2310	2337	2010	1740	1540	1416	1022	880								
5197	IB	23.9									2346	2373	2280	1854	1805	1536	1184	1080
6149	H1N1	23.4	2310	2323	2010	1747	1540	1429	1022	880								
6496	H1N1	23.5					1439	1172	1013	880								
6651	IB	22									2346	2366	2280	1855	1805	1536	1185	1061
6707	IB	22									2346	2373	2280	1853	1788	1536	870	898
6807	H3N2	20.4	2337	2337	2030	1762	1540	1461	1022	880								
7139	H3N2	19.7	2312	2337	2030	1758	1530	1443	1022	848								
7721	H3N2	22.4	2302	2300	2006	1738	1526	1395	1022	833								
8020	H1N1	16.3	2310	2337	2010	1747	1540	1417	1022	880								
9111	IB	23.3									2346	2373	2280	1864	1785	1536	1125	893
12712	H1N1	19	2310	2277	2010	1745	1561	1416	1022	880								
13278	H3N2	23.7	2310	2337	2028	1750	1540	1422	1013	816								
13428	H1N1	15.5	2302	2337	2010	1738	1563	1422	1022	880								
13496	H1N1	23					1329	826	1002	821								
13665	IB	18.8									2346	2373	2280	1864	1805	1536	1188	1042
17004	IB	20.1									2346	2373	2280	1854	1805	1536	1185	1100
17041	H3N2	19.7	2336	2337	2030	1761	1540	1461	1022	880								
17098	H1N1	19.2	2310	2325	2010	1745	1540	1429	1022	880								
17172	H3N2	21.1	2312	2337	2030	1762	1547	1447	1022	831								
17281	H1N1	19.7	2337	2337	2030	1758	1538	1461	1022	880								
18506	IB	18									2346	2373	2280	1855	1816	1506	1188	1100
18755	H1N1	21.9	2310	2337	2010	1751	1540	1417	1022	880								
18873	IB	23.3									2346	2373	2280	1864	1805	1536	1159	1046
25821	IB	16.3									2346	2373	2260	1855	1805	1504	1188	1061
25836	IB	18									2346	2373	2280	1854	1807	1536	1188	1049
25957	H1N1	21.1	2310	2326	2010	1740	1540	1429	1022	880								
28482	H3N2	25.4	2295	2287	2021	1723	1529	1384	1020									
28628	H1N1	22.2	2169	2106	1798	1673	1513	1370	1019	880								
28708	IB	22.4									2346	2373	2280	1864	1805	1536	1188	1017
30464	H3N2	18.4	2337	2337	2030	1758	1540	1461	1022	880								
32561	H3N2	25.4	2158	2145	1624	1674	1483	1380	1000	780								
33047	H3N2	21.7	2312	2337	2030	1762	1530	1443	1013	880								
33105	IB	19.6									2346	2366	2279	1852	1796	1536	1159	1021
34150	IB	19.4									2346	2373	2259	1855	1805	1536	1188	987
34242	IB	21.2									2346	2373	2250	1855	1800	1536		
34616	H1N1	21.4	2310	2337	2010	1735	1538	1417	1022	880								
34684	H3N2	23.9	2310	2296	2026	1750	1524	1418	1022	854								
41304	H1N1	21.9	2310	2337	2010	1745	1562	1416	1022	880								
45637	H1N1	22.1	2300	2337	2010	1739	1540	1429	1022	880								
46283	IB	21.9									2331	2373	2280	1853	1797	1536	1162	958
49795	H1N1	21																
50405	H1N1	25.3						1270	998	860								
53409	IB	24.3									2346	2373	2280	1855	1805	1536	1137	927
55926	H1N1	24	2307	2269	2003	1737	1560	1396	1022	880								
55991	H3N2	24.7	2300	2316	2001	1747	1534	1285	1022	811								
56518	H1N1	21.6	2310	2337	2010	1746	1532	1421	1022	880								
57204	H3N2	18.7	2312	2337	2030	1758	1551	1461	1022	831								
63783	H1N1	22.4	2310	2315	2010	1739	1539	1424	1022	880								
63805	IB	20.6									2346	2373	2280	1864	1797	1536	1174	928
63984	H3N2	24.1	2336	2337	2030	1761	1540	1457	1022	846								
67956	IB	18									2346	2373	2280	1864	1805	1536	1188	1047
67971	IB	20.4									2346	2373	2280	1855	1800	1536		
68297	H3N2	22.3	2312	2337	2030	1758	1530	1443	1022	818								
68430	H1N1	25.3	2310	2337	2010	1750	1540	1413	1022	880								
75805	H1N1	23.1	2310	2324	2010	1749	1540	1429	1022	880								
76206	H3N2	19.1	2312	2337	2030	1762	1535	1444	1022	828								
76329	IB	17.8									2346	2373	2280	1855	1805	1536	1185	1056
76337	IB	24									2346	2373	2280	1864	1795	1536	1178	1005
76507	H1N1	22.2	2300	2335	2010	1737	1530	1422	1022	880								
76529	H3N2	17.7	2337	2337	2030	1758	1540	1443	1022	880								
76655	IB	23.2									2346	2373	2257	1855	1805	1536	1178	1055
77603	H1N1	23.6	2310	2337	2010	1747	1540	1429	1022	880								
77746	H1N1	16.5	2310	2337	2010	1748	1563	1424	1022	880								
77928	IB	23.9									2346	2373	2247	1842	1779	1536	1090	881
77929	H3N2	21.4	2336	2337	2030	1758	1540	1461	1022	827								
78159	H1N1	20.6	2300	2337	2010	1746	1563	1422	1022	880								
79887	H1N1	24			1805	1668	1488	1296	1001	880								
79947	H3N2	21	2312	2337	2030	1758	1532	1443	1022	880								
81184	IB	22.5									2346	2373	2250	1854	1805	1536	1180	953
86569	IB	18.8									2346	2373	2280	1855	1805	1536	1178	1100
87152	IB	20									2346	2373	2280	1855	1805	1536	1188	1100
87176	IB	22									2346	2373	2280	1852	1795	1536	1185	1041
87417	H1N1	23.2	2295	2337	2003	1739	1539	1422	1022	880								
87552	H3N2	22.1	2310	2332	2026	1697	1532	1355	1006	841								
87577	H1N1	20.3	2310	2337	2010	1748	1540	1429	1022	880								
94001	H3N2	22	2312	2337	2030	1758	1547	1461	1022	831								
94068	H1N1	21.7	2310	2337	2010	1741	1540	1429	1022	880								
94693	IB	18.1									2346	2373	2280	1855	1805	1536	1188	1006
95129	IB	22.9									2346	2327	2237	1836	1782	1536	1142	924

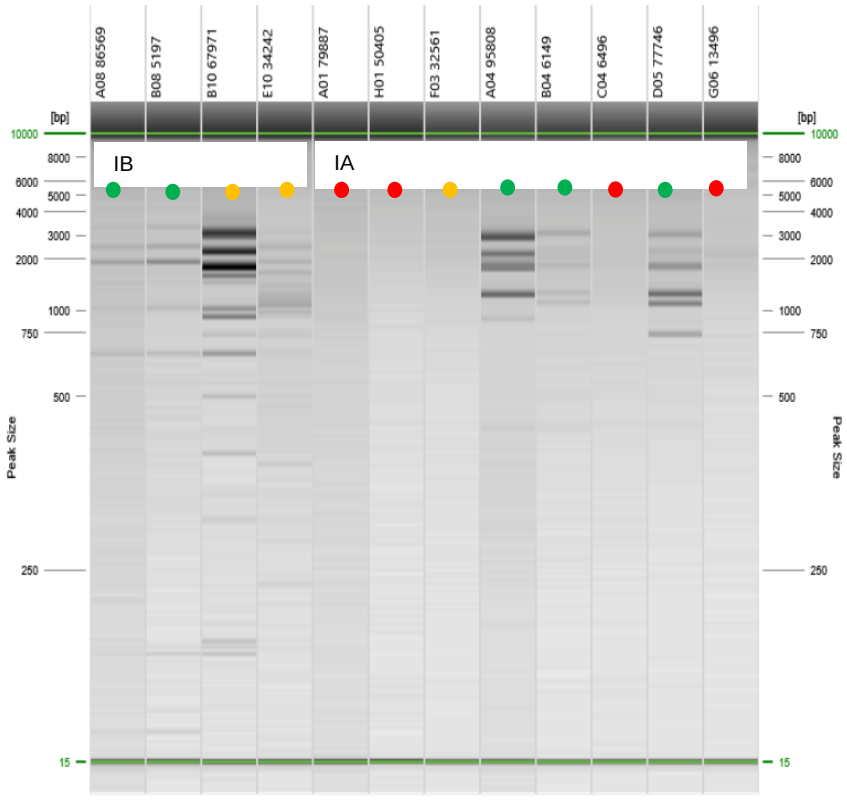


Figure 13. QIAxcel analysis of a selection of samples included in the robustness testing that showed incomplete genomic sequence data (red and orange dots). Some samples that showed complete genomic sequences were included in QIAxcel analysis and are marked with green dots.

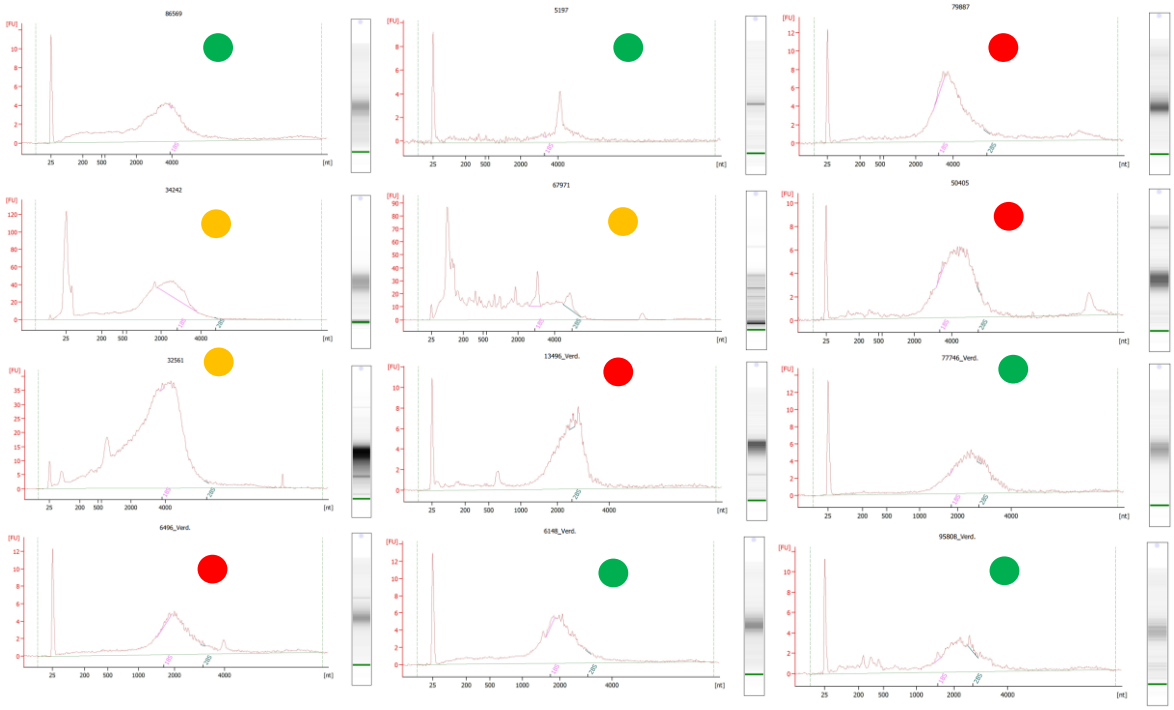


Figure 14. Bioanalyzer trace files of RNA samples performing poor and some samples with complete sequence information. Colour code as in Figure 12.

A third WGS run was performed following the same protocol as the second one. Eighteen zoonotic or antiviral resistant influenza samples, all of cell/egg culture origin, were included. Most of the samples were completely sequenced, except one with a Ct value >25 and 4 with low Ct values (Table 8).

Table 8. Influenza viruses included in the third NGS trial. Samples with one or more failed sequences and either HA or NA are absent are in red. In yellow, samples were at least one fragment is lost/incomplete but HA and NA genes are present

ID	Type	Ct	Remarks	Seg1	Seg2	Seg3	Seg4	Seg5	Seg6	Seg7	Seg8
Sent_1	H5N1	13	BPL Inactivated	x	95.0%	x	99.8%	✓	✓	✓	✓
Sent_2	H5N6	16	BPL Inactivated	x	x	x	x	✓	✓	✓	✓
Sent_3	H5N8	17	BPL Inactivated	x	x	x	x	✓	x	✓	✓
Sent_4	H5N1	25	-	✓	✓	99.9%	99.8%	✓	✓	✓	✓
Sent_5	H9N2	26	-	x	x	x	x	x	x	✓	✓
Sent_6	H3N2v	21	-	✓	✓	✓	✓	✓	✓	✓	✓
Sent_7	H1N1	18	D199E	✓	✓	✓	✓	✓	✓	✓	✓
Sent_8	H1N1	18	D199E	✓	✓	✓	99.6%	✓	✓	✓	✓
Sent_9	H3N2	20	E119V- del245-248	✓	✓	✓	✓	✓	✓	✓	99.6%
Sent_10	H3N2	20	E119V- del245-248	✓	✓	✓	✓	✓	✓	✓	99.6%
Sent_11	H1N1	16	mix 275H/Y*	✓	✓	✓	✓	✓	✓	✓	✓
Sent_12	H1N1	14	PA-I38T	✓	✓	✓	✓	✓	99.8%	✓	✓
Sent_13	H1N1	12	H275Y	✓	99.9%	✓	99.3%	✓	✓	✓	✓
Sent_14	H1N1	12	H275	✓	99.9%	✓	99.3%	✓	✓	✓	✓
Sent_15	H3N2	15	E119	✓	✓	✓	✓	✓	✓	✓	✓
Sent_16	H3N2	12	E119V	x	x	✓	✓	✓	✓	✓	✓
Sent_17	B	12	R152 (150)	✓	✓	✓	✓	✓	✓	✓	✓
Sent_18	B	11	R152K (150)	✓	99.7%	✓	✓	✓	✓	✓	✓

Failed sequencing for some fragments could be explained either by the samples' RNA quality/degradation, particularly for those that were β-propiolactone (BLP)-inactivated, or PCR-amplification inhibition due to high RNA concentration. The inhibition hypothesis could be tested by diluting the samples 1:10 and 1:50 and then running the PCR again.

In summary, 65 HA (90.3%), 63 NA (87.5%) and 62 PA (86.1%) influenza A genes out 72 samples could be fully sequenced. When looking at influenza B, all 35 HA, NA and PA genes were recovered. M and NS genes, the influenza diagnostic rRT-PCR targets are also available for a majority of the samples.

In conclusion, even if not perfect, the current influenza A and B WGS option at Microsynth AG is acceptable for epidemiological surveillance purposes. In addition, we are able to obtain information on segments that is not available by Sanger sequencing at all at the NRCI.

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

1. Shared material: *Influenza H3N2 isolates and a H3N2 clinical sample (MTA).*

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

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

2. Shared material: *Quantified Influenza A and B M and NS RNA transcripts, respectively.*

   With whom: *Prof. Alexandre Kuhn / Gordana Pistoletti, HES-SO Valais-Wallis, Institut des Technologies du Vivant.*

   Project*: Development of detection methods for influenza A and B.*

3*.* Shared material: *MDCK ATCC/63*

   With whom: *Dr Benjamin Meyer, Department of Paediatrics, Gynaecology and Obstetrics*, *Faculty of Medicine, University of Geneva*.

   Project*: Development of immunological assays for influenza*

10.3 Collaborative projects/publications

As for 2020, the NRCI continued to support the laboratory of virology and the National Reference Centre for Emerging Viral Infections (CRIVE), particularly for SARS-CoV-2 variant detection.

Support for national surveillance in the context of influenza:

An Online Influenza Surveillance System for Primary Care Workers in Switzerland: Observational Prospective Pilot Study. Martin S, Maeder MN, Gonçalves AR, Pedrazzini B, Perdrix J, Rochat C, Senn N, Mueller Y. JMIR Public Health Surveill. 2020 Sep 10;6(3):e17242. doi: 10.2196/17242.

Support for national surveillance and clinics in the context of SARS-CoV-2:

SARS-CoV-2 N501Y Introductions and Transmissions in Switzerland from Beginning of October 2020 to February 2021-Implementation of Swiss-Wide Diagnostic Screening and Whole Genome Sequencing. Goncalves Cabecinhas AR, Roloff T, Stange M, Bertelli C, Huber M, Ramette A, Chen C, Nadeau S, Gerth Y, Yerly S, Opota O, Pillonel T, Schuster T, Metzger CMJA, Sieber J, Bel M, Wohlwend N, Baumann C, Koch MC, Bittel P, Leuzinger K, Brunner M, Suter-Riniker F, Berlinger L, Søgaard KK, Beckmann C, Noppen C, Redondo M, Steffen I, Seth-Smith HMB, Mari A, Lienhard R, Risch M, Nolte O, Eckerle I, Martinetti Lucchini G, Hodcroft EB, Neher RA, Stadler T, Hirsch HH, Leib SL, Risch L, Kaiser L, Trkola A, Greub G, Egli A. Microorganisms. 2021 Mar 25;9(4):677. doi: 10.3390/microorganisms9040677.

Prevalence of Immunoglobulin G (IgG) Against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and Evaluation of a Rapid MEDsan IgG Test in Children Seeking Medical Care. Posfay-Barbe KM, Andrey DO, Virzi J, Cohen P, Pigny F, Goncalves AR, Pinosch S, Lacroix L, Stringhini S, Kaiser L, Vuilleumier N, L'Huillier AG. Clin Infect Dis. 2021 Apr 8;72(7):e192-e195. doi: 10.1093/cid/ciaa1702.

10.4 Work in progress

During 2020/2021, new assay and platform validations were prioritized over research.

10.4.1 Sanger sequencing of influenza PA genes

In 2020, the PA cap-dependent endonuclease activity inhibitor baloxavir marboxil (Xofluza®) has been authorized in Switzerland for treatment of patients ≥12 years old with uncomplicated influenza. PA I38T/M/F/N, E23G/K, A37T, and E199G substitutions have been identified as being associated with reduced susceptibility to this new drug.[88] It is therefore meaningful to have a test that allows their identification.

Sanger sequencing is currently used at the NRCI for the identification of mutations associated with reduced susceptibility to neuraminidase inhibitors. It was logical to use the same platform for baloxavir marboxil resistance assessment requests in the context of a clinical cases. Of note, whole genome sequencing will be preferred in the context of epidemiological resistance follow-up.

We tested three different forward and reverse primers. Some were designed in-house and others were recovered from literature.

For influenza A, we use for a final reaction volume of 50 µl: 25 µl of the 2X Reaction Mix (SuperScript™ III One-Step RT-PCR System, Invitrogen™), 1 µl of SuperScript III RT/Platinum Taq High Fidelity Enzyme Mix, 2 µl of PCR-grade water, 4 µl of 5 µM forward primer “PA InfA 14 For M13”(*TGTAAAACGACGGCCAGTTGCGACAATGCTTCAATCC*), 8 µl of 5 µM reverse primer “A-PA-I-M13R”(*CAGGAAACAGCTATGACCGGYTCTTTCCAKCCAAAG*)[86,89] and 10 µl of test RNA. Temperature cycling was inspired by Zhou et al[86,90] and was the following: 42°C for 60 min, 94°C for 2 min, then 5 cycles (94°C for 30s, 44°C for 30s, and 68°C for 3min 30s), followed by 35 cycles (94°C for 30s, 52°C for 30s, and 68°C for 3min 30s), 68°C 10min, and hold at 4°C.

For influenza B, we used the same mix and cycling temperatures conditions as for influenza A but with “InfB PA F10”(*GGTGCGTTTGATTTRTC*) as the forward primer and “InfB PA R945”(*CTAGACATTCTTTGGC*) as the reverse.

According to recent results (Figure 15 left panel), the influenza A primer combination described above will most probably be used at the NRCI during 2021/2022. The first amplification trial for the influenza B/Victoria/2/87-lineage sample looked good (Figure 15 right panel). However, the influenza B/Yamagata/16/88-lineage sample was not

correctly amplified (not shown). We suspect sample degradation rather than a problem of primer and cycling conditions.

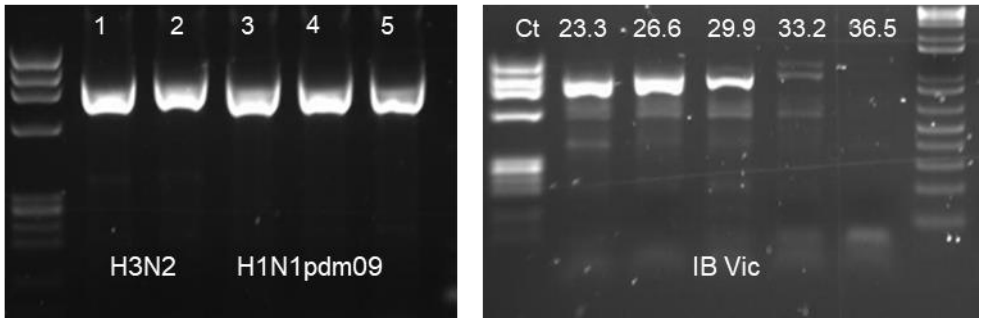


Figure 15. Gel electrophoresis showing influenza A (left, ≈964bp) and B (right, ≈951bp) PA fragment PCR amplification. Left panel: 1. A/Louisiana/50/2017, PA-I38 (GISIAD: EPI_ISL_315857), 2: A/Louisiana/49/2017, PA-I38M (GISIAD: EPI_ISL_315858), 3. A/Illinois/08/2018, PA-I38 (GISAID: EPI_ISL_315855), 4. A/Illinois/37/2018, PA-I38L (GISAID: EPI_ISL_315856). 5. A/Illinois/08/2018, PA-I38T (GISIAD: EPI_ISL_348120). Right panel: 10-fold dilution of a B/Victoria/2/87-lineage positive sample (xxxx6849).

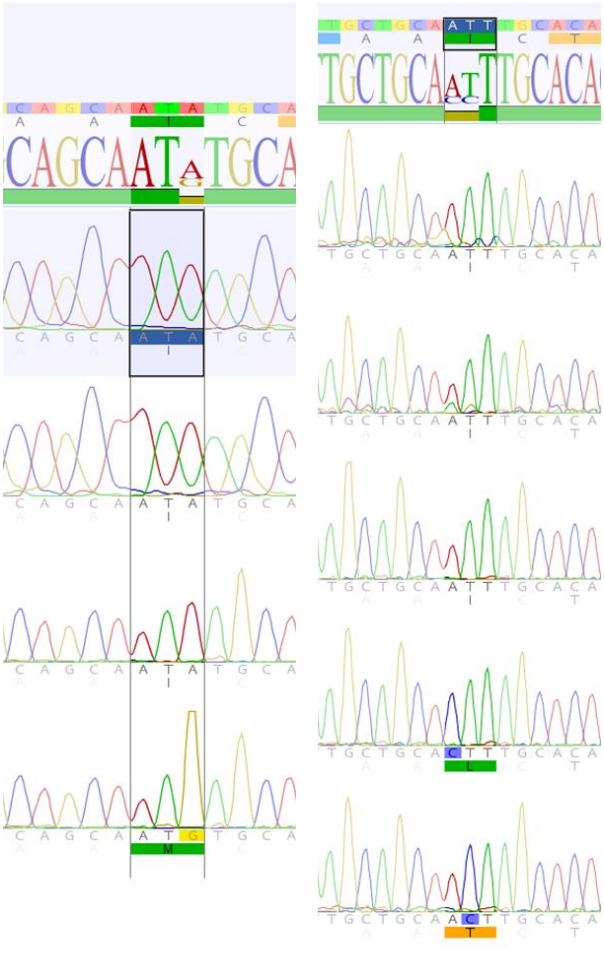


Figure 16: Sanger sequencing chromatographs showing the successful sequencing of PA positions A37 and I38 of influenza. Left panel: from top to bottom A(H3N2) samples (xxxx5103, 31.2 and 34.5 Ct), A/Louisiana/50/2017, PA-I38 (GISIAD: EPI_ISL_315857) and A/Louisiana/49/2017, PA-I38M (GISIAD: EPI_ISL_315858). Right panel: A(H1N1) samples (xxxx5686, 32.5 and 35.8 Ct), A/Illinois/08/2018, PA-I38 (GISAID: EPI_ISL_315855), A/Illinois/37/2018, PA-I38L (GISAID: EPI_ISL_315856) and A/Illinois/08/2018, PA-I38T (GISIAD: EPI_ISL_348120).

If possible, the same cycling temperature conditions for both influenza A and B PA will be used. We will also try to shorten the cycling time as much as possible.

10.4.2 Design and production of an human H5 RNA transcript

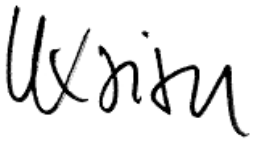
In order to always have meaningful/recent rRT-PCR controls, notably for influenza strains with pandemic potential, we have designed and ordered a plasmid construct containing a T7 promotor for RNA transcripts production of full length A(H5) of the recently the identified human A/Astrakhan/3212/2020 (H5N8, reference sequence: EPI_ISL_1038924), using the GeneArt gene synthesis service of Thermofisher (Germany). An Xho I site was introduced for plasmid linearization. The construct is under production and will be linearized, reverse transcribed and quantified upon future arrival by the NRCI.

Geneva, July 29th 2021

Dr Ana Rita Gonçalves Cabecinhas

Mrs Patricia Suter-Boquete

Professor Laurent Kaiser



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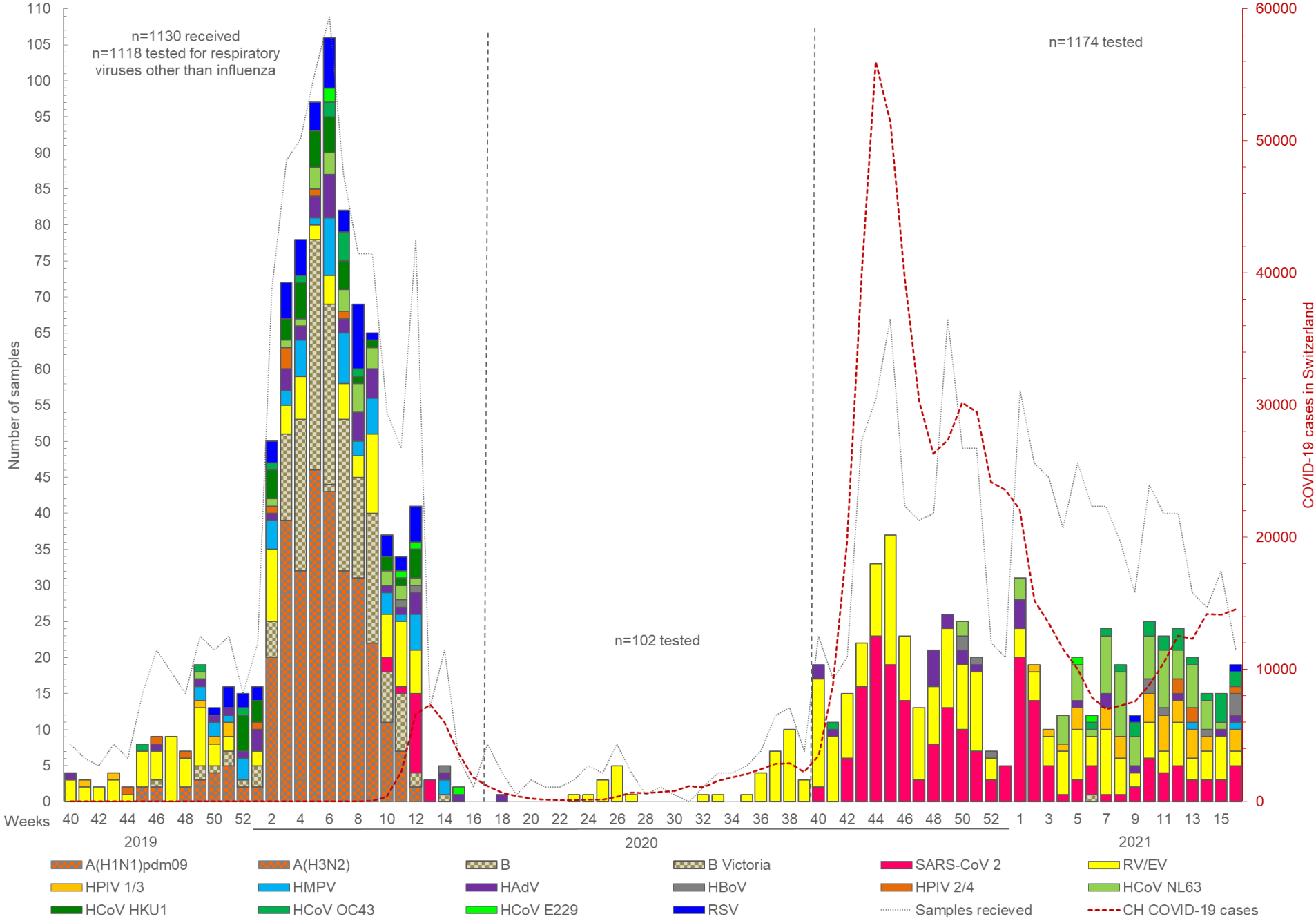
Annex 1: Overview of the respiratory viruses detected at the NCRI from week 40/2020 to week 16/2021



Annex 2: Lists of SARS-CoV-2 isolates submitted to GISAID (40/2020-16/2021)

Isolate name	Pangolin lineage (v.3.0.5, 05.06.2021)	Collection date	GISAID ID
hCoV-19/Switzerland/BE-SNRCI-6109/2020	C.36	20200928	EPI_ISL_860745
hCoV-19/Switzerland/BE-SNRCI-4089/2020	B.1.177	20201012	EPI_ISL_860746
hCoV-19/Switzerland/BE-SNRCI-7858/2020	B.1.1.232	20201013	EPI_ISL_860747
hCoV-19/Switzerland/VD-SNRCI-1387/2020	B.1.160.16	20201014	EPI_ISL_860748
hCoV-19/Switzerland/BL-SNRCI-5004/2020	B.1.474	20201015	EPI_ISL_860749
hCoV-19/Switzerland/BE-SNRCI-83/2020	B.1.177.44	20201016	EPI_ISL_860750
hCoV-19/Switzerland/TI-SNRCI-265/2020	B.1.177.51	20201016	EPI_ISL_860751
hCoV-19/Switzerland/BE-SNRCI-4612/2020	B.1.1.232	20201019	EPI_ISL_860753
hCoV-19/Switzerland/GR-SNRCI-4719/2020	B.1.177.44	20201019	EPI_ISL_860733
hCoV-19/Switzerland/GR-SNRCI-5726/2020	B.1.177.44	20201019	EPI_ISL_860755
hCoV-19/Switzerland/LU-SNRCI-5417/2020	B.1.177.44	20201019	EPI_ISL_860754
hCoV-19/Switzerland/ZH-SNRCI-4555/2020	B.1.177.23	20201019	EPI_ISL_860752
hCoV-19/Switzerland/BE-SNRCI-8433/2020	B.1.160	20201020	EPI_ISL_860756
hCoV-19/Switzerland/GR-SNRCI-1135/2020	B.1.1.39	20201020	EPI_ISL_860757
hCoV-19/Switzerland/ZH-SNRCI-1241/2020	B.1.177.44	20201020	EPI_ISL_860758
hCoV-19/Switzerland/GR-SNRCI-1337/2020	B.1.1.39	20201021	EPI_ISL_860739
hCoV-19/Switzerland/TI-SNRCI-1223/2020	B.1.177	20201021	EPI_ISL_860734
hCoV-19/Switzerland/VD-SNRCI-1410/2020	B.1.177	20201021	EPI_ISL_860759
hCoV-19/Switzerland/GR-SNRCI-4687/2020	B.1.177	20201022	EPI_ISL_860735
hCoV-19/Switzerland/BE-SNRCI-2110/2020	B.1.177.44	20201023	EPI_ISL_860736
hCoV-19/Switzerland/LU-SNRCI-4535/2020	B.1.177.23	20201023	EPI_ISL_860760
hCoV-19/Switzerland/ZH-SNRCI-6958/2020	B.1.1.39	20201024	not submited but available
hCoV-19/Switzerland/BE-SNRCI-6649/2020	B.1.258	20201026	EPI_ISL_860731
hCoV-19/Switzerland/VS-SNRCI-3876/2020	B.1.160.10	20201026	EPI_ISL_860767
hCoV-19/Switzerland/BE-SNRCI-2603/2020	B.1.258	20201027	EPI_ISL_860743
hCoV-19/Switzerland/BE-SNRCI-4734/2020	B.1.177	20201027	EPI_ISL_860771
hCoV-19/Switzerland/BL-SNRCI-3977/2020	B.1.177	20201027	EPI_ISL_860768
hCoV-19/Switzerland/VD-SNRCI-6/2020	B.1.160.14	20201027	EPI_ISL_860762
hCoV-19/Switzerland/VS-SNRCI-4140/2020	B.1.177.44	20201027	EPI_ISL_860769
hCoV-19/Switzerland/BE-SNRCI-3399/2020	B.1.160.16	20201028	EPI_ISL_860763
hCoV-19/Switzerland/BE-SNRCI-3473/2020	B.1.177	20201028	EPI_ISL_860764
hCoV-19/Switzerland/BE-SNRCI-3521/2020	B.1.160.16	20201028	EPI_ISL_860765
hCoV-19/Switzerland/BE-SNRCI-3747/2020	B.1.367	20201028	EPI_ISL_860740
hCoV-19/Switzerland/BE-SNRCI-4517/2020	B.1.160	20201028	EPI_ISL_860770
hCoV-19/Switzerland/VD-SNRCI-3593/2020	B.1.160.16	20201028	EPI_ISL_860766
hCoV-19/Switzerland/BL-SNRCI-4938/2020	B.1.177	20201029	EPI_ISL_860772
hCoV-19/Switzerland/ZH-SNRCI-2348/2020	B.1.177.23	20201029	EPI_ISL_860773
hCoV-19/Switzerland/SG-SNRCI-2687/2020	B.1.160.14	20201030	EPI_ISL_860732
hCoV-19/Switzerland/ZH-SNRCI-2363/2020	B.1.160.16	20201030	EPI_ISL_860774
hCoV-19/Switzerland/BE-SNRCI-7400/2020	B.1.177.23	20201031	EPI_ISL_860776
hCoV-19/Switzerland/SG-SNRCI-3430/2020	B.1.177.81	20201031	EPI_ISL_860775
hCoV-19/Switzerland/BL-SNRCI-1755/2020	B.1.416.1	20201102	EPI_ISL_860737
hCoV-19/Switzerland/GE-SNRCI-7587/2020	B.1.160	20201102	EPI_ISL_860777
hCoV-19/Switzerland/GE-SNRCI-8178/2020	B.1.160	20201102	EPI_ISL_860779
hCoV-19/Switzerland/GR-SNRCI-1898/2020	B.1.221	20201102	EPI_ISL_860781
hCoV-19/Switzerland/VD-SNRCI-8030/2020	B.1.177.44	20201102	EPI_ISL_860742
hCoV-19/Switzerland/ZH-SNRCI-1707/2020	B.1.160.16	20201102	EPI_ISL_860780
hCoV-19/Switzerland/BE-SNRCI-7899/2020	B.1.160.14	20201103	EPI_ISL_860778
hCoV-19/Switzerland/NE-SNRCI-1796/2020	B.1.160.14	20201103	EPI_ISL_860741
hCoV-19/Switzerland/NE-SNRCI-5822/2020	B.1.160	20201103	EPI_ISL_860758

Isolate name	Pangolin lineage (v.3.0.5, 05.06.2021)	Collection date	GISAID ID
hCoV-19/Switzerland/BE-SNRCI-1777/2020	B.1.258	20201104	EPI_ISL_860744
hCoV-19/Switzerland/BL-SNRCI-5725/2020	B.1.177.44	20201105	EPI_ISL_860730
hCoV-19/Switzerland/BL-SNRCI-7887/2020	B.1.177.23	20201105	EPI_ISL_857489
hCoV-19/Switzerland/NE-SNRCI-4080/2020	B.1.160.32	20201105	EPI_ISL_857490
hCoV-19/Switzerland/ZH-SNRCI-4303/2020	B.1.177	20201105	EPI_ISL_857491
hCoV-19/Switzerland/BE-SNRCI-8344/2020	B.1.160	20201106	EPI_ISL_857518
hCoV-19/Switzerland/ZH-SNRCI-3848/2020	B.1.160.15	20201106	EPI_ISL_857516
hCoV-19/Switzerland/BE-SNRCI-8083/2020	B.1.160	20201109	EPI_ISL_857517
hCoV-19/Switzerland/BE-SNRCI-8047/2020	B.1.177.44	20201110	EPI_ISL_857492
hCoV-19/Switzerland/VS-SNRCI-942/2020	B.1.177.44	20201110	EPI_ISL_857493
hCoV-19/Switzerland/BE-SNRCI-5531/2020	B.1.177	20201112	EPI_ISL_857519
hCoV-19/Switzerland/BL-SNRCI-5550/2020	B.1.177.44	20201112	EPI_ISL_857494
hCoV-19/Switzerland/NE-SNRCI-1683/2020	B.1.177.44	20201113	EPI_ISL_857495
hCoV-19/Switzerland/AG-SNRCI-2816/2020	B.1.160.22	20201116	EPI_ISL_857540
hCoV-19/Switzerland/ZH-SNRCI-7533/2020	B.1.160	20201116	EPI_ISL_857520
hCoV-19/Switzerland/BE-SNRCI-6124/2020	B.1.177.44	20201117	EPI_ISL_857496
hCoV-19/Switzerland/GR-SNRCI-9213/2020	B.1.221	20201119	EPI_ISL_857497
hCoV-19/Switzerland/AG-SNRCI-6047/2020	B.1.221	20201123	EPI_ISL_857521
hCoV-19/Switzerland/AG-SNRCI-6138/2020	B.1.160.16	20201123	EPI_ISL_857522
hCoV-19/Switzerland/GR-SNRCI-6169/2020	B.1.221	20201123	EPI_ISL_857523
hCoV-19/Switzerland/BE-SNRCI-8217/2020	B.1.177.23	20201124	EPI_ISL_857498
hCoV-19/Switzerland/GR-SNRCI-8061/2020	B.1.160	20201124	EPI_ISL_857524
hCoV-19/Switzerland/BE-SNRCI-77/2020	B.1.160.14	20201126	EPI_ISL_857525
hCoV-19/Switzerland/BE-SNRCI-2380/2020	B.1.1.39	20201128	EPI_ISL_857526
hCoV-19/Switzerland/NE-SNRCI-2588/2020	B.1.177.44	20201128	EPI_ISL_857499
hCoV-19/Switzerland/BE-SNRCI-8076/2020	B.1.177	20201130	EPI_ISL_857501
hCoV-19/Switzerland/BE-SNRCI-8130/2020	B.1.1.39	20201130	EPI_ISL_857502
hCoV-19/Switzerland/NE-SNRCI-8703/2020	B.1.177.44	20201130	EPI_ISL_857503
hCoV-19/Switzerland/VS-SNRCI-5743/2020	B.1	20201130	EPI_ISL_857500
hCoV-19/Switzerland/ZH-SNRCI-1153/2020	B.1.1.70	20201130	EPI_ISL_857527
hCoV-19/Switzerland/BE-SNRCI-8768/2020	B.1.177.44	20201201	EPI_ISL_882648
hCoV-19/Switzerland/BL-SNRCI-9619/2020	B.1.221	20201202	EPI_ISL_857504
hCoV-19/Switzerland/BE-SNRCI-1189/2020	B.1.160.14	20201203	EPI_ISL_857528
hCoV-19/Switzerland/BE-SNRCI-1263/2020	B.1.160.12	20201203	EPI_ISL_857529
hCoV-19/Switzerland/BE-SNRCI-1283/2020	B.1.160.16	20201203	EPI_ISL_857530
hCoV-19/Switzerland/BE-SNRCI-1302/2020	B.1.177.44	20201203	EPI_ISL_857505
hCoV-19/Switzerland/SG-SNRCI-3473/2020	B.1.1	20201203	EPI_ISL_857507
hCoV-19/Switzerland/GR-SNRCI-3453/2020	B.1.406	20201204	EPI_ISL_857506
hCoV-19/Switzerland/BE-SNRCI-8907/2020	B.1.221	20201207	EPI_ISL_857532
hCoV-19/Switzerland/BL-SNRCI-8347/2020	B.1.177.43	20201207	EPI_ISL_857531/857509
hCoV-19/Switzerland/ZH-SNRCI-6623/2020	B.1	20201207	EPI_ISL_857508
hCoV-19/Switzerland/BE-SNRCI-8484/2020	B.1.177	20201208	EPI_ISL_857510
hCoV-19/Switzerland/SH-SNRCI-2552/2020	B.1.258.17	20201208	EPI_ISL_857533
hCoV-19/Switzerland/ZH-SNRCI-2798/2020	B.1.177	20201208	EPI_ISL_857512
hCoV-19/Switzerland/BL-SNRCI-2577/2020	B.1.221	20201209	EPI_ISL_857511/857534
hCoV-19/Switzerland/SH-SNRCI-3323/2020	B.1.1.39	20201209	EPI_ISL_857535
hCoV-19/Switzerland/BE-SNRCI-5303/2020	B.1.160	20201211	EPI_ISL_882649
hCoV-19/Switzerland/ZH-SNRCI-9747/2020	B.1.177.43	20201211	EPI_ISL_857536
hCoV-19/Switzerland/GE-SNRCI-9913/2020	B.1.1.317	20201214	EPI_ISL_857539
hCoV-19/Switzerland/NE-SNRCI-9813/2020	B.1.160.16	20201214	EPI_ISL_857537

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hCoV-19/Switzerland/NE-SNRCI-9837/2020	B.1.160.16	20201214	EPI_ISL_857538
hCoV-19/Switzerland/ZH-SNRCI-5931/2020	B.1.149	20201214	EPI_ISL_857514
hCoV-19/Switzerland/BE-SNRCI-5823/2020	B.1.160.16	20201216	EPI_ISL_857513
hCoV-19/Switzerland/BE-SNRCI-7838/2020	B.1.177.43	20201217	EPI_ISL_857541
hCoV-19/Switzerland/ZH-SNRCI-4677/2020	B.1.1.70	20201222	not submited but available
hCoV-19/Switzerland/BE-SNRCI-5554/2020	B.1.177.44	20201228	EPI_ISL_857515
hCoV-19/Switzerland/ZH-SNRCI-32927909/2021	B.1.1.7 (alpha)	20210107	EPI_ISL_864737
hCoV-19/Switzerland/BL-SNRCI-32927853/2021	B.1.221	20210108	EPI_ISL_864596
hCoV-19/Switzerland/LU-SNRCI-32927500/2021	B.1.1.519	20210108	EPI_ISL_864717
hCoV-19/Switzerland/VS-SNRCI-32927480/2021	B.1.177	20210108	EPI_ISL_864736
hCoV-19/Switzerland/BE-SNRCI-32938514/2021	B.1.160.16	20210111	EPI_ISL_864595
hCoV-19/Switzerland/AG-SNRCI-32950124/2021	B.1.177.86	20210112	EPI_ISL_897688
hCoV-19/Switzerland/BE-SNRCI-32961174/2021	B.1.160	20210112	EPI_ISL_897716
hCoV-19/Switzerland/GR-SNRCI-32961384/2021	B.1.160.26	20210112	EPI_ISL_897903
hCoV-19/Switzerland/ZH-SNRCI-32994252/2021	B.1.160.9	20210112	EPI_ISL_953666
hCoV-19/Switzerland/VD-SNRCI-32961024/2021	B.1.1.7 (alpha)	20210113	EPI_ISL_897603
hCoV-19/Switzerland/VS-SNRCI-32961189/2021	B.1.160.14	20210113	EPI_ISL_897984
hCoV-19/Switzerland/BE-SNRCI-32972889/2021	B.1.258.17	20210114	EPI_ISL_897680
hCoV-19/Switzerland/LU-SNRCI-32986144/2021	B.1	20210114	EPI_ISL_897904
hCoV-19/Switzerland/ZH-SNRCI-32961210/2021	B.1.160.9	20210114	EPI_ISL_897985
hCoV-19/Switzerland/ZH-SNRCI-32994297/2021	B.1.177.43	20210114	EPI_ISL_953667
hCoV-19/Switzerland/VD-SNRCI-33019591/2021	B.1.177.44	20210119	EPI_ISL_953698
hCoV-19/Switzerland/LU-SNRCI-33031086/2021	B.1.258.17	20210120	EPI_ISL_953627
hCoV-19/Switzerland/FR-SNRCI-33099045/2021	B.1.258	20210126	EPI_ISL_953644
hCoV-19/Switzerland/BE-SNRCI-33170670/2021	B.1.160.14	20210203	EPI_ISL_981720
hCoV-19/Switzerland/VD-SNRCI-33216514/2021	B.1.1.7 (alpha)	20210206	EPI_ISL_1040279
hCoV-19/Switzerland/ZH-SNRCI-33229054/2021	B.1.1.7 (alpha)	20210209	EPI_ISL_1040296
hCoV-19/Switzerland/ZH-33253640/2021	B.1.160	20210210	EPI_ISL_1084994
hCoV-19/Switzerland/VD-33265552/2021	B.1.1.7 (alpha)	20210212	EPI_ISL_1084879
hCoV-19/Switzerland/LU-SNRCI-33311161/2021	B.1.258	20210216	EPI_ISL_1084932
hCoV-19/Switzerland/BE-SNRCI-33356712/2021	B.1.1.7 (alpha)	20210222	EPI_ISL_1166783
hCoV-19/Switzerland/BE-SNRCI-33450564/2021	B.1.1.519	20210303	EPI_ISL_1228888
hCoV-19/Switzerland/ZH-33497776/2021	B.1.1.7 (alpha)	20210304	EPI_ISL_1296140
hCoV-19/Switzerland/AG-33497723/2021	B.1.1.7 (alpha)	20210308	EPI_ISL_1296150
hCoV-19/Switzerland/ZH-SNRCI-33522344/2021	B.1.1.7 (alpha)	20210308	EPI_ISL_1296141
hCoV-19/Switzerland/LU-SNRCI-33521494/2021	B.1.1.7 (alpha)	20210310	EPI_ISL_1296101
hCoV-19/Switzerland/SH-SNRCI-33521750/2021	B.1.1.7 (alpha)	20210310	EPI_ISL_1296192
hCoV-19/Switzerland/BE-SNRCI-33533460/2021	B.1.1.7 (alpha)	20210311	not submited but available
hCoV-19/Switzerland/BE-SNRCI-33533521/2021	B.1.1.7 (alpha)	20210311	EPI_ISL_1372462
hCoV-19/Switzerland/BE-33568047/2021	B.1.1.7 (alpha)	20210315	EPI_ISL_1372463
hCoV-19/Switzerland/ZH-SNRCI-33603898/2021	B.1.1.7 (alpha)	20210315	EPI_ISL_1448537
hCoV-19/Switzerland/ZH-SNRCI33651886/2021	B.1.160	20210319	EPI_ISL_1448709
hCoV-19/Switzerland/ZH-SNRCI-33662435/2021	B.1.1.7 (alpha)	20210323	EPI_ISL_1448749
hCoV-19/Switzerland/ZH-SNRCI-33675032/2021	B.1.1.7 (alpha)	20210324	EPI_ISL_1533376
hCoV-19/Switzerland/ZH-SNRCI-33698410/2021	B.1.1.7 (alpha)	20210327	EPI_ISL_1533377
hCoV-19/Switzerland/BE-SNRCI-33724649/2021	B.1.1.7 (alpha)	20210330	EPI_ISL_1533312
hCoV-19/Switzerland/BE-SNRCI-33824142/2021	B.1.1.7 (alpha)	20210409	EPI_ISL_1708027
hCoV-19/Switzerland/ZH-SNRCI-33847474/2021	B.1.1.7 (alpha)	20210410	EPI_ISL_1708231
hCoV-19/Switzerland/TG-SNRCI-33896062/2021	B.1.1.7 (alpha)	20210411	EPI_ISL_1811072
hCoV-19/Switzerland/VD-33847407/2021	B.1.1.7 (alpha)	20210412	EPI_ISL_1708118

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hCoV-19/Switzerland/ZH-SNRCI-33896040/2021	B.1.1.7 (alpha)	20210414	EPI_ISL_1811168
hCoV-19/Switzerland/BE-SNRCI-33945550/2021	B.1.1.7 (alpha)	20210421	EPI_ISL_1810976
hCoV-19/Switzerland/BE-SNRCI-33968828/2021	B.1.1.7 (alpha)	20210423	EPI_ISL_1963042
hCoV-19/Switzerland/ZH-SNRCI-33979431/2021	B.1.1.7 (alpha)	20210423	EPI_ISL_1963241