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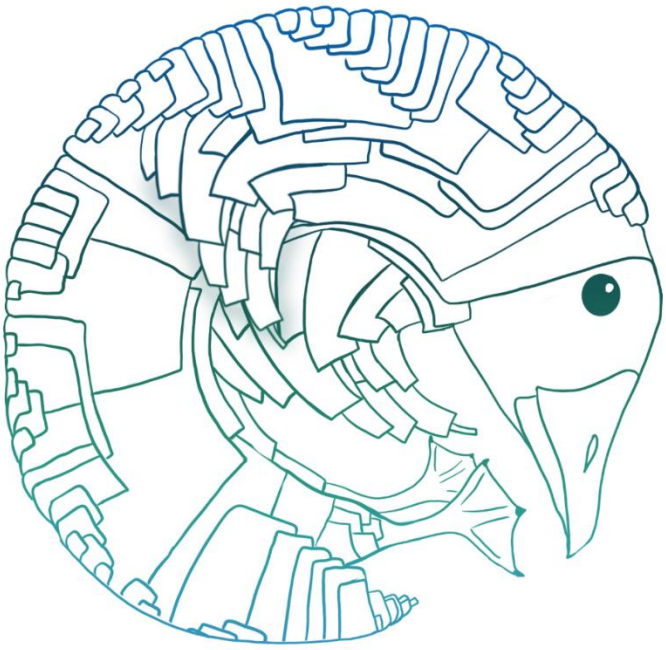
Addendum to the Influenza virus surveillance in Switzerland for the season 2021-2022

(Week 17/2022 to week 39/2022)

National Reference Centre of Influenza
Laboratory of Virology
Geneva University Hospitals
4 Rue Gabrielle-Perret-Gentil
1211 GENEVA 14 – SWITZERLAND



Cover image credit and description: Digital illustration using Procreate® by Lisa Spedaliero
Phylogenetic tree representing a wild goose.
Image definition: 2000x2000 pixels

Contacts

Ms Tania Spedaliero	Tel: +41/22 372 40 82 ✉ : tania.spedaliero@hcuge.ch
Dr Ana Rita Gonçalves Cabecinhas	Tel: +41/22 372 40 81 ✉ : agnv@hcuge.ch
Ms Patricia Boquete-Suter	Tel: +41/22 372 40 94 ✉ : patricia.suter@hcuge.ch
Dr Manuel Schibler	Tel: +41/22 372 40 96 ✉ : manuel.schibler@hcuge.ch
Professor Laurent Kaiser	Tel: +41/22 372 98 01 ✉ : laurent.kaiser@hcuge.ch

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Abbreviations and Acronyms

cDNA	complementary deoxyribonucleic acid
GISAID	global initiative on sharing all influenza data
HA	hemagglutinin
HAI	hemagglutinin inhibition
HAdV	human adenovirus
HBoV	human bocavirus
HCoV	human coronavirus
H/LPAI	high/low pathogenic avian influenza
HMPV	human metapneumovirus
HPIV	human parainfluenza virus
IA, IB	influenza A, B
MDCK	Madin-Darby canine kidney cells
MDCK-SIAT1	sialic acid-enriched MDCK cells
MUNANA	20-(4-methylumbelliferyl)-a-D-N-acetylneuraminic acid
NA	neuraminidase
NAI	neuraminidase inhibitor
NCBI	national center for biotechnology information
NPS	nasopharyngeal sample/swab
NH	northern Hemisphere
NRCI	national reference centre of influenza
PA	acidic protein
RV/EV	rhinovirus/enterovirus
RSV	respiratory syncytial virus
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SH	southern Hemisphere
rRT-PCR	real-time reverse-transcription polymerase chain reaction
Vic, Yam	victoria, yamagata
VOC	variant of concern
WIC	worldwide influenza centre

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Summary

A total of 1044 nasopharyngeal swabs were tested in the context of the Swiss Sentinel surveillance network from week 17/2022 to week 39/2022.

Out of the 1044 samples tested, 31 influenza viruses were detected, which represent 4.4% of the positive samples. As observed until week 17/2022, influenza A(H3N2) viruses were predominant. The viruses that were antigenically characterized belonged to the 3C.2a1b.2a.2 genetic group and reacted well with the antiserum raised against the 2022 southern hemisphere vaccine strain A/Darwin/9/2021 (3C.2a1b.2a.2). Only few influenza A(H1N1)pdm09 and influenza B viruses were detected in Switzerland. The A(H1N1)pdm09 viruses characterized antigenically were best recognized by the antiserum raised against the previous 2020/2021 vaccine strain, A/Guangdong-Maonan/SWL1536/2019. Two influenza B viruses were identified at the NRCI, from week 17/2022 to 39/2022. Only one had sufficient viral load to be further subtyped. It belonged to the B-Victoria lineage and was antigenically similar to the 2022 southern hemisphere vaccine strain B/Austria/1359417/2021.

During the summer 2022, influenza viruses circulated at higher levels compared to the same period in 2020 and 2021. However, influenza activity in general remained low. No zoonotic influenza infections were observed in Switzerland.

In contrast to 2021, high levels of avian influenza were detected throughout June and August in 2022. This season was the largest highly pathogenic avian influenza epidemic observed in Europe so far.

1 Identification of influenza and other respiratory viruses

Data gathered in the present report correspond to Sentinel samples received at the National Reference Centre of Influenza (NRCI) from April 23rd, 2022 (week 17/2022) to September 30th, 2022 (week 39/2022). Samples were collected and processed as during week 40/2021 to week 16/2022. Data are available at https://www.hug.ch/sites/interhug/files/structures/laboratoire_de_virologie/document/nrci_annual_report2021_2022.pdf.

2 Detection of respiratory viruses in nasopharyngeal samples

From week 17/2022 to week 39/2022, 1044 nasopharyngeal samples (NPS) were screened for influenza virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), respiratory syncytial virus (RSV), human coronavirus (HCoV) NL63, HKU1, OC43, 229E, human parainfluenza virus (HPIV), human bocavirus (HBoV), human adenovirus (HAdV), rhinovirus/enterovirus (RV/EV) and human metapneumovirus (HMPV).

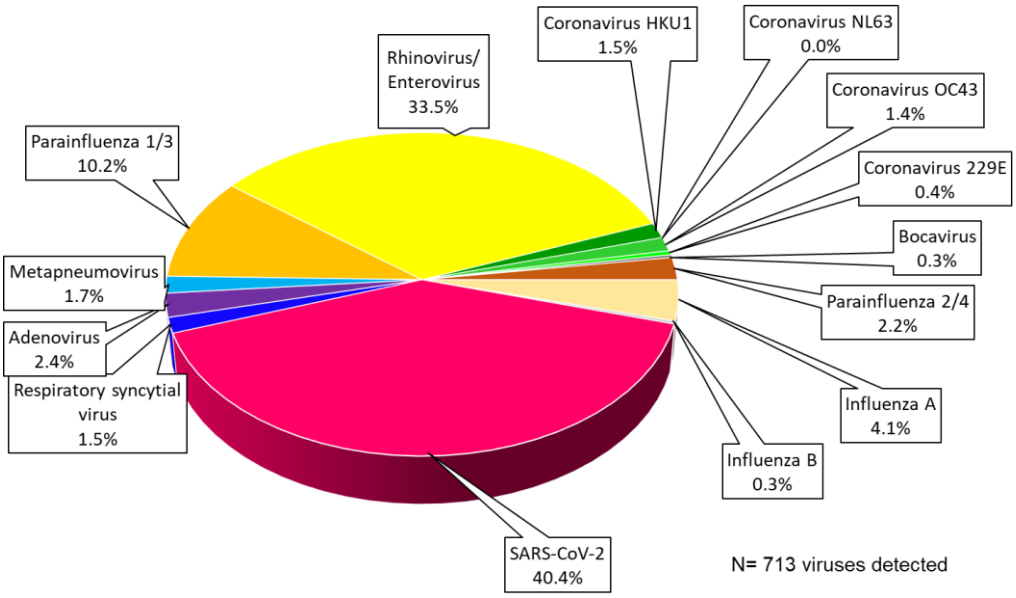
Six hundred and sixty samples (63.2%) were positive for at least one respiratory virus and 713 viruses were detected in total. Among these, the following pathogens were identified: 288 SARS-CoV-2 (40.4%), 239 RV/EV (33.5%), 73 HPIV 1/3 (10.2%), 29 influenza A viruses (4.1%), , 17 HAdV (2.4%), 16 HPIV 2/4 (2.2%), 12 HMPV (1.7%), 11 RSV (1.5%), 11 HCoV HKU1 (1.5%), 10 HCoV OC43 (1.4%), 3 HCoV 229E (0.4%), 2 HBoV (0.3%) and two influenza B viruses (0.3%) (Figure 1a). Of note, no HCoV NL63 virus was detected.

A maximum positivity rate of 81% during week 39/2022 and a minimum positivity rate of 36% during week 30/2022 (median positivity rate: 64.4%, range [36 to 81%]; 95% CI (60.1-68.8 %)) were observed (Figure 1b).

SARS-CoV-2 and RV/EV were regularly detected from week 17/2022 to week 39/2022. The proportion of positive RV/EV was even higher than that of SARS-CoV-2 during week 37/2022. Since week 17/2022, HPIV1/3 and human coronaviruses (HKU1, OC43, 229E), although at lower numbers, were also regularly detected until week 31/2022 and 29/2022, respectively. In contrast, HMPV, HAdV, and HPIV2/4 were only sporadically detected. Interestingly, influenza viruses were detected almost every week from week 17/2022 to week 37/2022.

From week 17/2022 to week 39/2022, more than one virus was detected in 49 (7.4%) out of 660 positive samples (Appendix 1). The highest number of co-detections (5) was observed in week 19/2022. Not surprisingly, among those, 28 concerned RV/EV (57.1%); which was often observed with SARS-CoV-2 (42.8%), HAdV (21.4%), HPIV1/3 (21.4%), and IA (7.1%).

a.



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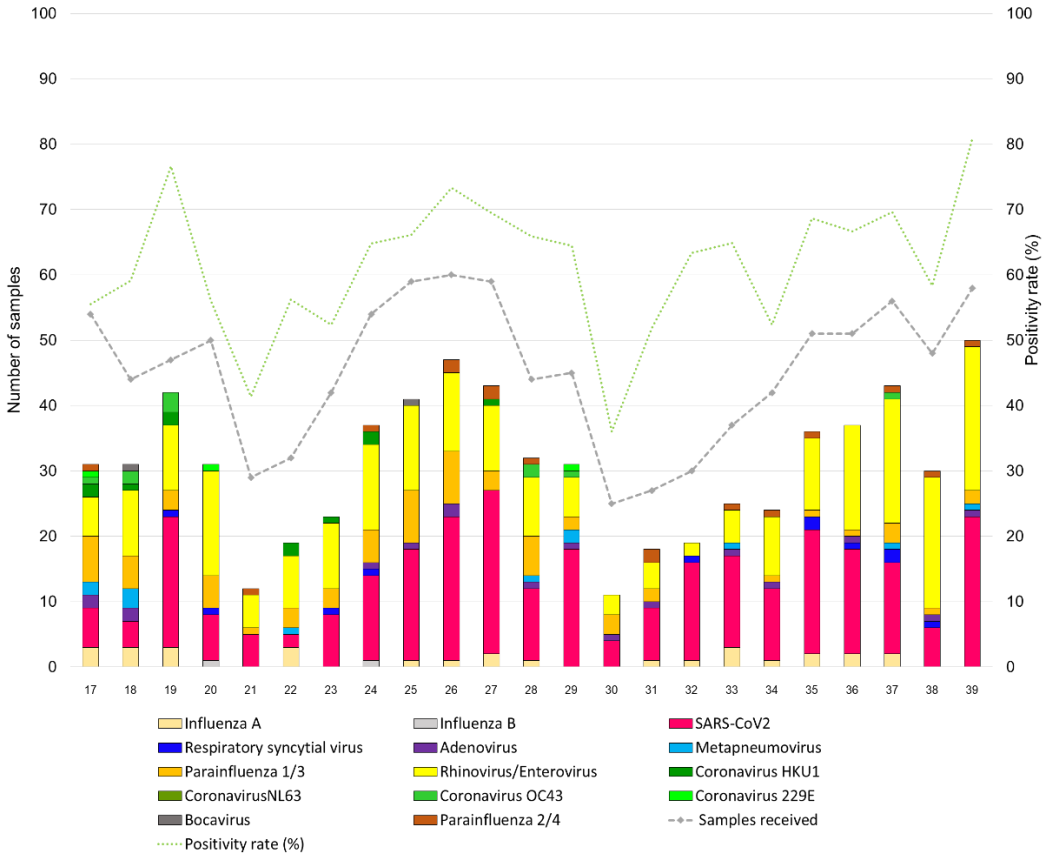
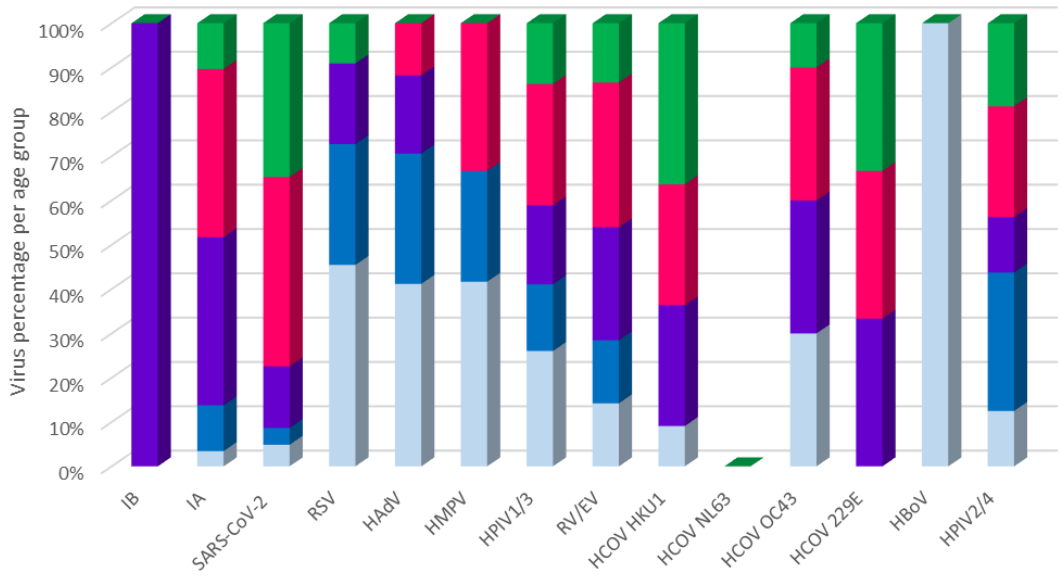


Figure 1. Percentage and temporal distribution of respiratory viruses detected in NPS collected from week 17/2022 to 39/2022. a. Percentages of the different respiratory viruses (N=713) detected in 1044 NPS. **b**. Distribution of the samples tested and the detected pathogens throughout the surveillance period. Positivity rate is based on the number of positive samples per total number of samples received each week.

When stratifying positive samples by age groups, a majority of RSV, HMPV and HAdV were observed in infants and toddlers (Figure 2a, b). Indeed, in the 0-4 year-old group, they represented 45.5%, 41.7% and 41.2%, of the detected viruses respectively (Figure 2a). The two HBoV positive samples identified during this period were also found in the 0-4 year-old group. HPIV2/4 was frequent in older children in whom they accounted for 31.3% of the positive samples. SARS-CoV-2 was present mostly in adults (Figure 2b) with 42.7%, 34.7% and 13.9% for the 30-64, ≥65 and 15-29 year-old groups, respectively. The 0-4 and 5-14 age groups accounted for 4.9% and 3.8% of the positive SARS-CoV-2 samples, respectively. Influenza A virus detection was equally distributed in the 30-64 and 15-29 groups, corresponding to 37.9%. The only two influenza B viruses were observed in the 15-29 years old group. RV/EV, HCoV OC43 and HPIV1/3 positive samples were more evenly distributed throughout all age groups.

a.



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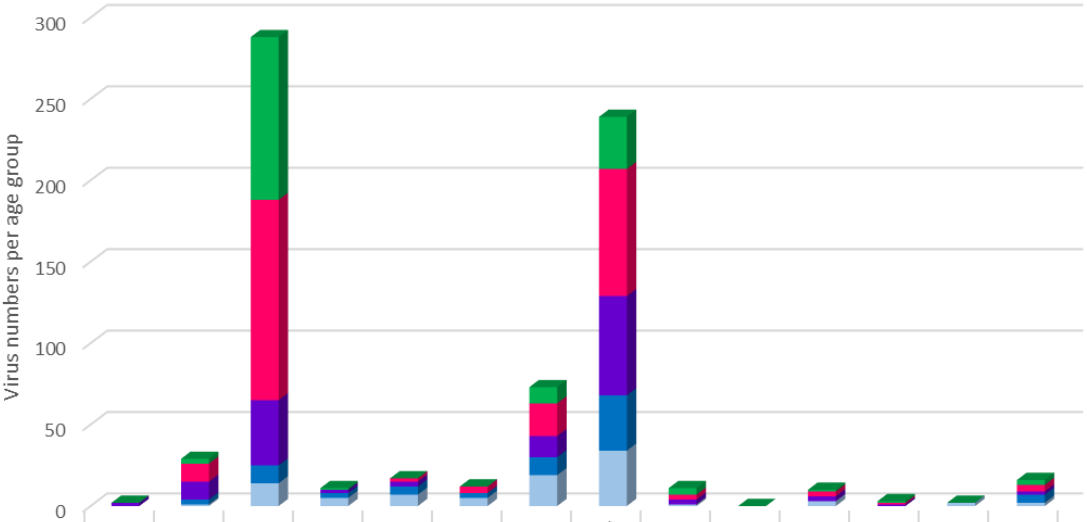


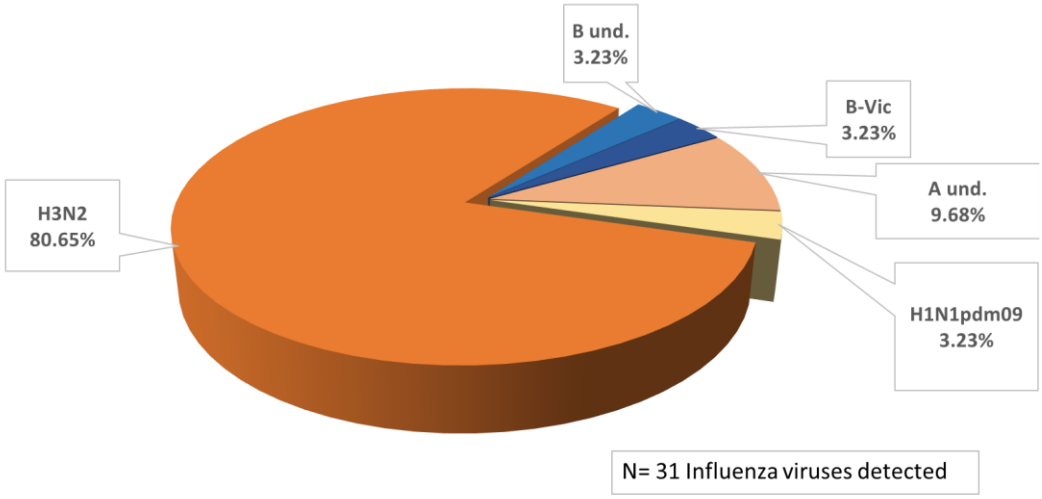
	IB	IA	SARS-CoV-2	RSV	HAdV	HMPV	HPIV1/3	RV/EV	HCOV HKU1	HCOV NL63	HCOV OC43	HCOV 229E	HBoV	HPIV2/4
≥65	0	3	100	1	0	0	10	32	4	0	1	1	0	3
30-64	0	11	123	0	2	4	20	78	3	0	3	1	0	4
15-29	2	11	40	2	3	0	13	61	3	0	3	1	0	2
5-14	0	3	11	3	5	3	11	34	0	0	0	0	0	5
0-4	0	1	14	5	7	5	19	34	1	0	3	0	2	2

Figure 2. Respiratory viruses' distribution: a. per age group in percent; **b.** in absolute numbers of positive samples.

2.1 Detection of influenza viruses

Among the 31 influenza viruses detected, 29 influenza A and 2 influenza B were identified (Figure 3a, b). Twenty-five influenza A were subtyped as A(H3N2) (80.7%) and one as A(H1N1)pdm09 (3.2%). Three samples (9.7%) could not be further subtyped due to low viral load. Of the two influenza B viruses, only one was further characterized as a B-Victoria lineage (3.2%) (Figure 3a). The median positivity rate for influenza was about 2.4% (range [0 to 9.4%]; 95% CI (1.2-3.5%)) (Figure 3b); with a peak at 9.4% during week 22/2022.

a.



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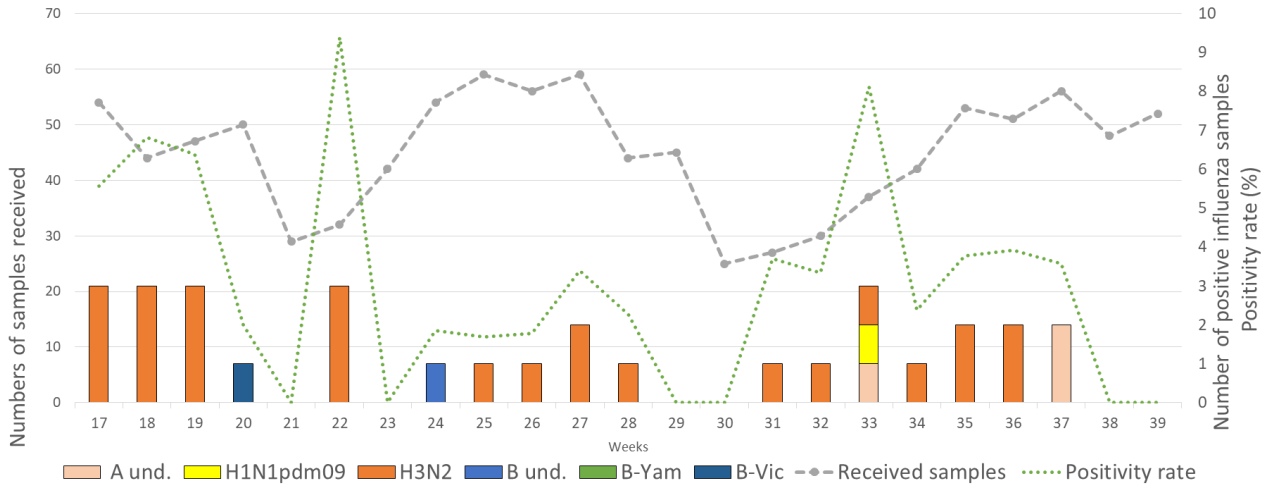

Figure 3. Percentage and temporal distribution of Influenza viruses detected in NPS collected from week 17/2022 to week 39/2022. a. Percentages of influenza viruses, subtypes (FluA) and lineages (FluB). N=31 influenza viruses. **b.** Distribution of the detected influenza viruses throughout the surveillance period. Influenza viruses typing and subtyping done by real-time rRT-PCR. A und. and B und.: influenza A and B viruses that could not be further subtyped. H1N1pdm09 and H3N2 refer to influenza A(H1N1)pdm09 and influenza A(H3N2), respectively. B-Yam: influenza B virus of Yamagata lineage. B-Vic: influenza B virus of Victoria lineage. Positivity rate is based on the number of weekly positive influenza samples per the number of samples received each week.

2.2 SARS-CoV-2 and influenza viruses characterization

2.2.1 SARS-CoV-2 genetic analysis and variants identification

Among the 288 SARS-CoV-2 positive samples identified from week 17/2022 to week 39/2022, 97 had Ct values lower than 32 and were further characterized by whole genome sequencing. Sequences for 90 samples were submitted to the global initiative on sharing all influenza data (GISAID) database and fell into 20 distinct Pangolin[111] lineages (Table 1). All the sequences were identified as belonging to variant of concern (VOC) Omicron, with a majority of BA.5.2 and BA.5.1 subvariants. First cases of VOC Omicron were detected in Switzerland beginning December 2021.

Table 1. List of the different SARS-CoV-2 Pangolin lineages within which Sentinel isolates were distributed

Pangolin[1] lineages (VOC)	Number of isolates (n=97)
BA.2	1
BA.2.12.1	1
BA.4	1
BA.4.1	1
BA.4.6	2
BA.5	3
BA.5.1	23
BA.5.1.1	1
BA.5.1.10	1
BA.5.1.5	1
BA.5.2	27
BA.5.2.1	13
BA.5.3.1	1
BA.5.9	1
BE.1	3
BE.1.1	5
BF.1	1
BF.2	1
BF.5	1
BF.7	2
NA	7

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

2.3 Antigenic and genetic characterization of influenza viruses

Cell culture isolation was performed for 96 negative-SARS-CoV-2 samples. Among those 96 samples, 22 were known to be influenza positive, and 13 grew on MDCK and/or MDCK-SIAT1 cells. Twelve of them further underwent antigenic characterization. Eleven out of 12 were antigenically characterized.

Ninety-six samples from March 2022 were submitted for genetic characterization. Ninety HA sequences were successfully recovered. Among these, 85 were A(H3N2) and 5 A(H1N1)pdm09 (Figures 4 and 5). Ninety NA sequences were also successfully recovered: 85 were A(H3N2), and 5 A(H1N1)pdm09 (Data not shown). Isolates with collection dates from January 2022 were shared with the Worldwide Influenza Centre (WIC)[2] for additional analysis and characterization (Appendixes 2 to 4). Furthermore, the ninety isolates which were successfully recovered were then submitted to GISAID (Appendix 5).

2.3.1 Characterization of influenza A (H3N2) viruses

Nine out of the 11 samples antigenically characterized were A(H3N2). Five isolates were well recognized by the antiserum raised against A/Darwin/9/2021 (recommended vaccine strain 2022/2023) (subclade 3C.2a1b.2a.2), 2 isolates were poorly (≥8-fold titers reduction compared to the homologous titer) recognized by both the A/Cambodia/e0826360/2020 and A/Darwin/9/2021 reference antisera, but reacted well with the antiserum raised against an A/HongKong/2671/2019 (subclade 3C.2a1b.1b) virus. Two isolates were poorly (≥8-fold titers reduction compared to the homologous) recognized by both the A/Cambodia/e0826360/2020 and A/Darwin/9/2021 reference antisera, but surprisingly showed reactivity within 2- to 4-fold the homologous titer in presence of the antiserum raised against an A/England/538/2018 virus, a “recent” 3C.3a1 (Data not shown).

According to the WIC report, most of the shared samples tested were recognized at reasonable levels by the antiserum raised against egg-propagated vaccine 2022/2023 A/Darwin/9/2021 (subclade 3C.2a1b.2a.2). All the isolates belong to the subclade 3C.2a1b.2a.2 (Appendix 2).

At the genetic level, 85 A(H3N2) HA1 (Figure 4) and 85 NA (A(H3N2)) (Data not shown) sequences were successfully recovered. All viruses fell into the genetic group 3C.2a1b.2a.2 represented by A/Bangladesh/4005/2020-like and carried this subclade

typical substitutions: Y159N, T160I, L164Q, G186D and D190N in HA1. We observed several sub-clusters characterised by specific mutations (D53N, G78D, D53G, D104G, I140M, H156S, R220K, and R299K) within the 3C.2a1b.2a.2 subclade (Figure 4).

As observed at the NRCI, the samples sequenced by the WIC belonged to the subclade 3C.2a1b.2a.2. They were also distributed along this 3C.2a1b.2a.2 subclade with the same specific mutations (Figure 4; appendix 2).

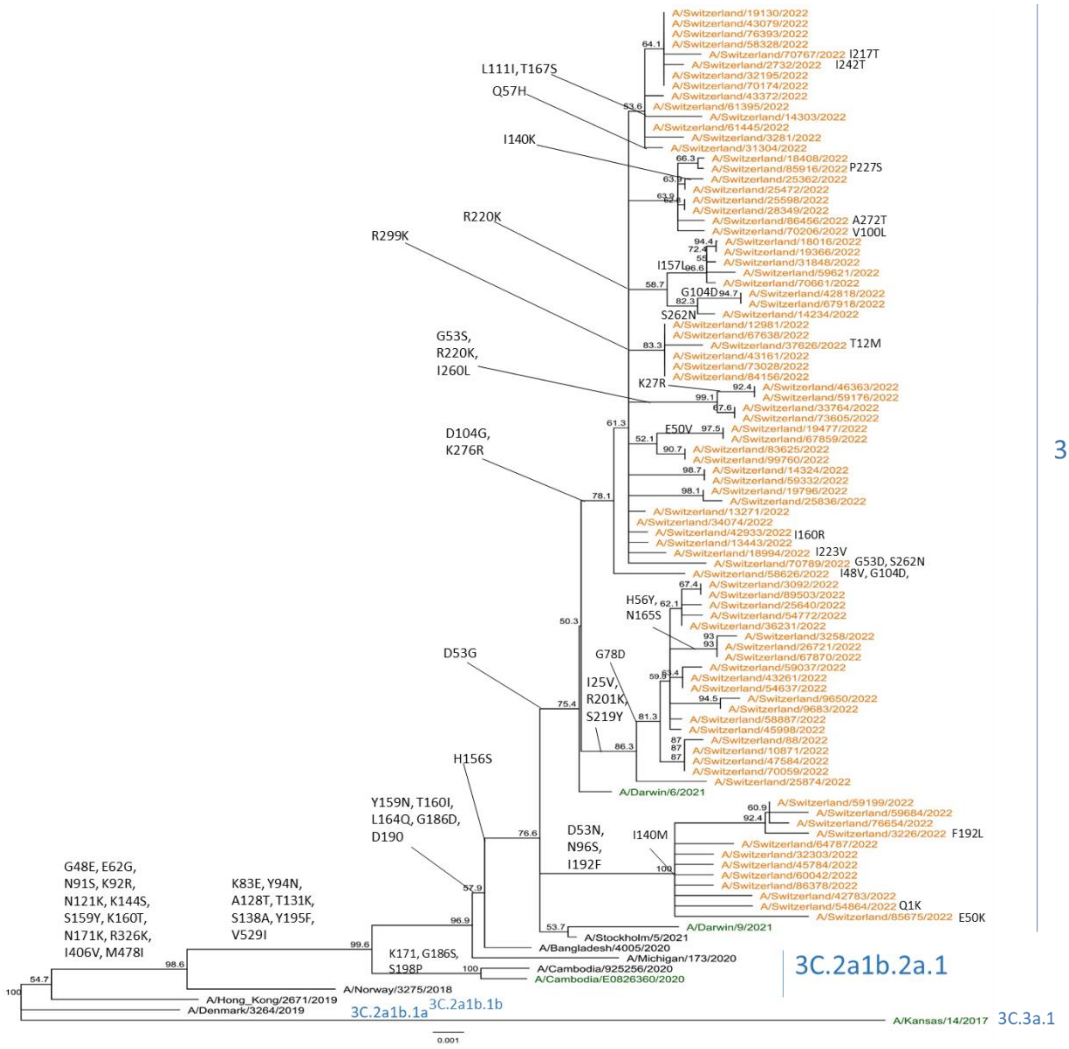

Figure 4. Phylogenetic analysis of the HA gene of A(H3N2) viruses. Orange: influenza viruses detected in the Sentinel network during the 2021/2022 season. Green: vaccine strains for NH 19/20 A/Kansas/14/2017, 21/22 A/Cambodia/E0826360/2020, SH 22 A/Darwin/9/2021 (egg-based), SH 22 A/Darwin/6/2021 (cell-based). Black: reference strains. Some typical substitutions characterizing the respective clusters described by the WIC and Nexclade V.2.8.1 are displayed in black. Blue: genetic groups/sub-groups. Sequences were aligned using Geneious Prime 2022.1.1 MAFT alignment with default settings. A consensus tree was built from 1000 original trees in maximum likelihood (50% support threshold) using Geneious Prime 2022.1.1 PHYML default settings.

2.3.2 Characterization of influenza A(H1N1pdm09) viruses

One A(H1N1)pdm09 virus was antigenically characterized. The isolate was well recognized by the antiserum raised against the recommended vaccine from northern hemisphere 2022/2023 A/Victoria/2570/2019 antiserum, but reacted poorly (>32x the homologous titre) with antiserum raised against the egg-based vaccine strain 2020/2021 A/Guangdong-Maonan/SWL1536/2019 (Data not shown).

Regarding the WIC analysis, 10 A(H1N1)pdm09 isolates collected from January 2022 were characterized. Nine viruses showed good recognition with the antiserum from the recommended vaccine 2020/2021 A/Guangdong-Maonan/SWL1536/2019 (Appendix 3).

At the genetic level, 5 HA and 5 NA genes were successfully sequenced (Figure 5; data not shown). All A(H1N1)pdm09 viruses sequenced at the NRCI fell into the 6B.1A.5a.1 subclade and carried the amino acid substitutions D187A and Q189E in HA1 (Figure 5). The A/Switzerland/36111/2022 isolate was separated from the others with specific subclade mutations S128T, P137S, G155E and belonged to the subgroup of the reference A/Lyon/820/2021 virus according to the WIC report. [2]

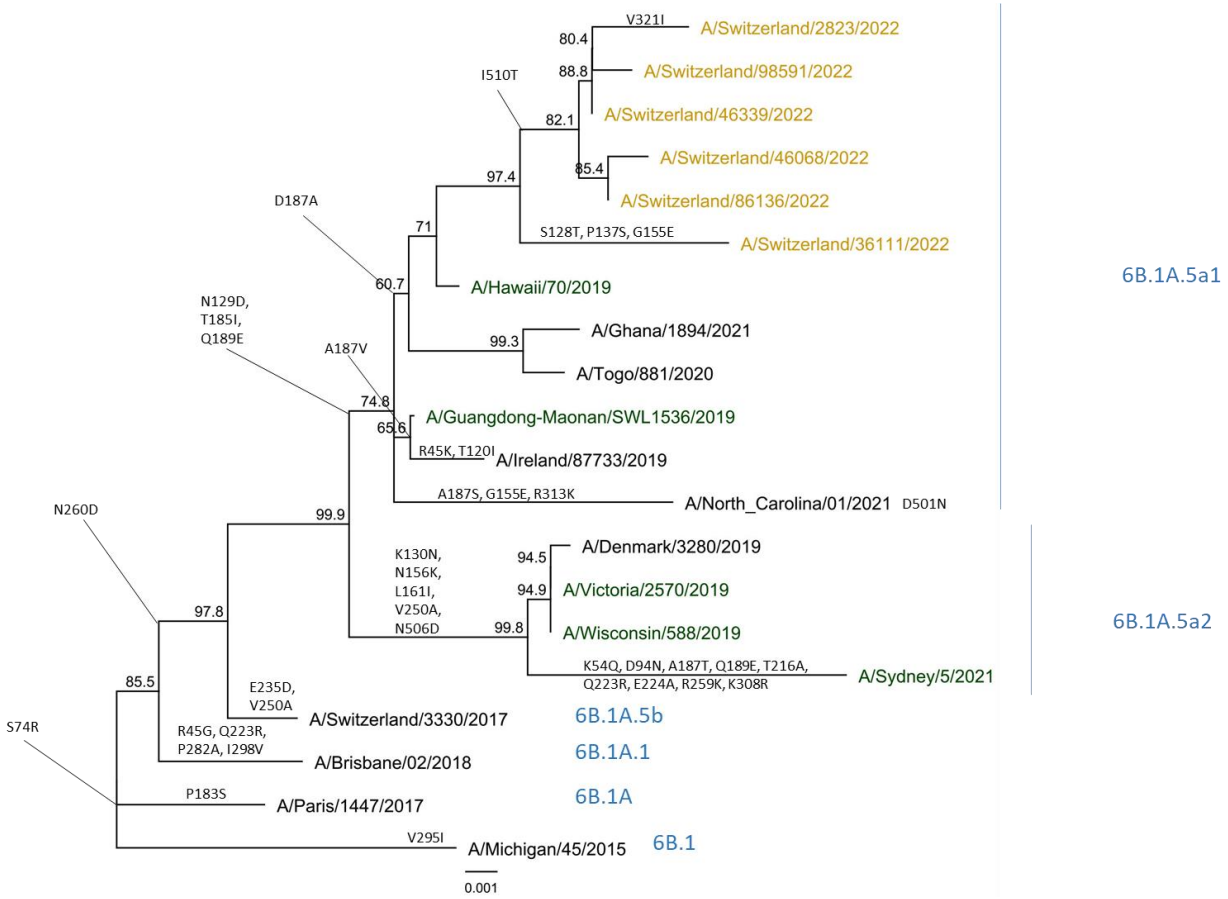

Figure 5. Phylogenetic analysis of the HA gene of A(H1N1)pdm09 viruses. Yellow: influenza viruses detected in the Sentinel network during the 2021/2022 season. Green: vaccine strains for 2020/2021 A/Guangdong-Maonan/SWL1536/2019 (egg-based), 2020/2021 A/Hawaii/70/2019 (cell-based), 2022/2023 A/Victoria/2570/2019 (egg-based), 2022/2023 A/Wisconsin/588/2019 (cell-based), 2023 A/Sydney/5/2021 (egg/cell-based). Black: reference strains. Some typical mutations characterizing the respective clusters described by the WIC and Nexclade V.2.8.1 are displayed in black. Blue: genetic groups/sub-groups. Sequences were aligned using Geneious Prime 2022.1.1 MAFT alignment with default settings. A consensus tree was built from 1000 original trees in maximum likelihood (50% support threshold) using Geneious Prime 2022.1.1 PHYML default settings.

2.3.3 Characterization of influenza B-Victoria viruses

Two influenza B viruses were identified by the NRCI from week 17/2022 to week 39/2022. Only one was characterized by HAI assay. The isolate reacted well with the antiserum raised against B/Austria/1359417/2021 virus (recommended vaccine southern hemisphere 2022, subclade V1A.3a.2) (Data not shown).

No influenza B sequence analysis was carried out and no isolate was analyzed by the WIC.

2.4 Antiviral resistance

Ninety (85 A(H3N2) and 5 A(H1N1)pdm09) samples were submitted to NA, and PA genes sequencing analysis to search for the antiviral resistance-associated mutations. None of the isolates displayed any mutations associated with a decreased

susceptibility to Baloxavir marboxil (Data not shown). Seventy-nine A(H3N2) and all the A(H1N1)pdm09 isolates did not display any mutations in the NA gene associated with reduced susceptibility to Oseltamivir (Data not shown). Four A(H3N2) samples exhibited the S331R mutation that can be associated with normal inhibition to reduced inhibition to antiviral treatment. Of note, S331R substitution has been associated with reduced sialidase activity based on the MUNANA assay, this altered enzyme characteristics render the use standard MUNANA-based assays unreliable for assessing susceptibility to neuraminidase inhibitors. Isolate A/Switzerland/67859/2022 bearing mutation S331R and tested phenotypically by the WIC, for oseltamivir and zanamivir susceptibility, exhibited normal inhibition by both antivirals (Appendix 9).

Twenty-four (14 A(H3N2) and 10 A(H1N1)pdm09) of 39 sentinel influenza viruses sent to the WIC had sufficient neuraminidase activity and were therefore eligible for phenotypic antiviral resistance testing. They were all sensitive to both oseltamivir and zanamivir (Appendix 9).

3 Worldwide

In the southern hemisphere, influenza viruses' detection raised up from week 17/2022 to week 25/2022, and then from week 34/2022 up to 39/2022 resulting in an extended late influenza activity compared to the 2020/2021 period. Influenza A of subtype A(H3N2) was overall dominant compared to A(H1N1)pdm09 except in some regions of Asia (eg. China) where influenza B virus of B-Victoria lineage from subclade 1A.3 dominated.[3] In the northern hemisphere, from week 30/2022, influenza activity remained low and at inter-seasonal levels in most European countries.[4] Globally few A(H1N1)pdm09 viruses were detected during the 2021/2022 season. They were attributed to 6B.1A.5a.1 and 6B.1A.5a.2 genetic subgroups, which were observed in several European countries as well as in Australia.[5] Influenza A(H3N2) viruses were detected worldwide and the vast majority fell into the 3C.2a1b.2a.2 subclade (referent A/Bangladesh/4005/2020-like), except in China where a majority of viruses were attributed to the 3C.2a1b.2a.1 (referent A/Cambodia/925256/2020) genetic subgroup.[5]

4 Zoonotic influenza infections

From week 17/2022 to week 39/2022, no samples were sent to the NRCI for a suspicion of zoonotic influenza transmission.

At the global scale and since 31rst of August 2022, 2 new infections of avian influenza A(H5N6) and A(H10N3) viruses from China and 3 new cases of human infection with swine influenza A(H1N2)v and A(H3N2)v viruses from the USA have been reported to the WHO.[6,7]

Since 28th September 2022, two new human cases of A(H5N1) were identified in the Eastern Asian region, and one asymptomatic case was detected in Spain in a poultry farmer.[8-10]

5 Avian influenza A[11]

During 2021/2022 season, the prevalence of avian influenza circulating, in wild birds and poultry, was the highest ever observed in Europe. Since 22nd of June 2022, a total of 739 HPAI outbreaks and no LPAI outbreaks have been reported.[12] A(H5N1) virus was dominant in 2022 compared to 2021 and, along with the A(H5N8) subtypes, was considered to be in persistent circulation. In Switzerland, four cases of HPAI A(H5N1) have been identified in a Grey Heron, a black-headed gull and 2 mute swans since 14 November 2022.[13]

6 Ongoing project

In order to develop alternative testing methods in case of conventional real-time polymerase chain reaction regents' shortage and/or the emergence of a new influenza variants requiring new diagnostic and/or subtyping tests, we started to investigate the "Respiratory Virus Oligo Panel with Illumina RNA Prep with Enrichment" next-generation sequencing. This is a collaborative project with the Heath 2030 Genome Center DNA Sequencing and Data Analytics and Interpretation Platforms' teams.

Methods based on target enrichment via hybrid-capture, as the one we have chosen, were developed to allow for a high sensitive detection and full genome sequencing of the targeted pathogen(s) directly from clinical samples. In addition, the oligo probes were designed in order to cope with microorganisms evolving rapidly.

The tested panel covers :human coronaviruses 229E, NL63, OC43, as well as SARS-CoV-2, human adenoviruses (B1, C2 and E4), human bocaviruses (1, 2c,3 and 4), human parainfluenza viruses (1, 2, 3 and 4a), human metapneumoviruses (CAN97-83), Respiratory syncytial viruses (type A and B), influenza A (H1N1, H1N1pdm09, H2N2, H3N2, H7N9, H9N2 and H5N1) and B (Victoria and Yamagata), polyomaviruses (KI and WU), human parechoviruses (1 and 6), Human rhinoviruses (A89, C, B14) and human enteroviruses (C104, C109). See for more details: https://www.illumina.com/products/by-type/sequencing-kits/library-prepkits/respiratory-virus-oligo-panel.html

Reverse transcription converted RNA into complementary DNA (cDNA), which was fragmented and then PCR amplified. Viral sequence-specific biotinylated probes combined with magnetic beads were used to capture region of interest. The resulting libraries were sequenced and the results analysed KrakenUniq to classify the reads to the respective viral genomes (based NCBI reference sequences). The consensus sequences of the detected viruses were reconstructed using the pipeline developed at the Health 2030 Genome Center used for SARS-CoV-2 analysis.

The panel was initially tested by the Heath 2030 Genome Center, using Twist Biosciences synthetic influenza A and B viruses as well as other Twist Biosciences synthetic respiratory viruses. The encouraging results obtained in this first experiment allowed us to proceed with true clinical samples (Data not shown). For the second and last experiment performed until now, we used 16 samples positive for influenza A and B, our main targets of interest, in order to have a rough idea of the panel sensitivity

(Table 2). We also included 14 samples positive for other respiratory viruses, including 7 with more than one virus, to draw a first picture of the panel specificity (Data not shown).

The first experiment using real clinical samples gave very promising results. Indeed, the expected viral genomes were detected at low concentrations and with a high specificity. Of note, the detection performance was dependent on the viral strain. The percent of mapping reads and coverage (80-100%, Table 2) correlated well with the viral load and, in most cases, a consensus sequence could also be reconstructed, even for samples with more than one pathogen (Figures 6 and 7).

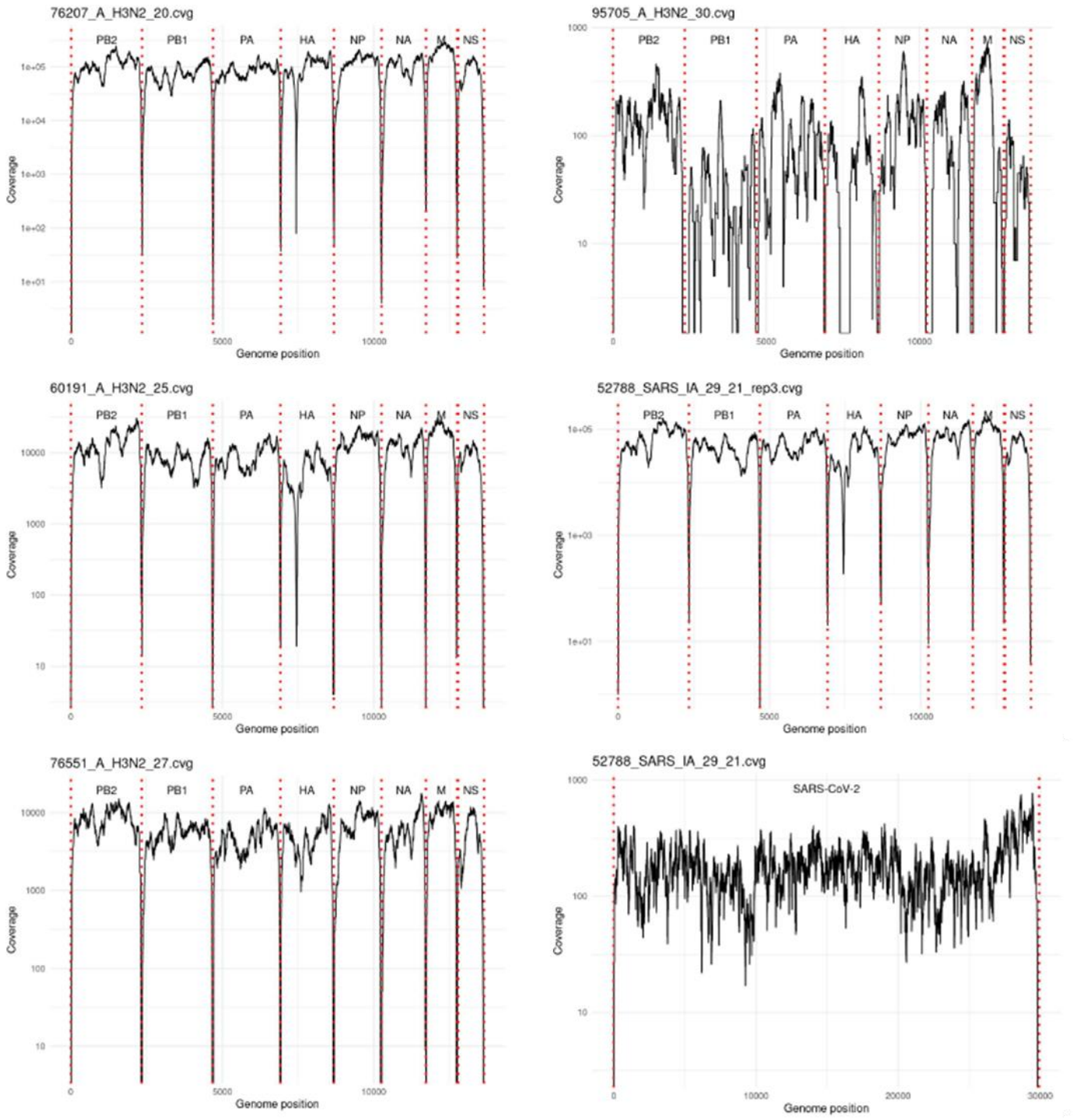

Figure 6. Genomic coverage obtained for influenza A(H3N2) and SARS-CoV-2. Coverage correlates well with the viral load.

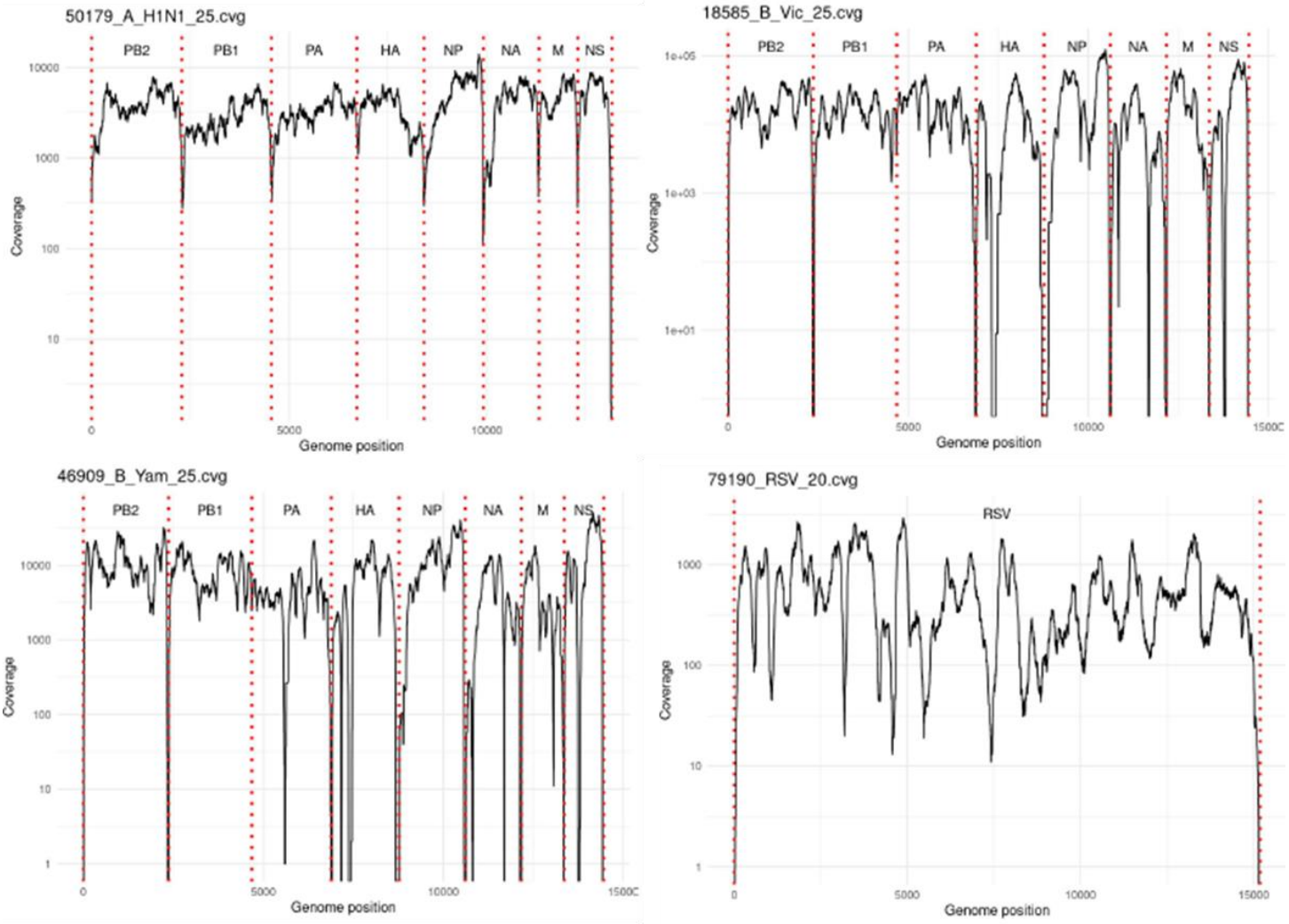

Figure 7. Genomic coverage obtained for influenza A (H1N1pdm09), influenza B and RSV. Coverage correlates well with the viral load.

To conclude, despite the fact that the sensitivity and specificity of the panel are encouraging, further testing is still required prior to panel implementation in our laboratory.

Table 2. Results of the Respiratory Virus Oligo Panel analysis for samples positive for SARS-CoV-2 and Influenza

	Respiratory Virus Oligo Panel results									
Coverage (0-1)	SARS-CoV-2		A (H3N2)		A (H1N1pdm09)		B (1940)		Homo sapiens	Number of raw reads
Sample pathogen(s) ID (Ct)	KrakenUniq	Mapping	KrakenUniq	Mapping	KrakenUniq	Mapping	KrakenUniq	Mapping	KrakenUniq	
12288 SARS (16) RVS (27) run in triplicates	0.96	1					0.01		0.02	24 569 107.00
52788 SARS (29) A_H3N2 (21) run in triplicates	0.9	0.99	0.59	1	0.04				0.01	4 620 833.00
76207 A_H3N2 (20)	0.03		0.67	1	0.05				0.06	10 849 475.00
60191 A_H3N2 (25)			0.45	1	0.03				0.04	2 996 790.00
76551 A_H3N2 (27)			0.43	0.98	0.02				0.08	19 541 485.00
95705 A_H3N2 (30) run in triplicates	0.01		0.27	0.91					0.14	14 046 523.00
37805 A_H1N1 (20)	0.01		0.03		0.66	0.99			0.08	8 586 977.00
50179 A_H1N1 (25)	0.02		0.01		0.43	0.99			0.09	7 106 606.00
95790 A_H1N1 (27)	0.02				0.34	0.98			0.11	8 372 382.00
34338 A_H1N1 (30)					0.35	0.93			0.03	1 245 244.00
40555 B_Vic (20)	0.21						0.22	0.98	0.08	194 153 429.00
18585 B_Vic (25)	0.02						0.18	0.96	0	12 341 516.00
29442 B_Vic (27)							0.14	0.95	0.05	6 156 569.00
74630 B_Vic (30) run in triplicates							0.09	0.94	0.07	6 449 141.00
40747 B_Yam (20)	0.01						0.2	0.97	0	6 971 620.00
46909 B_Yam (25)							0.17	0.97	0.03	4 560 362.00
47032 B_Yam (27)	0.02						0.14	0.96	0.03	3 082 733.00
67672 B_Yam (30) run in triplicates							0.09	0.91	0.02	1 658 014.00

For samples run in triplicates only one of them is shown.

7 External quality controls

In order to assess its analytical and methodological performance, the NRCI participates yearly to different external quality assessment programs for the detection of Influenza, SARS-CoV-2 and other respiratory viruses.

The tables below resume the results of our external quality control assessments from 2020 to 2022.

Table 3. External Quality Assessment Programme panel 21 (2022) for influenza

Sample No.	Intended results		Obtained results	Remarks		
V01-2022	IA(H9)		IA(H9)	Certificate of completion pending		
V02-2022	IB		IB (Victoria lineage)			
V03-2022	IA(H1)pdm09		IA(H1N1)pdm09			
V04-2022	IA/IB negative		Negative			
V05-2022	IA(H9)		IA(H9)			
V06-2022	IB		IB (Yamagata lineage)			
V07-2022	IA(H3)		IA(H3N2)			
V08-2022	IA(H5)		IA(H5N8)			
V09-2022	IA(H7)		IA(H7N9)			
V10-2022	IA/IB negative(+)		SARS-CoV-2	***Genotypic testing***	***Phenotypic testing***	
Sample No.	**Type/Subtype**	**Results**	**amino acid substitution**	**Associated with (Highly) reduced inhibition**	**Oseltamivir**	**Zanamivir**
NAI01P-2022	IA(H1N1)pdm09	Intended	H275Y (C823T)	Yes	(Highly) reduced inhibition	Normal inhibition
NAI01G-2022		Obtained	H275Y (C823T)	Yes	(Highly) reduced inhibition	Normal inhibition
NAI02P-2022	IA(H3N2)	Intended	**E119V (A356T)	Yes	(Highly) reduced inhibition	Normal inhibition
NAI02G-2022		Obtained	**E119V (A356T)	Yes	(Highly) reduced inhibition	Normal inhibition
NAI03P-2022	IA(H3N2)	Intended	NA-no mutation; ^PA I38M	No	Normal inhibition	Normal inhibition
NAI03G-2022		Obtained	NA-no mutation; ^PA I38M	No	Normal inhibition	Normal inhibition

(+) Sample contains SARS-CoV-2 for educational purpose
* Residue position in N1 Neuraminidase numbering
** Residue position in N2 neuraminidase numbering
^Sample contains PA I38M substitution associated with reduced inhibition by baloxavir (PA resul for educational purpose)

Table 4. External Quality Assessment Programme panel 21 (2022) for SARS-CoV-2

Sample No.	Intended results	Obtained results	Remarks
2022-01	SARS-CoV-2	SARS-CoV-2	Certificate of completion pending
2022-02	SARS-CoV-2	SARS-CoV-2	
2022-03	SARS-CoV-2 negative/(+)IB	IB	
2022-04	SARS-CoV-2 negative/HCoV OC43	HcoV OC43	
2022-05	SARS-CoV-2	SARS-CoV-2	

(+)Sample contains influenza B for educational purpose

Table 4. Quality Control for Molecular Diagnostics (QCMD) 2022 Respiratory I/II Program (QAV164188_89_2)

Sample No.	Intended results	Obtained results	Detection frequency	Detection score	Remarks
RESPI22C1-01	IB (Victoria lineage)	IB (Victoria lineage)	Frequently detected	highly satisfactory	Certificate of completion obtained
RESPI22C1-02	IB (Yamagata lineage)	IB (Yamagata lineage)	Frequently detected	highly satisfactory	
RESPI22C1-03	IA (H1N1)pdm09	IA	Frequently detected	highly satisfactory	
RESPI22C1-04	IA; RSV	IA; RSV	Detected	highly satisfactory	
RESPI22C1-05	RSV	RSV	Frequently detected	highly satisfactory	
RESPI22C2-01	IA(H3N2)	IA	Frequently detected	highly satisfactory	
RESPI22C2-02	Negative	Negative	Negative	highly satisfactory	
RESPI22C2-03	IB (Victoria lineage)	IB	Frequently detected	highly satisfactory	
RESPI22C2-04	RSV	RSV	Frequently detected	highly satisfactory	
RESPI22C2-05	IA(H3N2)	IA	Frequently detected	highly satisfactory	
RESPII22C1-01	EV	EV	Detected	highly satisfactory	
RESPII22C1-02	hCoV	HCoV NL63	Detected	highly satisfactory	
RESPII22C1-03	HPIV	HPIV1/3	Frequently detected	highly satisfactory	
RESPII22C1-04	HMPV	HMPV	Frequently detected	highly satisfactory	
RESPII22C1-05	AdV	AdV	Frequently detected	highly satisfactory	
RESPII22C2-01	Negative	Negative	Negative	highly satisfactory	
RESPII22C2-02	AdV	AdV	Detected	highly satisfactory	
RESPII22C2-03	RV	RV	Detected	highly satisfactory	
RESPII22C2-04	HPIV	HPIV1/3	Frequently detected	highly satisfactory	
RESPII22C2-05	HCoV OC43	HCoV OC43	Detected	highly satisfactory	

Table 5. External Quality Assessment Programme panel 20 (2021) for influenza

Sample No.	Intended results	Obtained results	Remarks
V01-2021	IB	IB (Victoria lineage)	Certificate of completion obtained
V02-2021	negative IA/B (+)	SARS-CoV-2	
V03-2021	IA(H3)	IA(H3N2)	
V04-2021	IA(H9)	IA(H9)	
V05-2021	IA(H1)pdm09	IA(H1N1)pdm09	
V06-2021	IA(H7)	IA(H7N9)	
V07-2021	IA(H5)	IA(H5N6)	
V08-2021	IA(H9)	IA(H9)	
V09-2021	IA(H5N6)	IA(H5N6)	
V10-2021	IB	IB (Yamagata lineage)	

				Genotypic testing	*Phenotypic testing*	
Sample No.	Type/Subtype	Results	amino acid substitution	Associated with (Highly) reduced inhibition	Oseltamivir	Zanamivir
NAI01P-2021	IA(H1N1)pdm09	Intended	NA-no mutation; ^PA I38M	No	Normal inhibition	Normal inhibition
NAI01G-2021		Obtained	NA-no mutation; [PA-I38T (T113C)]	No [I38T is associated with reduced inhibition by baloxavir]	Normal inhibition	Normal inhibition
NAI02P-2021		Intended	Mixture of wild-type and H275Y (C823T)	Yes	(Highly) reduced inhibition	Normal inhibition
NAI02G-2021		Obtained	H275Y (C823T)	Yes-Oseltamivir and Peramivir	(Highly) reduced inhibition	Normal inhibition
NAI03P-2021	IB (Yamagata)	Intended	Wild-type	No	Normal inhibition	Normal inhibition
NAI03G-2021		Obtained	Wild-type	No	Normal inhibition	Normal inhibition

(+) Sample contains SARS-CoV-2 for educational purpose

* Residue position in N1 Neuraminidase numbering

** Residue position in N2 neuraminidase numbering

^Sample contains PA I38M substitution associated with reduced inhibition by baloxavir (PA resul for educational purpose)

Table 6. External Quality Assessment Programme panel 20 (2021) for SARS-CoV-2

Sample No.	Intended results	Obtained results	Remarks
2021-01	Negative SARS-CoV-2 /HCoV OC43	HCoV OC43	Certificate of completion obtained
2021-02	SARS-CoV-2	SARS-CoV-2	
2021-03	Negative SARS-CoV-2 /(+)IA	IA(H1N1)pdm09	
2021-04	SARS-CoV-2	SARS-CoV-2	
2021-05	SARS-CoV-2	SARS-CoV-2	

(+)Sample contains influenza A for educational purpose

Table 7. Quality Control for Molecular Diagnostics (QCMD) 2021 Respiratory I/II programme (QAV164188_89_2)

Sample No.	Intended results	Obtained results	Detection frequency	Detection score	Remarks
RESPI21C1-01	IA(H1N1)pdm09	IA	Frequently detected	highly satisfactory	Certificate of completion obtained
RESPI21C1-02	IB(Victoria lineage)	IB	Frequently detected	highly satisfactory	
RESPI21C1-03	IB (Yamagata lineage)	IB	Frequently detected	highly satisfactory	
RESPI21C1-04	RSV	RSV	Detected	highly satisfactory	
RESPI21C1-05	Negative	Negative	Negative	highly satisfactory	
RESPI21C2-01	IA (H3N2)	IA	Frequently detected	highly satisfactory	
RESPI21C2-02	IA (H3N2)	IA	Detected	highly satisfactory	
RESPI21C2-03	RSV	RSV	Detected	highly satisfactory	
RESPI21C2-04	IB(Victoria lineage)	IB	Frequently detected	highly satisfactory	
RESPI21C2-05	IA; RSV	IA; RSV	Detected	highly satisfactory	
RESPII21C1-01	EV	EV	Detected	highly satisfactory	
RESPII21C1-02	ADV	ADV	Detected	highly satisfactory	
RESPII21C1-03	HPIV1	HPIV1	Frequently detected	highly satisfactory	
RESPII21C1-04	ADV	ADV	Frequently detected	highly satisfactory	
RESPII21C1-05	Negative	Negative	Negative	highly satisfactory	
RESPII21C2-01	HCoV NL63	HCoV NL63	Detected	highly satisfactory	
RESPII21C2-02	HMPV	HMPV	Frequently detected	highly satisfactory	
RESPII21C2-03	RV	RV	Detected	highly satisfactory	
RESPII21C2-04	HMPV	HMPV	Frequently detected	highly satisfactory	
RESPII21C2-05	HCoV OC43	HCoV OC43	Detected	highly satisfactory	

Table 8. External Quality Assessment Programme panel 19 (2020) for influenza

Sample No.	Intended results	Obtained results	Remarks
V01-2020	IA(H7)	IA(H7N9)	Certificate of completion obtained
V02-2020	IA(H1)pdm09	IA(H1N1)pdm09	
V03-2020	IB	IB (Yamagata lineage)	
V04-2020	IA(H5)	IA(H5N1)	
V05-2020	IB	IB (Victoria lineage)	
V06-2020	IA(H9)	IA(H9)	
V07-2020	IA(H5)	IA(H5N6)	
V08-2020	IA(H3)	IA(H3N2)	
V09-2020	Negative	Negative	
V10-2020	IA(H5)	IA (H5N6)	

				Genotypic testing	*Phenotypic testing*	
Sample No.	Type/Subtype	Results	amino acid substitution	Associated with (Highly) reduced inhibition	Oseltamivir	Zanamivir
NAI01P-2020	IA(H1N1)pdm09	Intended	Mixture of wild-type and H275Y (C823T)	Yes	(Highly) reduced inhibition	Normal inhibition
NAI01G-2020		Obtained	H275Y (C823T)	Yes	(Highly) reduced inhibition	Normal inhibition
NAI02P-2020	IA(H1N1)pdm09	Intended	NA-no mutation; ^PA I38T	No	Normal inhibition	Normal inhibition
NAI02G-2020		Obtained	Wild-type	No	Normal inhibition	Normal inhibition
NAI03P-2020	IB (Victoria)	Intended	Wild-type	No	Normal inhibition	Normal inhibition
NAI03G-2020		Obtained	Wild-type	No	Normal inhibition	Normal inhibition

^Sample contains PA-I38T mutation associated with reduced inhibition to Baloxavir

Table 9. External Quality Assessment Programme panel 19 (2020) for SARS-CoV-2

Sample No.	Intended results	Obtained results	Remarks
2020-01	SARS-CoV-2	SARS-CoV-2	Certificate of completion obtained
2020-02	SARS-CoV-2	SARS-CoV-2	
2020-03	Negative SARS-CoV-2	Negative SARS-CoV-2	
2020-04	Negative SARS-CoV-2/HCoV OC43	HCoV OC43	
2020-05	SARS-CoV-2	SARS-CoV-2	

Table 10. Panel EEQIAP 2020 molecular detection, antigenic and genetic characterization

Sample No.	Results	Subtype/Lineage	Antigenic Category	Genetic category
	Intended	A(H3N2)	A(H3) A/Hong Kong/4801/2014-like	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016
EISN_INF20-1	Obtained	A(H3N2)	A(H3) A/Hong Kong/4801/2014-like	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016
	Intended	NA	NA	NA
EISN_INF20-2	Obtained	NA	NA	NA
	Intended	A(H1N1)pdm09	A(H1)pdm09 A/Brisbane/02/2018-like but also very similar to A/Michigan/45/2015	A(H1)pdm09 clade 6B.1A5A representative A/Norway/3433/2018
EISN_INF20-3	Obtained	A(H1N1)pdm09	A(H1)pdm09 A/California/7/2009-like	A(H1)pdm09 clade 6B.1A5A representative A/Norway/3433/2018
	Intended	A(H3N2)	A(H3) A/South Australia/34/2019-like	A(H3) clade 3C.2a1b +131K A/South Australia/34/2019
EISN_INF20-4	Obtained	A(H3N2)	A(H3) A/Singapore/INFIMH-16-0019/2016-like	A(H3) clade 3C.2a1b +131K A/South Australia/34/2019
	Intended	B/Victoria	B(Vic) lineage not attributed to category; low reacto B/Brisbane/60/2008	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008
EISN_INF20-5	Obtained	B/Victoria	B(Vic) B/Colorado/06/2017-like	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008
	Intended	B/Yamagata	B(Yam) B/Phuket/3073/2013, but still considered B/Yam B/Phuket/3073/2013-like	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013
EISN_INF20-6	Obtained	B/Yamagata	B(Yam) B/Phuket/3073/2013	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013
	Intended	A(H3N2)	A(H3) A/Kansas/14/2017-like; similar to A/England/538/2018	A(H3) clade 3C.3a representative A/Kansas/14/2017
EISN_INF20-7	Obtained	A(H3N2)	A(H3) A/Kansas/14/2017-like	A(H3) clade 3C.3a representative A/Kansas/14/2017
	Intended	B/Victoria	B(Vic) B/Colordado/06/2017-like	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017
EISN_INF20-8	Obtained	B/Victoria	B(Vic) B/Colordado/06/2017-like	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017
	Intended	A(H1N1)pdm09 (provided)	NA	NA
EISN_AV20-1	Obtained	NA	NA	NA
	Intended	A(H3N2) (provided)	NA	NA
EISN_AV20-2	Obtained	NA	NA	NA

Table 11. Panel EEQIAP 2020 antiviral susceptibility

Sample No.	Results	Phenotypic testing		Genotypic testing	
		Oseltamivir	Zanamivir	Oseltamivir	Zanamivir
	Inended	NI	NI	AANI	AANI
EISN_INF20-1	Obtained	NA	NA	AANI	AANI
	Inended	NA	NA	NA	NA
EISN_INF20-2	Obtained	NA	NA	NA	NA
	Inended	NI	NI	AANI	AANI
EISN_INF20-3	Obtained	NA	NA	AANI	AANI
	Inended	NI	NI	AANI	AANI
EISN_INF20-4	Obtained	NI	NI	AANI	AANI
	Inended	NI	NI	AANI	AANI
EISN_INF20-5	Obtained	NI	NI	AANI	AANI
	Inended	NI	NI	AANI	AANI
EISN_INF20-6	Obtained	NA	NA	AANI	AANI
	Inended	NI	NI	AANI	AANI
EISN_INF20-7	Obtained	NA	NA	AANI	AANI
	Inended	NI	NI	AANI	AANI
EISN_INF20-8	Obtained	NA	NA	AANI	AANI
	Inended	RI	NI	AARI NA D199E	AANI
EISN_AV20-1	Obtained	NI	NI	AANI	AANI
	Inended	HRI	HRI	AAHRI NA-E119V + aa del245-248	AAHRI NA-E119V + aa del245-248
EISN_AV20-2	Obtained	HRI	RI	AAHRI NA-E119V + aa del245-248	AAHRI NA-E119V + aa del245-248

NI= normal inhibition (fold-change IC50; A<10; B<5)
RI= reduced inhibition (fold-change IC50; A≥10 & ≤ 100; B ≥ 5 & ≤ 50)
HRI= highly reduced inhibited (fold-change IC50; A >100; B>50)
AANI= no amino acid substitutions previously associated with RI or HRI
AARI= amino acid substitutions previously associated with RI
AAHRI= amino acid substitutions previously associated with HRI
NA= not applicable

8 References

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Geneva, December 22nd 2022

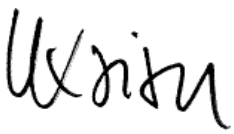
Mrs Tania Spedaliero

Dr Ana Rita Gonçalves Cabecinhas

Mrs Patricia Suter-Boquete

Dr Manuel Schibler

Professor Laurent Kaiser



Appendix 1: Detailed description of the observed co-detections, n=49 (17/2022-39/2022)

Weeks	Co-infections					
17	HMPV/SARS-CoV2					1
18	HPIV1-3/HAdV	HPIV1-3/SARS-CoV2/RV-EV	HBoV/RV-EV	HCoVOC43/HCoVHKU1		4
19	IA/HCoVOC43	SARS-CoV2/HCoVHKU1	RV-EV/SARS-CoV2	RV-EV/SARS-CoV2	VRS/SARS-CoV2	5
20	VRS/SARS-CoV2	HPIV1-3/IB	HPIV1-3/SARS-CoV2			3
22	HCoVHKU1/RV-EV					1
23	HPIV1-3/RV-EV					1
24	HPIV1-3/RV-EV	IB/SARS-CoV2				2
25	RV-EV/HAdV	RV-EV/SARS-CoV2				2
26	HPIV1-3/RV-EV	RV-EV/HAdV	RV-EV/SARS-CoV2			3
27	RV-EV/SARS-CoV2	HPIV2-4/ RV-EV				2
28	HPIV1-3/RV-EV	HCoVOC43/HAdV	RV-EV/IA			3
29	HCoVOC43/SARS-CoV2	SARS-CoV2/ HAdV				2
30	RV-EV/HAdV	HPIV1-3/SARS-CoV2				2
31	IA/HPIV2-4/ SARS-CoV2	HPIV1-3/SARS-CoV2	RV-EV/SARS-CoV2			3
33	IA/SARS-CoV2					1
34	RV-EV/HAdV	RV-EV/SARS-CoV2				2
35	RV-EV/SARS-CoV2					1
36	RV-EV/HAdV	RV-EV/SARS-CoV2	IA/VRS			3
37	HPIV1-3/IA	HCoVOC43/HPIV2-4	VRS/RV-EV	RV-EV/IA		4
38	HAdV /VRS					1
39	RV-EV/SARS-CoV2	RV-EV/SARS-CoV2	RV-EV/HAdV			3
Total						**49**

Appendix 2: Antigenic analyses of influenza A(H3N2) viruses (with 20nM Oseltamivir) 2022-06-24, WIC

Viruses	Other information	Collection date	Passage history	Haemagglutination inhibition titre								
				Post-infection ferret antisera								
				A/Denmark	A/HK	A/Camb	A/Camb	A/Bang	A/Darwin	A/Stock	A/Eng	A/Kansas
				3264/19	2671/19	e0826360/20	925256/20	4005/20	9/21	5/21	214191723/21	14/17
	Passage history			SIAT	Cell	Egg	SIAT	SIAT	Egg	SIAT	SIAT	SIAT
	Ferret number			F19/20[*1]	St Judes F21/20[*1]	F10/21[*1]	F03/21[*1]	F07/21[*1]	F38/21[*1]	F35/21[*1]	F07/22[*1]	F17/19[*1]
	Genetic group			3C.2a1b.1a	3C.2a1b.1b	3C.2a1b.2a.1	3C.2a1b.2a.1	3C.2a1b.2a.2	3C.2a1b.2a.2	3C.2a1b.2a.2	3C.2a1b.2a.2	3C.3a1
REFERENCE VIRUSES												
A/Denmark/3264/2019	3C.2a1b.1a	2019-10-25	SIAT3/SIAT4	320	160	160	640	160	320	160	40	80
A/Hong Kong/2671/2019	3C.2a1b.1b	2019-06-17	MDCK1/SIAT4	320	320	160	640	160	160	160	40	160
A/Cambodia/e0826360/2020	3C.2a1b.2a.1	2020-07-16	E5/E2	80	<	1280	160	160	320	160	320	80
A/Cambodia/925256/2020	3C.2a1b.2a.1	2020-09-25	SIAT5	160	160	320	640	160	320	160	40	160
A/Bangladesh/4005/2020	3C.2a1b.2a.2	2020-10-04	SIAT3	160	40	320	320	640	640	640	640	320
A/Darwin/9/2021	3C.2a1b.2a.2	2021-04-17	E3/E4	160	<	640	160	640	2560	1280	640	80
A/Stockholm/5/2021	3C.2a1b.2a.2	2021-04-16	SIAT0/SIAT3	160	<	160	160	320	1280	640	320	40
A/England/214191723/2021	3C.2a1b.2a.2	2021-10-12	MDCK1/SIAT3	80	<	80	80	320	640	320	640	40
A/Kansas/14/2017	3C.3a1	2017-12-14	SIAT3/SIAT2	40	<	80	80	40	80	80	40	320
TEST VIRUSES												
A/Switzerland/09683/2022		2022-03-30	MDCK0/SIAT2	320	<	640	80	320	1280	640	640	160
A/Switzerland/18559/2022		2022-03-08	SIAT1	160	<	320	80	320	1280	640	640	80
A/Switzerland/60042/2022		2022-03-10	SIAT1	40	<	80	40	160	640	160	640	<
A/Switzerland/12981/2022		2022-03-14	SIAT1	40	<	80	40	160	640	320	320	<
A/Switzerland/73028/2022		2022-03-16	SIAT1	40	<	80	40	160	640	160	320	<
A/Switzerland/34074//2022		2022-03-18	SIAT1	40	<	80	40	160	640	320	320	<
A/Switzerland/45784/2022		2022-03-21	SIAT1	40	<	80	40	160	640	320	640	<
A/Switzerland/10871/2022		2022-03-28	SIAT1	80	<	160	80	320	640	640	640	40
A/Switzerland/18016/2022		2022-03-28	SIAT1	80	<	160	40	160	320	320	320	40
A/Switzerland/19130/2022		2022-03-28	SIAT1	40	<	80	40	320	640	320	320	<
A/Switzerland/54637/2022		2022-03-30	SIAT1	320	40	640	320	640	1280	1280	640	160
A/Switzerland/76393/2022		2022-03-31	SIAT1	80	<	80	40	160	640	320	320	<
A/Switzerland/89503/2022		2022-03-31	SIAT1	160	<	320	160	320	1280	1280	640	80
A/Switzerland/64395/2022		2022-04-02	SIAT1	80	<	80	40	160	320	320	320	40
A/Switzerland/83625/2022		2022-04-05	SIAT1	40	<	80	40	160	320	320	320	40
A/Switzerland/83466/2022		2022-04-07	SIAT1	40	<	80	40	160	320	320	320	40
A/Switzerland/84156/2022		2022-04-12	SIAT1	80	<	80	40	160	320	320	320	40
A/Switzerland/67859/2022		2022-04-19	SIAT1	40	<	40	40	160	320	320	320	40
A/Switzerland/03092/2022		2022-04-25	SIAT1	160	<	320	160	640	1280	640	640	80
						Vaccine NH 2021			Vaccine NH 2022			

* Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used)
1 < = <40, ND = Not Done

Appendix 3: Antigenic analyses of influenza A(H1N1)pdm09 viruses 2022-06-28, WIC

Viruses	Other information	Passage history / Ferret number / Genetic group	Collection date	Passage history	Haemagglutination inhibition titre: Post-infection fereret antisera							
					A/Ire 87733/19 Egg St Jude's F18/20*1 6B.1A.5a.1	A/G-M SWL1536/19 Egg F12/20*1 6B.1A.5a.1	A/G-M SWL1536/19 MDCK F09/20*1 6B.1A.5a.1	A/Ghana 1894/21 Egg F02/22*1 6B.1A.5a.1	A/Lyon 820/21 Egg F06/22*1 6B.1A.5a.1	A/Denmark 3280/19 MDCK F08/20*1 6B.1A.5a.2	IVR-215 A/Vic/2570/19 Egg F37/21*1 6B.1A.5a.2	A/Sydney 5/21 Egg F04/22*1 6B.1A.5a.2
REFERENCE VIRUSES												
A/Ireland/87733/2019		6B.1A.5a.1	2019-11-03	E4	320	1280	1280	320	160	<	40	<
A/Guangdong-Maonan/SWL1536/2019		6B.1A.5a.1	2019-06-17	E3/E2	640	1280	1280	640	160	<	40	<
A/Guangdong-Maonan/SWL1536/2019		6B.1A.5a.1	2019-06-17	C2/MDCK1	640	1280	1280	640	320	<	80	40
A/Ghana/1894/2021		6B.1A.5a.1	2021-07-21	E2/E1	640	1280	1280	640	160	<	80	40
A/Lyon/820/2021		6B.1A.5a.1	2021-11-16	E1/E2	80	320	320	160	320	<	40	40
A/Denmark/3280/2019		6B.1A.5a.2	2019-11-10	MDCK4/MDCK6	<	80	40	40	80	640	1280	640
IVR-215 (A/Victoria/2570/2019)		6B.1A.5a.2	2018-11-22	E4/D7/E2	40	40	80	40	80	640	1280	1280
A/Sydney/5/2021		6B.1A.5a.2		E3/E1	<	40	80	40	40	640	1280	1280
TEST VIRUSES												
A/Switzerland/50178/2022			2022-01-25	MDCK1	640	1280	1280	320	320	<	40	40
A/Switzerland/95789/2022			2022-02-14	MDCK1	320	1280	1280	640	320	<	40	40
A/Switzerland/17908/2022			2022-02-17	MDCK1	320	1280	1280	640	160	<	40	40
A/Switzerland/17941/2022			2022-02-17	MDCK1	320	1280	1280	640	160	<	40	40
A/Switzerland/36111/2022			2022-03-10	MDCK1	80	160	640	320	320	<	40	40
A/Switzerland/86136/2022			2022-03-15	MDCK1	640	2560	1280	1280	320	<	80	40
A/Switzerland/98591/2022			2022-03-16	MDCK1	640	1280	1280	1280	320	<	40	40
A/Switzerland/46339/2022			2022-03-18	MDCK1	640	1280	1280	1280	320	<	40	40
A/Switzerland/46068/2022			2022-03-22	MDCK1	640	2560	2560	1280	320	<	80	40
A/Switzerland/58218/2022			2022-03-22	MDCK2	320	1280	1280	640	160	<	40	<
						Vaccine NH 2020-21					Vaccine SH 2021 NH 2021-22 SH 2022	

Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used)
1 < = <40; 2 < = <80; ND = Not Done

Appendix 4: Antiviral susceptibility testing of Influenza A viruses, WIC (2022)

Collection date	Virus name	Type/Subtype	OS IC50	OS sensitivity	Zan IC50	Zan sensitivity
14/02/2022	A/Switzerland/95940/2022	H1pdm				
05/04/2022	A/Switzerland/02713/2022	H1pdm				
05/04/2022	A/Switzerland/02823/2022	H1pdm				
25/01/2022	A/Switzerland/50178/2022	H1pdm	1.44	Normal inhibition	0.41	Normal inhibition
14/02/2022	A/Switzerland/95789/2022	H1pdm	1.40	Normal inhibition	0.39	Normal inhibition
17/02/2022	A/Switzerland/17908/2022	H1pdm	1.63	Normal inhibition	0.39	Normal inhibition
17/02/2022	A/Switzerland/17941/2022	H1pdm	3.04	Normal inhibition	1.58	Normal inhibition
10/03/2022	A/Switzerland/36111/2022	H1pdm	1.92	Normal inhibition	0.63	Normal inhibition
15/03/2022	A/Switzerland/86136/2022	H1pdm	1.51	Normal inhibition	0.47	Normal inhibition
16/03/2022	A/Switzerland/98591/2022	H1pdm	1.30	Normal inhibition	0.53	Normal inhibition
18/03/2022	A/Switzerland/46339/2022	H1pdm	1.35	Normal inhibition	0.45	Normal inhibition
22/03/2022	A/Switzerland/46068/2022	H1pdm	3.05	Normal inhibition	0.57	Normal inhibition
22/03/2022	A/Switzerland/58218/2022	H1pdm	3.02	Normal inhibition	0.73	Normal inhibition
01/04/2022	A/Switzerland/26721/2022	H3				
31/03/2022	A/Switzerland/54772/2022	H3				
01/04/2022	A/Switzerland/37626/2022	H3				
04/04/2022	A/Switzerland/61445/2022	H3				
20/04/2022	A/Switzerland/67870/2022	H3				
22/04/2022	A/Switzerland/67918/2022	H3				
22/04/2022	A/Switzerland/02732/2022	H3				
31/03/2022	A/Switzerland/89503/2022	H3	0.00	Failed	0.00	Failed
28/03/2022	A/Switzerland/19130/2022	H3		Insufficient Titre		Insufficient Titre
30/03/2022	A/Switzerland/09683/2022	H3		Insufficient Titre		Insufficient Titre
08/03/2022	A/Switzerland/18559/2022	H3		Insufficient Titre		Insufficient Titre
10/03/2022	A/Switzerland/60042/2022	H3		Insufficient Titre		Insufficient Titre
30/03/2022	A/Switzerland/54637/2022	H3	0.81	Normal inhibition	0.80	Normal inhibition
31/03/2022	A/Switzerland/76393/2022	H3	1.40	Normal inhibition	1.69	Normal inhibition
02/04/2022	A/Switzerland/64395/2022	H3	1.41	Normal inhibition	1.22	Normal inhibition
05/04/2022	A/Switzerland/83625/2022	H3	0.94	Normal inhibition	0.84	Normal inhibition
07/04/2022	A/Switzerland/83466/2022	H3	0.95	Normal inhibition	0.93	Normal inhibition
12/04/2022	A/Switzerland/84156/2022	H3	0.71	Normal inhibition	0.67	Normal inhibition
19/04/2022	A/Switzerland/67859/2022	H3	3.90	Normal inhibition	3.25	Normal inhibition
25/04/2022	A/Switzerland/03092/2022	H3	0.67	Normal inhibition	0.74	Normal inhibition
14/03/2022	A/Switzerland/12981/2022	H3	0.83	Normal inhibition	0.63	Normal inhibition
16/03/2022	A/Switzerland/73028/2022	H3	0.70	Normal inhibition	0.57	Normal inhibition
18/03/2022	A/Switzerland/34074//2022	H3	0.81	Normal inhibition	1.11	Normal inhibition
21/03/2022	A/Switzerland/45784/2022	H3	0.53	Normal inhibition	0.76	Normal inhibition
28/03/2022	A/Switzerland/10871/2022	H3	0.50	Normal inhibition	0.54	Normal inhibition
28/03/2022	A/Switzerland/18016/2022	H3	0.80	Normal inhibition	0.82	Normal inhibition

Appendix 5: Lists of Influenza isolates submitted to GISAID (2022)

Collection date	Isolate-ID	Isolate name
2022-Mar-31	EPI_ISL_14328700	A/Switzerland/54637/2022
2022-Mar-31	EPI_ISL_14326692	A/Switzerland/67918/2022
2022-Mar-31	EPI_ISL_14328702	A/Switzerland/89503/2022
2022-Mar-30	EPI_ISL_14326681	A/Switzerland/42783/2022
2022-Mar-30	EPI_ISL_14326682	A/Switzerland/42818/2022
2022-Mar-30	EPI_ISL_14326683	A/Switzerland/42933/2022
2022-Mar-30	EPI_ISL_14326685	A/Switzerland/43161/2022
2022-Mar-30	EPI_ISL_14326686	A/Switzerland/43261/2022
2022-Mar-30	EPI_ISL_14326687	A/Switzerland/43372/2022
2022-Mar-30	EPI_ISL_14328703	A/Switzerland/54772/2022
2022-Mar-30	EPI_ISL_14326688	A/Switzerland/54864/2022
2022-Mar-30	EPI_ISL_14326891	A/Switzerland/9683/2022
2022-Mar-29	EPI_ISL_14326677	A/Switzerland/31304/2022
2022-Mar-29	EPI_ISL_14326678	A/Switzerland/31848/2022
2022-Mar-29	EPI_ISL_14326680	A/Switzerland/32303/2022
2022-Mar-29	EPI_ISL_14326684	A/Switzerland/43079/2022
2022-Mar-28	EPI_ISL_14326645	A/Switzerland/18408/2022
2022-Mar-28	EPI_ISL_14326646	A/Switzerland/18994/2022
2022-Mar-28	EPI_ISL_14328699	A/Switzerland/19130/2022
2022-Mar-28	EPI_ISL_14326647	A/Switzerland/19366/2022
2022-Mar-28	EPI_ISL_14326648	A/Switzerland/19477/2022
2022-Mar-28	EPI_ISL_14326649	A/Switzerland/19796/2022
2022-Mar-28	EPI_ISL_14326679	A/Switzerland/32195/2022
2022-Mar-24	EPI_ISL_14326634	A/Switzerland/70206/2022
2022-Mar-23	EPI_ISL_14326587	A/Switzerland/58328/2022
2022-Mar-23	EPI_ISL_14328727	A/Switzerland/58887/2022
2022-Mar-22	EPI_ISL_14328690	A/Switzerland/46068/2022
2022-Mar-22	EPI_ISL_14328728	A/Switzerland/59037/2022
2022-Mar-22	EPI_ISL_14326589	A/Switzerland/70059/2022
2022-Mar-22	EPI_ISL_14326633	A/Switzerland/70174/2022

Collection date	Isolate-ID	Isolate name
2022-Mar-22	EPI_ISL_14326635	A/Switzerland/70661/2022
2022-Mar-22	EPI_ISL_14326636	A/Switzerland/70767/2022
2022-Mar-21	EPI_ISL_14328697	A/Switzerland/45784/2022
2022-Mar-21	EPI_ISL_14328724	A/Switzerland/45998/2022
2022-Mar-21	EPI_ISL_14328726	A/Switzerland/58626/2022
2022-Mar-21	EPI_ISL_14328729	A/Switzerland/59176/2022
2022-Mar-21	EPI_ISL_14326588	A/Switzerland/59199/2022
2022-Mar-21	EPI_ISL_14326637	A/Switzerland/70789/2022
2022-Mar-18	EPI_ISL_14328723	A/Switzerland/33764/2022
2022-Mar-18	EPI_ISL_14328696	A/Switzerland/34074/2022
2022-Mar-18	EPI_ISL_14328691	A/Switzerland/46339/2022
2022-Mar-18	EPI_ISL_14328725	A/Switzerland/46363/2022
2022-Mar-16	EPI_ISL_14328698	A/Switzerland/10871/2022
2022-Mar-16	EPI_ISL_14328718	A/Switzerland/85916/2022
2022-Mar-16	EPI_ISL_14328719	A/Switzerland/86378/2022
2022-Mar-16	EPI_ISL_14328722	A/Switzerland/88/2022
2022-Mar-15	EPI_ISL_14328717	A/Switzerland/85675/2022
2022-Mar-15	EPI_ISL_14326586	A/Switzerland/86136/2022
2022-Mar-15	EPI_ISL_14328720	A/Switzerland/86456/2022
2022-Mar-14	EPI_ISL_14327259	A/Switzerland/73028/2022
2022-Mar-14	EPI_ISL_14328716	A/Switzerland/73605/2022
2022-Mar-14	EPI_ISL_14328721	A/Switzerland/99760/2022
2022-Mar-10	EPI_ISL_14328693	A/Switzerland/36111/2022
2022-Mar-10	EPI_ISL_14328715	A/Switzerland/36231/2022
2022-Mar-10	EPI_ISL_14328694	A/Switzerland/60042/2022
2022-Mar-09	EPI_ISL_14328711	A/Switzerland/13271/2022
2022-Mar-09	EPI_ISL_14328714	A/Switzerland/25472/2022
2022-Mar-08	EPI_ISL_14328695	A/Switzerland/12981/2022
2022-Mar-08	EPI_ISL_14328712	A/Switzerland/13443/2022
2022-Mar-08	EPI_ISL_14328746	A/Switzerland/28349/2022

Collection date	Isolate-ID	Isolate name
2022-Mar-07	EPI_ISL_14328713	A/Switzerland/25362/2022
2022-Mar-04	EPI_ISL_14328710	A/Switzerland/76654/2022
2022-Mar-03	EPI_ISL_14328709	A/Switzerland/64787/2022
2022-Apr-25	EPI_ISL_14328707	A/Switzerland/83625/2022
2022-Apr-24	EPI_ISL_14326795	A/Switzerland/9650/2022
2022-Apr-22	EPI_ISL_14328705	A/Switzerland/61395/2022
2022-Apr-22	EPI_ISL_14328706	A/Switzerland/61445/2022
2022-Apr-20	EPI_ISL_14328704	A/Switzerland/37626/2022
2022-Apr-11	EPI_ISL_14326792	A/Switzerland/59332/2022
2022-Apr-11	EPI_ISL_14326793	A/Switzerland/59621/2022
2022-Apr-11	EPI_ISL_14326794	A/Switzerland/59684/2022
2022-Apr-08	EPI_ISL_14326791	A/Switzerland/47584/2022
2022-Apr-07	EPI_ISL_14326787	A/Switzerland/25598/2022
2022-Apr-07	EPI_ISL_14328708	A/Switzerland/84156/2022
2022-Apr-06	EPI_ISL_14326784	A/Switzerland/14234/2022
2022-Apr-06	EPI_ISL_14326785	A/Switzerland/14303/2022
2022-Apr-06	EPI_ISL_14326786	A/Switzerland/14324/2022
2022-Apr-06	EPI_ISL_14326788	A/Switzerland/25640/2022
2022-Apr-06	EPI_ISL_14326789	A/Switzerland/25836/2022
2022-Apr-06	EPI_ISL_14326790	A/Switzerland/25874/2022
2022-Apr-05	EPI_ISL_14328692	A/Switzerland/2823/2022
2022-Apr-05	EPI_ISL_14326694	A/Switzerland/3092/2022
2022-Apr-05	EPI_ISL_14326696	A/Switzerland/3258/2022
2022-Apr-04	EPI_ISL_14326693	A/Switzerland/2732/2022
2022-Apr-04	EPI_ISL_14326695	A/Switzerland/3226/2022
2022-Apr-04	EPI_ISL_14326697	A/Switzerland/3281/2022
2022-Apr-02	EPI_ISL_14326690	A/Switzerland/67859/2022
2022-Apr-01	EPI_ISL_14326689	A/Switzerland/67638/2022
2022-Apr-01	EPI_ISL_14326691	A/Switzerland/67870/2022
2022-Apr-01	EPI_ISL_14328701	A/Switzerland/76393/2022