Wastewater-based Genomic Surveillance of Communicable Diseases 2024-25

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We summarize below our activities and achievements in the wastewater-based genomic surveillance of SARS-CoV-2, IAV, and RSV in the period Jan 2024 to Jan 2026. Collection and processing of wastewater samples was performed in collaboration with the teams of Tim Julian and Christoph Ort (Eawag). Sequencing was performed by the team of Catharine Aquino at the Functional Genomics Center Zurich (FGCZ). Processing of the sequencing data is performed in collaboration with the team of Franziska Singer of NEXUS Personalized Health Technologies at ETH Zurich.

SARS-CoV-2

Between January 2024 and January 2026, we have processed 2084 wastewater samples as part of our ongoing surveillance of SARS-CoV-2 genomic variants in wastewater (including a large batch delivered in January 2024, consisting of 195 samples from the preceding end-of-year 2023 break). We collected samples from six wastewater treatment plants: Basel (BS), Chur (GR), Geneva (GE), Laupen (BE), Lugano (TI), and Zurich (ZH). Samples had also been collected in the earlier part of the surveillance program during 2024 at four additional treatment plants: Altenrhein (SG), Bern (BE), Lausanne (VD), and Luzern (LU). Starting from 2025, each of the six WWTPs mentioned above was sampled twice per week, and sequencing was performed in biweekly batches. Until the end of 2024, WWTPs were sampled three times per week and the samples sequenced weekly.

After the sequencing of each batch (every two weeks starting from 2025, and weekly until end 2024), we processed and analyzed the data to reconstruct SARS-CoV-2 variants and sub-variants and to visualize the current landscape of SARS-CoV-2 variants in Switzerland. We published the resulting curves to be displayed on the covSPECTRUM platform and on the WISE dashboard (since July 2025), and they were shared with the Federal Office of Public Health (FOPH) for inclusion in the Infectious Disease Dashboard (IDD) platform.

We also designed and implemented the statistical and computational methodology for variant surveillance. Specifically, we developed Covvfit, a model for learning and forecasting selection dynamics of SARS-CoV-2 variants (https://doi.org/10.1101/2025.03.25.25324639), which we have included in the regular analysis since March 2025. We continuously expand and optimize our analysis methods to update graphical representations, provide in-depth quality control, and to enable the early detection of newly emerging variants. We have observed in wastewater the emergence of XEC, LP.8, NB.1.8.1 (Nimbus), XFG (Stratus), and BA.3.2, and we were able to produce selection modelling results for these variants, as well as forecasts of their spread.

Influenza

We have established a framework for whole-genome sequencing of human Influenza Virus A (IVA) from Swiss wastewater samples, in a joint effort with Seju Kang and Tim Julian (Eawag). Our approach targets segments of the surface proteins HA, NA, and M of subtypes H1N1 and H3N2. Using this panel we found, in a proof of principle study (https://doi.org/10.1016/j.watres.2025.124453), that wastewater-based abundance estimates of the dominant H1N1 clades 6B.1A.5a.2a and 6B.1A.5a.2a.1 correlated well with clinical-based estimates in the 2023/2024 season (Fig. **IVA-1**). Furthermore, wastewater-based sequencing allowed for robust identification of epitope-site mutations, which impact vaccine efficacy, and NA drug-target mutations. These mutations aligned with results from clinical surveillance during the time, highlighting the potential of wastewater-based IVA surveillance to inform public health interventions (Fig. **IVA-2**).

For the 2024/2025 winter season from November 2024 to March 2025, we extended our wastewater-based genomic surveillance approach to include Infulenza A and RSV (see below) sequencing. For both viruses we reported non-synonymous mutation frequencies on the GenSpectrum dashboard.

During the 2025/2026 winter season, we frequently observed the S247N mutation located on the NA segment of subtype H1N1. This mutation is associated with mild to normal reduction of oseltamivir, a neuraminidase inhibitor (NAI) (see full list of NAI resistance mutations by WHO). We observed the S247N mutation across different locations at high frequency throughout the season. Coverage in this region is variable but rarely below 1000x, given the mutation was detected. This substantiates the observed high frequency of the S247N mutation (see Fig. **IVA-3**). In addition, during the current 2025/2026 winter season, we focused on maintenance and optimisation of the IVA whole-genome sequencing protocol.

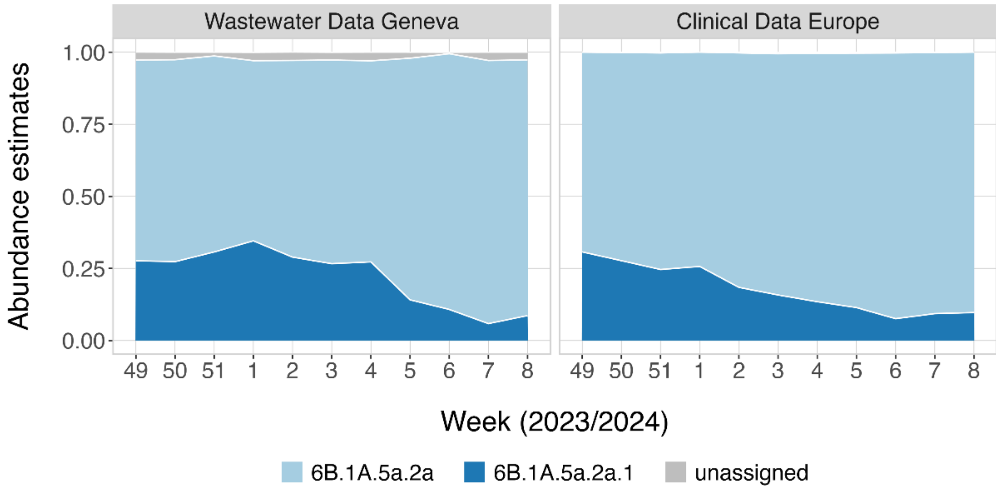

Figure IVA-1: Stacked area graph comparing the wastewater and clinical abundance estimates of the dominating H1N1 clades. As Swiss clinical data was limited, a comparison to clinical data from all of Europe was conducted. For wastewater data (STEP Aïre, Switzerland), the relative abundances of 6B.1A.5a.2a and 6B.1A.5a.2a.1 were estimated using LolliPop (Dreifuss et al. 2022). Wastewater samples with read depth < 5x at the signature mutations were excluded. The clinical data originated from GISAID (Khare et al. 2021) and comprised 9,333 samples.

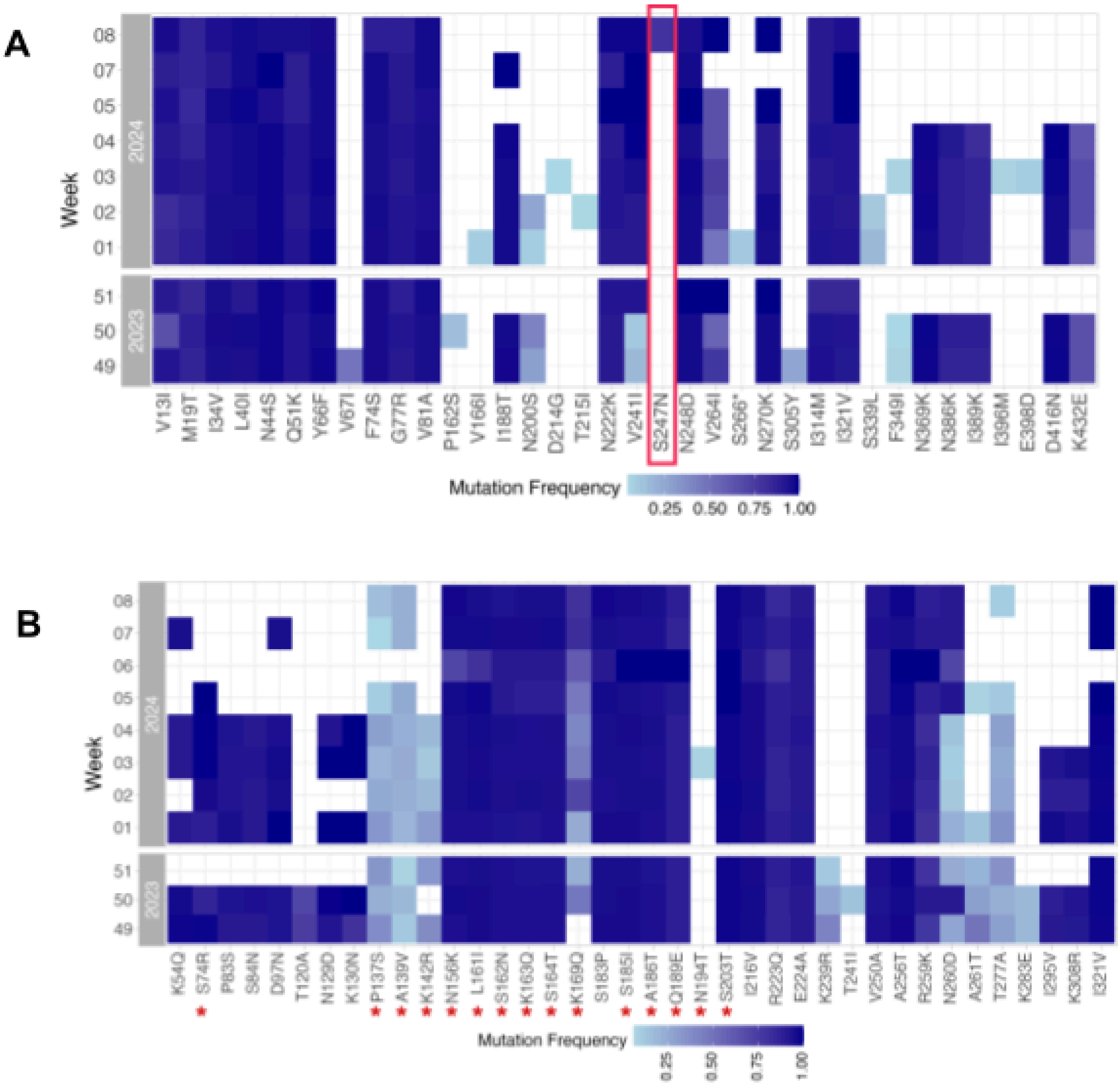

Figure IVA-2: Amino acid mutation frequencies at clinically relevant sites derived from 2023/2024 wastewater samples (STEP Aïre, Switzerland). (A) Mutation Frequencies for N1 segment from wastewater samples from one location in Switzerland. The absence of a mutation is marked as white. In red, the known drug resistance mutation S247N is marked. Mutations are relative to the A/California/07/2009 (CY121682.1) reference sequence. **(B)** H1 mutation frequencies over time relative to reference sequence A/California/07/2009 (CY121680.1) and gene HA0. Red stars mark HA epitope sites (Caton et al. 1982).

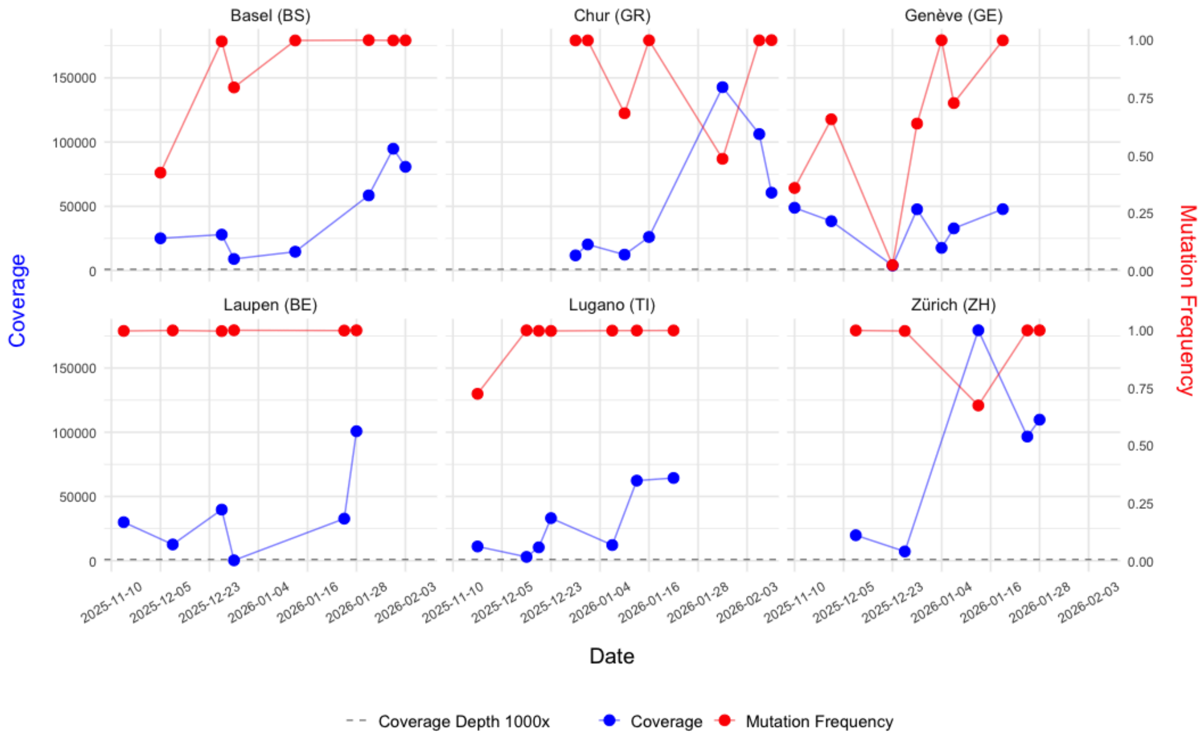

Figure IVA-3: Mutation Frequency and Coverage of the S247N mutation during the 2025/26 season. S247N is a N1 mutation known for mild oseltamivir inhibition. Data includes samples up to February 6 for Basel (BS), Chur (GR), Laupen (BE), Lugano (TI), Zurich (ZH) and up to February 7 for Geneva (GE). Samples with total drop-outs in the S247N region are not displayed.

RSV

We developed a sequencing approach for respiratory syncytial virus (RSV) in wastewater samples, in a joint effort with Jolinda de Korne and Tim Julian (Eawag). We optimized an amplicon-based RSV sequencing method, which was initially developed for clinical samples, and used it for the wastewater samples. For our pilot study (https://doi.org/10.1101/2025.02.28.25321637), we sequenced 28 wastewater time-series samples from Zurich and Geneva (2022/2023 season) and 36 RSV-A time-series samples from Zurich and Geneva (2023/2024 season).

Using whole-genome sequencing data from wastewater, we tracked RSV lineages over time. We showed that B.D.E.1 was the predominant RSV-B lineage in wastewater samples of the RSV-B-predominant 2022/2023 season, whereas in RSV-A predominant 2023/2024 season, multiple RSV-A lineages co-circulated together (Fig. **RSV-1**). Additionally, we identified potentially clinically relevant RSV-A and RSV-B mutations in the F gene which is the primary target of RSV immunoprophylaxis (Fig. **RSV-2**).

During the 2024/2025 season, a total of 328 wastewater samples were selected for RSV sequencing from six wastewater treatment plants: Basel (BS), Chur (GR), Genève (GE), Laupen (BE), Lugano (TI), and Zürich (ZH). Of these, 48 were sequenced for RSV-A, 15 for RSV-B, and 265 for both RSV-A and RSV-B. Until 19 Nov 2024, only the predominant subtype at each location was amplified and sequenced due to low concentrations. From this date onward, both subtypes were amplified and sequenced.

During the 2025/2026 season, wastewater samples are selected for sequencing of both RSV subtypes, RSV-A and RSV-B. Observed mutations and their relative frequencies are displayed as an interactive graph on the GenSpectrum platform (https://genspectrum.org/swiss-wastewater/rsv).

In the 2025/26 season, we observed two RSV-B F gene mutations that confer partial resistance to monoclonal antibodies (Fig. **RSV-3**). The K68N mutation is known to confer partial resistance to the monoclonal antibody nirsevimab and was observed in high frequencies in multiple locations. The K272R mutation, known to confer partial resistance to palivizumab, was observed at low frequency in the sample collected in Zurich on February 5. We also consistently observed the RSV-B F gene mutation S211N at high frequencies across all locations. This mutation can contribute to resistance to nirsevimab if present together with another F gene mutation, K65Q; however, we did not detect K65Q.

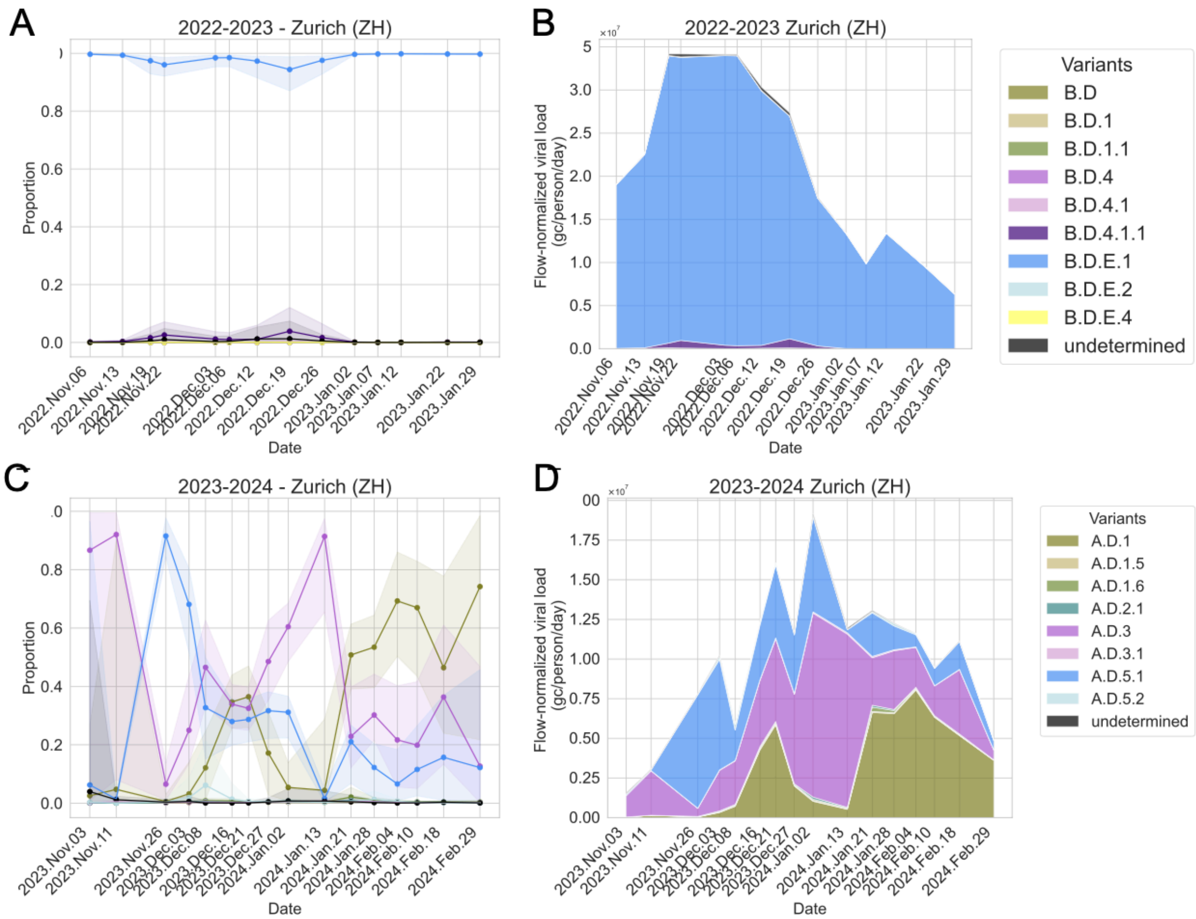

Fig. RSV-1. Deconvolution of signature mutation frequencies into lineages enabled tracking temporal dynamics of circulating RSV lineages. Estimated proportions and abundances of RSV-B lineages in wastewater collected from Zurich (A: proportions, B: abundances) in the 2022-2023 RSV season. Estimated proportions and abundances of RSV-A lineages in wastewater collected from Zurich (C: proportions, D: abundances) in the 2023-2024 RSV season. The shaded bands around the lines represent the 95% confidence intervals around the proportion estimates, based on bootstrapping.

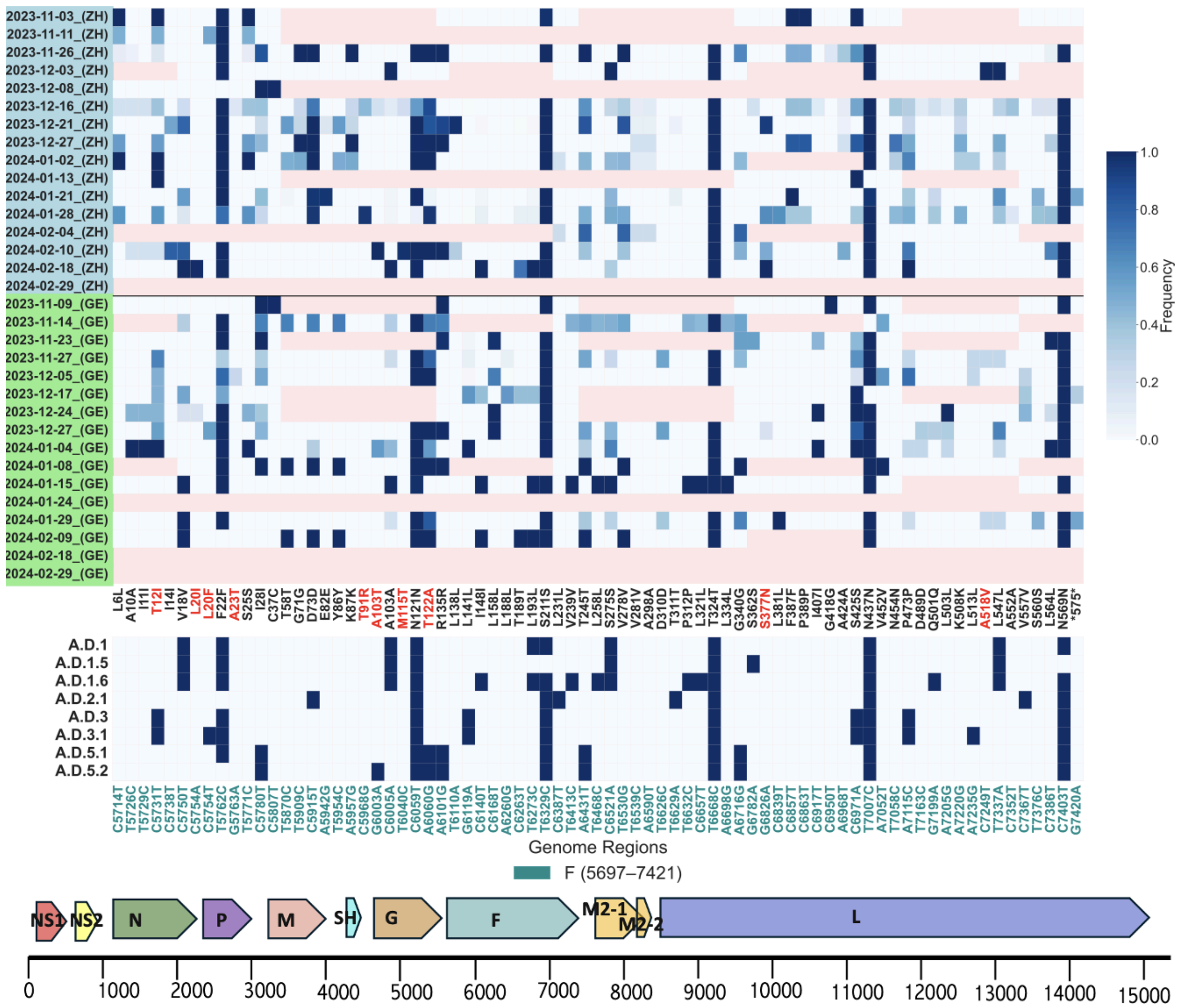

Fig RSV-2. RSV-A F gene amino acid substitutions identified in wastewater samples of the 2023-2024 season. Mutations in wastewater-derived RSV-A fusion (F) gene sequences, their frequencies, and their occurrence as signature mutations in RSV-A lineages.

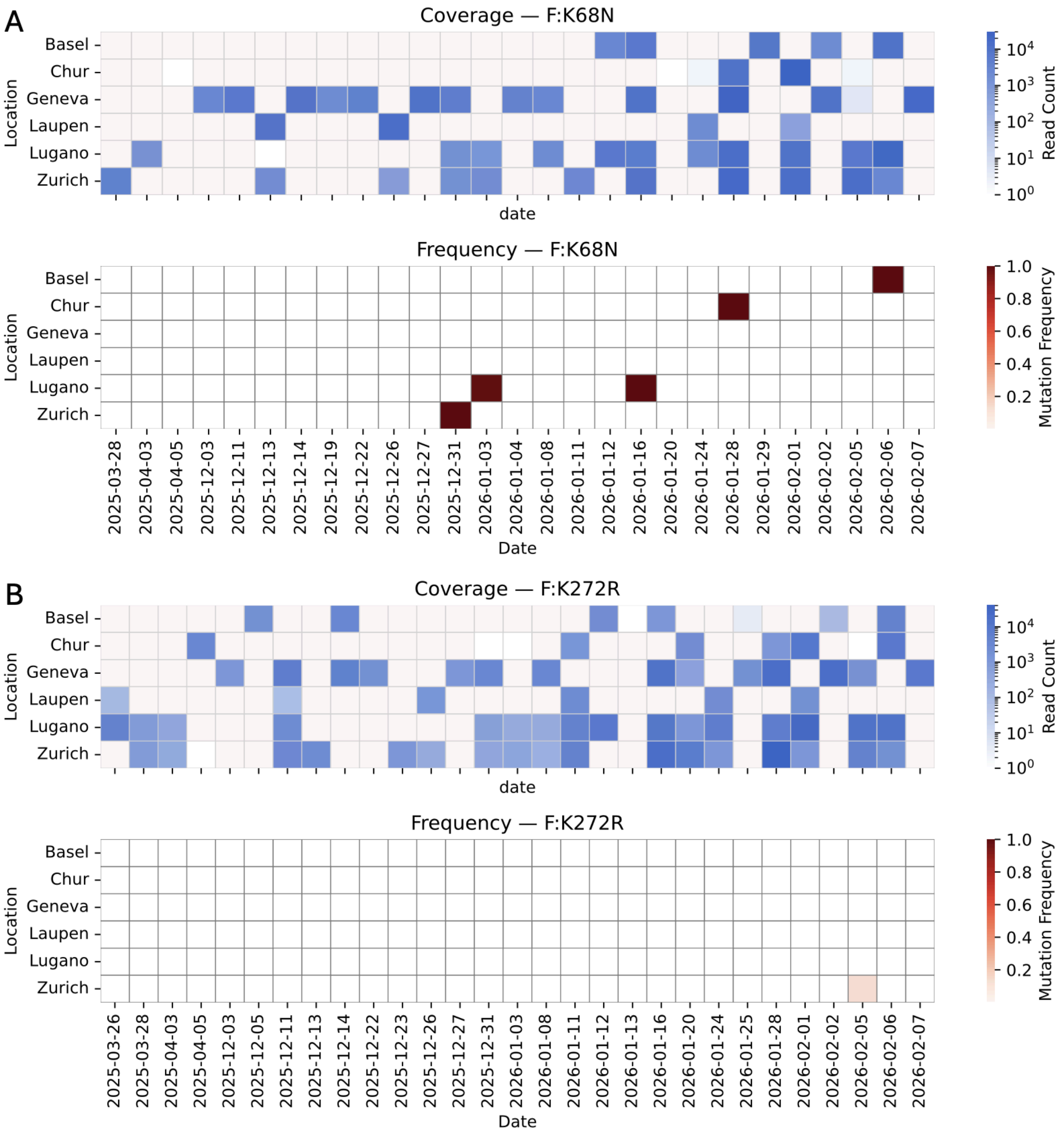

Fig RSV-3. RSV-B F gene resistance mutations coverage and frequency in wastewater samples during the 2025/26 season. (A) Upper panel: sequencing coverage at nucleotide position (5879) corresponding to mutation K68N. Coverage (read count) is encoded from white (lowest) to dark blue (highest), while coverage dropouts are shown in pink. Lower panel: frequency of mutation K68N. Frequencies are encoded from white (lowest, 0.0) to dark red (highest, 1.0). (B) Upper panel: sequencing coverage at nucleotide position (6490) corresponding to mutation K272R. Lower panel: the frequency of mutation K272R.

References

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