

Med, Volume 3

Supplemental information

**Monitoring of the SARS-CoV-2 Omicron BA.1/BA.2
lineage transition in the Swedish population
reveals increased viral RNA levels in BA.2 cases**

Antonio Lentini, Antonio Pereira, Ola Winqvist, and Björn Reinius

Figure S1

S:N211I & DEL212/212 S:214:EPE

a

```

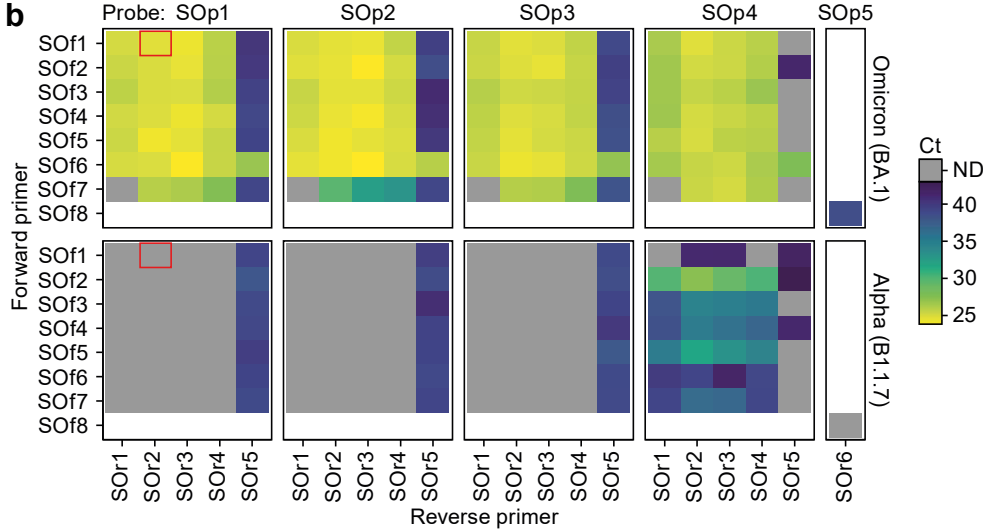
GAATATTGATGGTTATTTTAAAAATATATCTAAGCACACGCGCTATTATAGTGCCTGAGCCAGAAGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTGCCAATAGGT-Omicron BA.1
GAATATTGATGGTTATTTTAAAAATATATCTAAGCACACGCGCTATTATAGTGCCTGAGCCAGAAGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTGCCAATAGGT-sof1
GAATATTGATGGTTATTTTAAAAATATATCTAAGCACACGCGCTATTATAGTGCCTGAGCCAGAAGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTGCCAATAGGT-sof2
GAATATTGATGGTTATTTTAAAAATATATCTAAGCACACGCGCTATTATAGTGCCTGAGCCAGAAGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTGCCAATAGGT-sof3
GAATATTGATGGTTATTTTAAAAATATATCTAAGCACACGCGCTATTATAGTGCCTGAGCCAGAAGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTGCCAATAGGT-sof4
GAATATTGATGGTTATTTTAAAAATATATCTAAGCACACGCGCTATTATAGTGCCTGAGCCAGAAGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTGCCAATAGGT-sof5
GAATATTGATGGTTATTTTAAAAATATATCTAAGCACACGCGCTATTATAGTGCCTGAGCCAGAAGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTGCCAATAGGT-sof6
GAATATTGATGGTTATTTTAAAAATATATCTAAGCACACGCGCTATTATAGTGCCTGAGCCAGAAGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTGCCAATAGGT-sof7
GAATATTGATGGTTATTTTAAAAATATATCTAAGCACACGCGCTATTATAGTGCCTGAGCCAGAAGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTGCCAATAGGT-sof8
GAATATTGATGGTTATTTTAAAAATATATCTAAGCACACGCGCTATTATAGTGCCTGAGCCAGAAGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTGCCAATAGGT-sof1_right
GAATATTGATGGTTATTTTAAAAATATATCTAAGCACACGCGCTATTATAGTGCCTGAGCCAGAAGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTGCCAATAGGT-sof2_left
GAATATTGATGGTTATTTTAAAAATATATCTAAGCACACGCGCTATTATAGTGCCTGAGCCAGAAGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTGCCAATAGGT-sof1-Cy5
GAATATTGATGGTTATTTTAAAAATATATCTAAGCACACGCGCTATTATAGTGCCTGAGCCAGAAGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTGCCAATAGGT-sof2-Cy5
GAATATTGATGGTTATTTTAAAAATATATCTAAGCACACGCGCTATTATAGTGCCTGAGCCAGAAGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTGCCAATAGGT-sof3-Cy5
GAATATTGATGGTTATTTTAAAAATATATCTAAGCACACGCGCTATTATAGTGCCTGAGCCAGAAGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTGCCAATAGGT-sof4_right-Cy5
GAATATTGATGGTTATTTTAAAAATATATCTAAGCACACGCGCTATTATAGTGCCTGAGCCAGAAGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTGCCAATAGGT-sof5_left-Cy5
GAATATTGATGGTTATTTTAAAAATATATCTAAGCACACGCGCTATTATAGTGCCTGAGCCAGAAGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTGCCAATAGGT-sof1
GAATATTGATGGTTATTTTAAAAATATATCTAAGCACACGCGCTATTATAGTGCCTGAGCCAGAAGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTGCCAATAGGT-sof2
GAATATTGATGGTTATTTTAAAAATATATCTAAGCACACGCGCTATTATAGTGCCTGAGCCAGAAGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTGCCAATAGGT-sof3
GAATATTGATGGTTATTTTAAAAATATATCTAAGCACACGCGCTATTATAGTGCCTGAGCCAGAAGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTGCCAATAGGT-sof4_right
GAATATTGATGGTTATTTTAAAAATATATCTAAGCACACGCGCTATTATAGTGCCTGAGCCAGAAGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTGCCAATAGGT-sof5_right
GAATATTGATGGTTATTTTAAAAATATATCTAAGCACACGCGCTATTATAGTGCCTGAGCCAGAAGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTGCCAATAGGT-sof6_left
    
```

Forward primer

Probe

Reverse primer

b



c

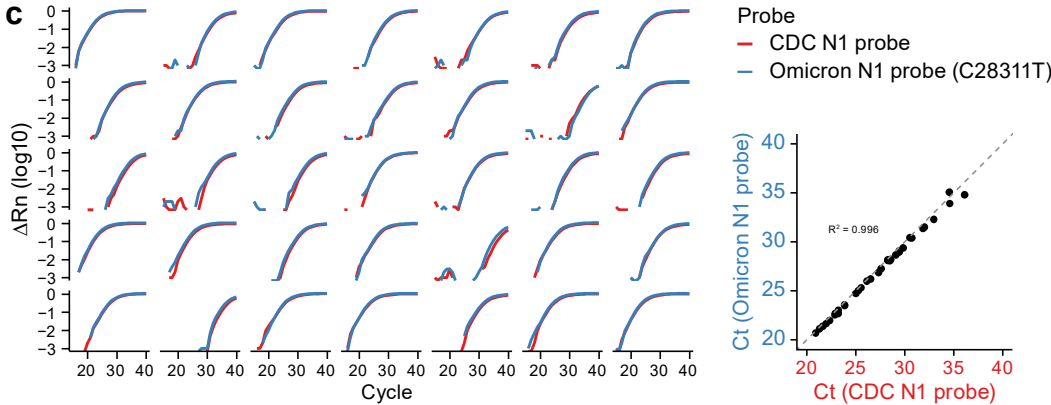


Figure S1. Extended analysis of Omicron BA.1-specific extraction-free RT-PCR, Related to Figure 1.

a. Evaluated primer-probe combinations targeting Omicron BA.1-specific mutations. Green: 3-nucleotide deletion in-between A[]T specifically in Omicron BA1. Purple: 9-nucleotide insertion specifically in Omicron BA1.

b. Performance of primer-probe combinations in **a.** by RT-PCR. Red box indicates the selected primer-probe set.

c. RT-PCR detection using the original CDC N1 probe (utilized in the diagnostic RT-PCR in this study) or a custom Omicron-specific N1 probe designed around the C28311T mutation (present in both Omicron BA.1 and BA.2) located in the 3rd base of the CDC N1 probe (See sequence in **Table S1c**). Amplification curves (left) and Ct values (right) shown for n = 35 clinical specimens classified as Omicron BA.1 by Thermo Fisher TaqMan SARS-CoV-2 Mutation Panel Assay and WGS.

Figure S2

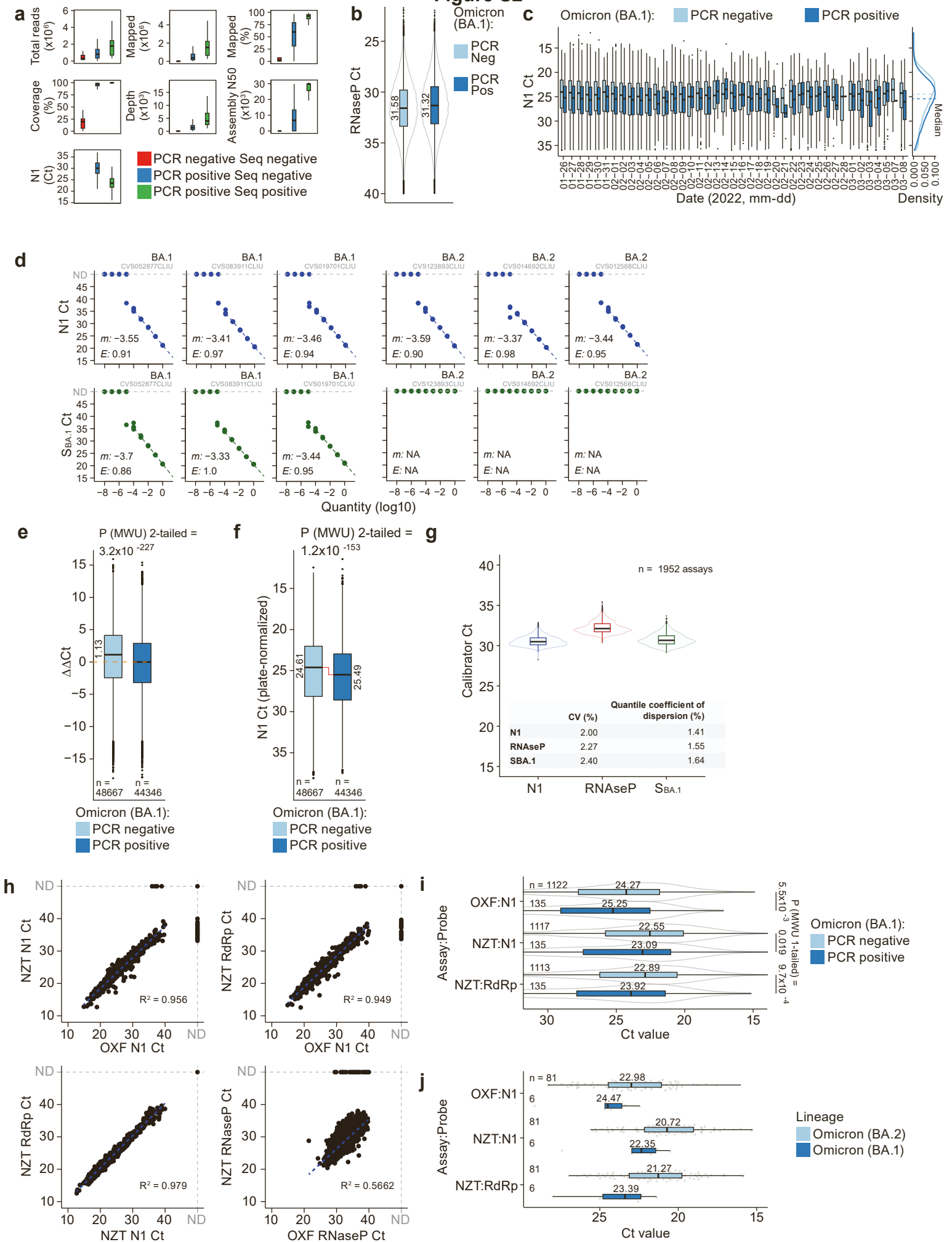


Figure S2. Extended analysis of WGS lineage calling results and viral load, Related to Figure 2.

- a. Sequencing quality metrics separated by SARS-CoV-2 positivity by RT-PCR and successful lineage assignment by WGS for $n = 1,153$ clinical specimens.
- b. RNaseP (human internal control) RT-PCR Ct values in samples stratified by BA.1 classification by RT-PCR for $n = 93,264$ SARS-CoV-2-positive cases (174,933 tests performed including negative cases). Boxplot show median, first, and third quartiles, and 1.5x inter-quartile range.
- c. Viral RNA load (N1 qPCR Ct) for BA.1-positive and negative SARS-CoV-2-positive samples based on RT-PCR calls over time for $n = 93,126$ clinical specimens. Density plot with marked medians shown to the right.
- d. Ct values from dilution series of three BA.1-positive (left) and three BA.2-positive (right) samples, for the N1 (upper) and BA.1-specific S (lower) primer-probe set. Each dilution point is represented by $n = 4$ technical replicates. Slope (m) and primer efficiency (E) calculated in the log-linear range for each sample.
- e. Boxplots of delta-delta Ct values, considering N1 as target gene and RNaseP as reference in each clinical specimen.
- f. Boxplots of N1 Ct values, normalized for RT-PCR plate variation using a positive control sample loaded on each plate as the calibrator sample.
- g. Boxplots of N1, RNaseP, and BA.1-specific S Ct values for the calibrator sample across 1952 separate plates and RT-PCR runs. Coefficient of variation (CV) and quantile coefficient of dispersion are shown the plots.
- h. Linear relationship between Omicron extraction-free ($N1+S_{BA1}$, OXF) and extraction-based ($N1+RdRp$, NZT) RT-PCR results for different primer-probe sets for $n = 3,323$ clinical specimens.
- i. Difference in viral RNA quantity (RT-PCR Ct for different primer-probe sets) for BA.1-positive and negative SARS-CoV-2 positive samples based on RT-PCR calls for $n = 1,252$ clinical specimens. OFX: Omicron extraction-free ($N1+S_{BA1}$). NZT: Extraction-based SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N Genes, IVD (NZYTech). P-values calculated using one-tailed Mann-Whitney U-tests.
- j. Same as i. but based on WGS lineage calls for $n = 87$ clinical specimens.

Figure S3

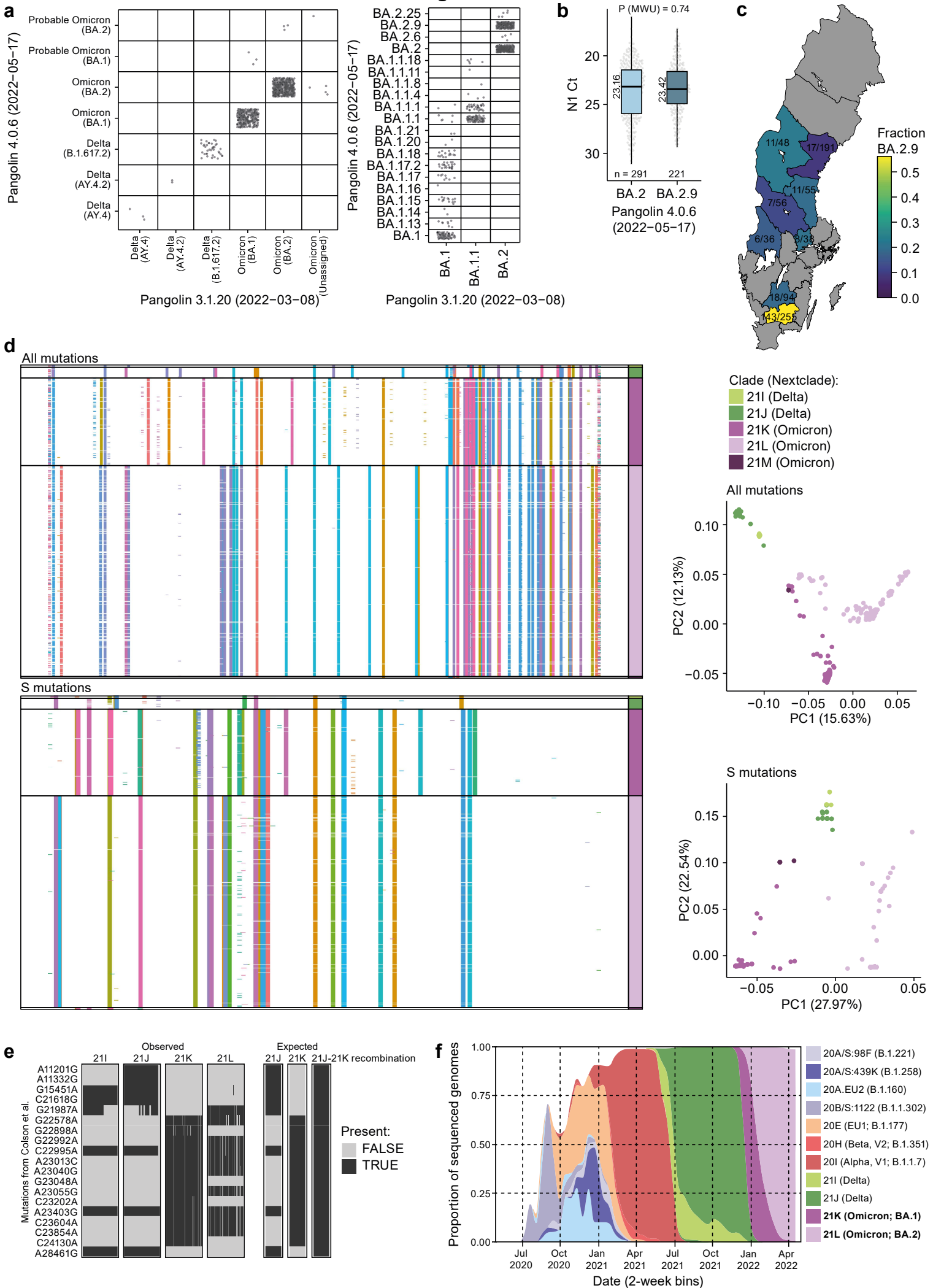
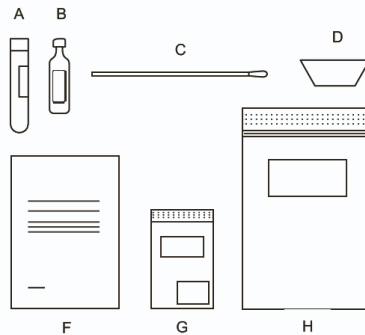


Figure S3. Extended analysis of SARS-CoV-2 mutations, Related to Figure 2.

- a.** Lineage assignment by WGS using the original lineage database (accessed 2022-03-08) or an updated version (2022-05-17) for n = 801 clinical specimens.
- b.** Viral load (N1 qPCR Ct) for BA.2 and BA.2.9 samples classified by WGS lineage calls using the updated (2022-05-17) lineage database. P-values calculated using two-tailed Mann-Whitney U-tests.
- c.** Prevalence of the BA.2.9 sublineage per healthcare district (compared to COVID-19 positive and lineage-classified samples).
- d.** Full SARS-CoV-2 mutational spectrum (top) or S-specific mutations (bottom) for n = 712 clinical specimens. Shown as heatmaps with arbitrarily coloured mutations on genomic coordinates as X-axis (left) or PCA analysis (right).
- e.** Heatmap of mutational signature associated with 21J-21K recombination from Colson et al. for samples in **d.**, excluding the 3 Omicron 21M samples.
- f.** Retrospective data of variant and lineage fraction relative to total sequenced genomes in Sweden over time, shown at 2-week resolution. Adapted from Covariants.org, accessed 2022-05-24.

The test kit contains:

- A Collection tube with barcode. Double check that the personal identification number on the barcode is correct.
- B Saline solution ampoule.
- C One swab.
- D Spittoon.
- E Absorbent (not used during swabbing, should be placed in small bag (G) after swabbing).
- F Swabbing instructions (this sheet of paper).
- G Small bag. Contains an absorbent not used during swabbing, should only be placed in a small bag (G) after swabbing.
- H Large bag (to throw away used swabs etc.)



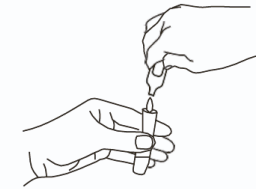
Instructions for self-sampling nasal, throat, and saliva specimen collection

See kit items and associated alphabetical markings in Kit Content on the next page. Please read Kit Content and the swabbing instructions below carefully before you start.

Preparation before the test

If a child is tested, parents should follow the instructions together with the child. If necessary, a parent can swab their child.

- Remove the small bag (G) and place it nearby. Place the rest of the contents of the large bag (H) on top of the small bag (G). Open the swab packaging (C).
- Take the saline solution ampoule (B) and open it by twisting off the top.
- Remove the lid from the collection tube (A) and stand the lid with the thread facing upwards.
- Empty the saline solution (B) into the tube (A). Hold the tube (A). Toss the empty saline solution packaging into the large bag (H).
- You will now do sampling in three different ways as described below.

**Throat - swab 1**

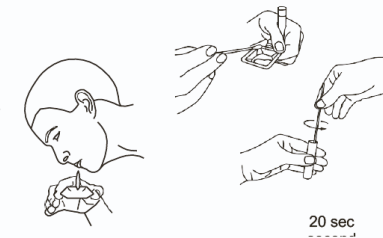
- Take the swab (C) and move it along the inside of your cheek to the back of the throat as far as you can.
- Scrape the cotton swab for about 20 seconds on each side of the back of your throat.
- Remove the swab (C) from the throat.

**Nose - swab 2**

- Now insert the cotton swab into your nostril, about 2-3 cm. Rub around the nostril for about 20 seconds. Do the same in both nostrils with the same swab.
- Remove the swab (C) from the nose, then hold the swab (C) in the same hand as the tube (A).

**Saliva - swab 3**

- Take the spittoon (D) and spit into it a few times so that the bottom surface is covered.
- Stir the swab (C) in the saliva for 20 seconds.
- Insert the swab (C) into the tube (A) and stir about 20 seconds. Remove the swab from the tube.
- Toss the swab (C) and spittoon (D) in the large bag (H).
- Carefully screw the lid onto the collection tube (A). Double check that the liquid stays in the tube and that the lid is firmly screwed on. Place the collection tube (A) in the small bag (G). The bag contains an absorbent that should be left untouched.

**After swabbing**

Deliver the small bag (G) to the place and in the way you were told at the time of booking.

Toss the large bag (H) with the swab, spittoon and the other rubbish into your household waste.

