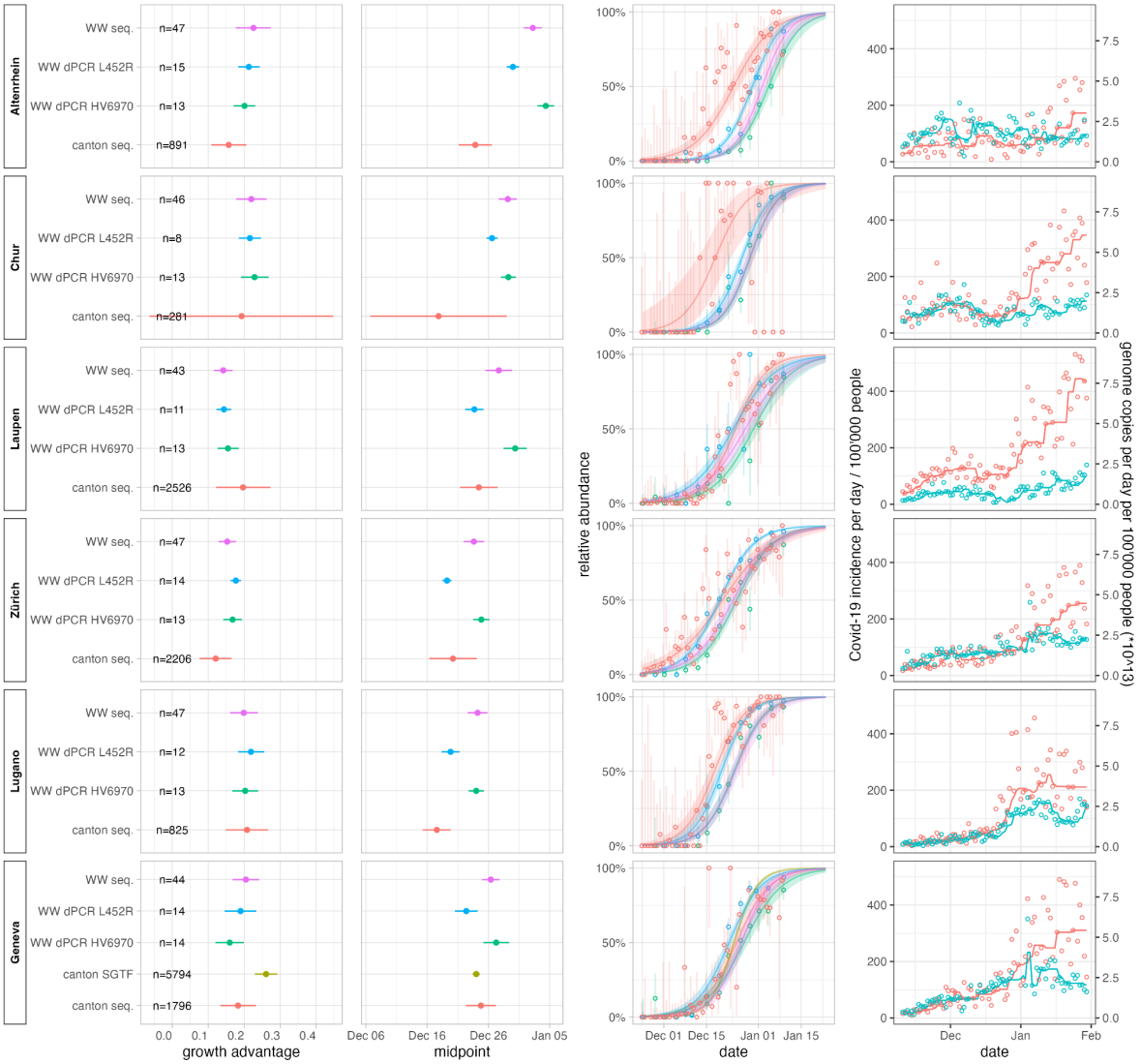
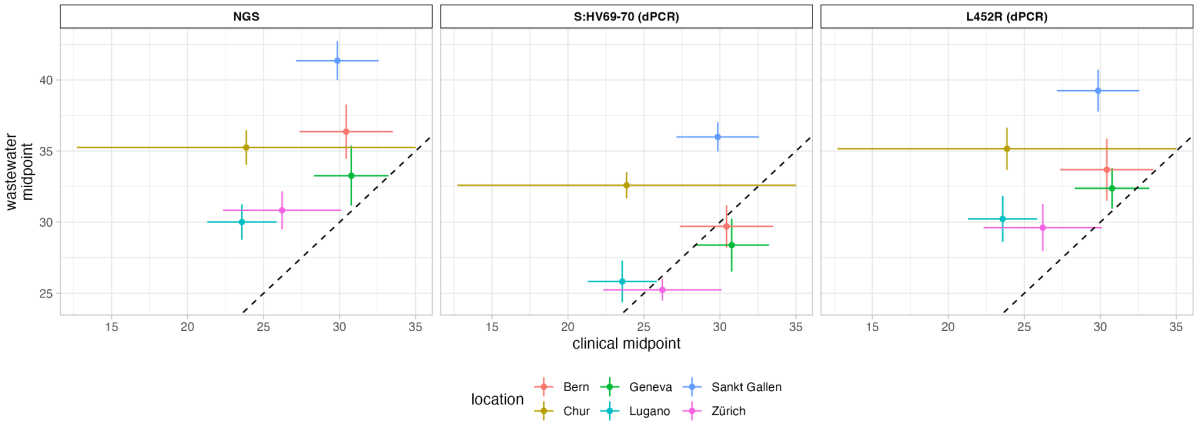
Supplementary material for « Estimated transmission dynamics of SARS-CoV-2 variants from wastewater are robust to differential shedding”



Supplementary Figure 1: Concordant estimation of the growth advantage of Omicron BA.1 in Switzerland using clinical and wastewater-derived data. Logistic growth fits of the relative abundance of BA.1 in the six WWTP regions, based on wastewater NGS (WW seq.), wastewater ddPCR duplex assays targeting S:L452R (WW dPCR L452R) and the S:HV69-70 deletion (WW dPCR HV6970), as well as clinical sequencing (canton seq.) and clinical SGTF data (cantong SGTF). Fourth column shows daily new case numbers measured in the surveyed regions, along with measured viral genome copies in wastewater. Third column shows the fitted curves along with 95% Wald confidence bands adjusted for overdispersion,

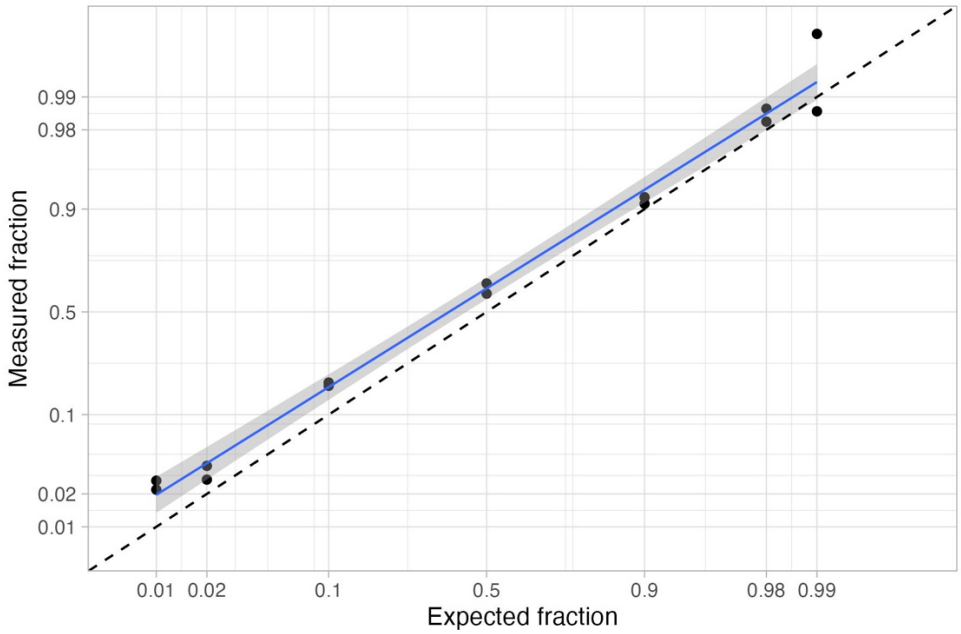
as well as the daily empirical estimates of relative abundance along with 95% confidence intervals. First and second columns respectively show estimates of the growth rate and midpoint of the logistic growth, along with 95% Wald confidence intervals adjusted for overdispersion.



Supplementary Figure 2: Midpoint parameter (in days after 2021-11-24) estimates for BA.1 in the six WWTP regions, based on wastewater NGS as well as wastewater ddPCR duplex assays targeting the S:HV69-70 deletion and S:L452R substitution, compared with estimates derived from clinical sequencing from the cantons surrounding the WWTPs. Error bars represent 95% Wald confidence intervals adjusted for overdispersion.

Benchmarking S:L452R RT-dPCR Assay

While the Δ69-70 assay was previously designed and evaluated[8], the S:L452R assay has been newly designed for this study. We assessed the performance of the new drop-off RT-dPCR assay against RNA extracts of the SARS-CoV-2 Wuhan-Hu-1 lineage (representing the wild-type) and synthetically assembled RNA fragments (gBlock, Integrated DNA Technologies, USA) representing the VOC following the protocol in Caduff et al. (2022). To assess the performance of the duplex assay estimating the ratio of VOC to wild-type in a mixed sample, we analyzed a series of concentrations containing 0.01, 0.02, 0.10, 0.50, 0.90, 0.98 and 0.99 proportions of VOC RNA (Supplementary Figure X). All samples were made with molecular grade and contained a total target concentration of 100 gc/µL. Measured proportion of VOC RNA follows the expected proportion, but the ratio of VOC to non-VOC RNA is overestimated by a factor of 1.68 (1.37 – 2.07).



Supplementary Figure 3: Benchmarking of the S:L452R drop-off RT-dPCR assay. Measured versus expected proportion of VOC RNA are reported here both on a logit scale, with a linear regression line along with a 95% confidence band.

Supplementary Table 1. Drop-off RT dPCR assay primer and probe sequences.

Target	*Type*	*DNA oligo name*	*DNA Sequence (5'-3')*	*Amplicon size*	*Reference*
Delta	*Primer forward*	*L452R_delta_F*	*TGATAGATTTCAGTTGAAATATCTCTCTCA*		*Current study*
	Primer reverse	*L452R_delta_R*	*AATCTTGATTCTAAGGTTGGTGGTAATTAT*		
	Probe mutation	*L452R_delta_P_mut*	*CTAAACAATCTATACCGGTAAT*		

	Probe Wildtype	*L452R_delta_P_wtcomp*	*CCTAAACAATCTATACAGGTAA*		
Alpha/Omicron	*Primer forward*	*Yale_69-70_F*	*TCAACTCAGGACTTGTTCTTACCT*		*Vogels et al. 2021, Caduff et al. 2022*
	Primer reverse	*Yale_69-70_R*	*TGGTAGGACAGGGTTATCAAAC*		
	Probe Wildtype	*Yale_69-70_Cy5_P*	*TTCCATGCTATACATGTCTCTGGGA*		
	Probe mutation	*LC_69-70_HEX_P*	*CCAATGGTACTAAGAG*		*Caduff et al., 2022*