Supplementary Materials

Table S1. Sequences of primers and probes.

Assay	Targeted gene	Primer sets	Ref.
1	N gene of SARS-CoV-2	2019-nCoV_N2-F: 5′-TTACAAACATTGGCCGCAAA-3′ 2019-nCoV_N2-R: 5′-GCGCGACATTCCGAAGAA-3′ 2019-nCoV_N2-P: 5′-JUN-ACAATTTGCCCCCAGCGCTTCAG-MGBNFQ-3′	[7,8, and the references there in]
	HRP3 gene fragment of AQHRPa	HRP3_F: 5′-AGATTTGGACCTGCGAGCG-3′ HRP3_R: 5′-GAGCGGCTGTCTCCACAAGT-3′ HRP3_P: 5′-6FAM-TTCTGACCTGAAGGCTCTGCGCG-MGBNFQ-3′	[7,8]
2	IPCb	IPC_F: 5′-ACGCACATATACGGGTAGCA IPC-R: 5′-TCCCGCCTATAGACCACCTT IPC-Probe: 5′-VIC-ACTTTCGGATGCATCTAGTGACA-MGBNFQ-3′	[7,8]

a Whole processing spike control, Armored RNA Quant RNase P standard (AQHRP)

b Inhibition assessment, internal RT-qPCR positive control (IPC)**Table S2**. Recipe, quantitative RNA standard, range of standard curve, and settings for threshold and baseline of RT-qPCR assays.

Assays	Recipe of RT-qPCR assays		Assay/Test	Quantitative RNA standard	Range of standard curve (gc/reaction)	Settings for threshold	Settings for baseline
	Component	Amount (volume) per 10-µL reaction					
N2	4X TaqPath™ 1-Step Master Mix (Thermo Fisher Scientific, CA, USA) Forward primer Reverse primer Probe RNA/Blank controls/Standards	2.5 µL 180 nM 180 nM 150 nM 2.5~ 5 µL	N2	Quantitative Synthetic SARSCoV2 RNA (ATCC, USA)	9.25E4, 9.25E3, 9.25E2, 9.25E1, 9.25E0, 5.29E0, 3.02E0, 1.73E0	0.04 0.04	auto auto
			*PMMoV	Synthetic RNA (IDT, USA)	5.00E8, 5.00E7, 5.00E6, 5.00E5, 5.00E4, 5.00E3, 5.00E2, 5.00E1	0.04	auto
			AQHRP	Extracted RNA from Armored RNA Quant RNase P (Asuragen, TX, USA)	2.50E4, 2.50E3, 2.50E2, 2.50E1, 6.25E0, 3.13E0, 1.56E0	0.04	auto
*FRNAPH-II, AQHRP (Duplex)	TaqPath™ 1-Step Master Mix (4X) HRP3-F HRP3-R HRP3-P VTB4-FphGIIf VTB4-FphGIIr VTB4-FphGIIprobe RNA/Blank controls/Standards	2.5 µL 180 nM 180 nM 150 nM 60 nM 60 nM 50 nM 2.5 ~ 5 µL	*FRNAPH-II	N.A.	N.A.	0.02	auto

Table S3. Performance of TaqMan RT-qPCR Assays.

Test	Assay	VOC	LOD (gc/reaction)	Y-intercept (mean ±SD)	Slope (mean± SD)	R2 (mean± SD)	Efficiency (mean ±SD)
SARS-CoV-2 N	N2		1.210	33.362 ± 0.660	-3.450 ± 0.103	0.994 ± 0.002	99.223 ± 4.301
AQHRP	HRP3		1.563	34.057 ± 0.927	−3.314 ± 0.167	0.990 ± 0.007	100.802 ± 6.771

$$\text{Gene copies / 100 mL} = \frac{\text{RNA concentration X RNA dilution volume}}{\text{Processed wastewater volume X 100 mL}}$$. ------

S1 Whole-genome sequencing of wastewater

Whole-genome sequencing (WGS) of wastewater samples was performed at the Division of Enteric Diseases, National Microbiology Laboratory, Public Health Agency of Canada (Winnipeg, MB, Canada). Briefly, after RNA extraction, the cDNA was synthesized using the SuperScript IV First-Strand Synthesis System (Invitrogen, USA). Tiled amplicons were amplified according to the ArticV4.1 protocol. Tiled amplicons were sequenced with MiSeq 300PE V3 chemistry (Illunima, USA). Mutations were identified on mapping files generated using SAMtools v 1.7 against a SARS-CoV-2 reference sequence (MN908947.3) [8]. SARS-CoV-2 lineage was assigned based on coverage of consensus mutations following the Pango Nomenclature proposal [8].

Table S4. Distribution of Omicron sub-variants in Saskatoon between January and September 2022.

Date Collected	% Breadth of coverage (≥ 5x depth)	Average depth of coverage	Median depth of coverage	VOC Detected (consensus)	Number of VOC mutations (consensus)	Average Frequencies of reads (cov >30) with VOC mutation (consensus)	B.1.1.529 (Omicron parent) 31 mutations	Pango lineage (consensus)/sublineage detected	Sub-consensus
9/12/2022	97.68	4744.51	1198	BA.5.2	36 / 38	0.88	30 (0.91)	BA.5.2	High presence of BA.5 Moderate presence of BA.4 Detection of mutations defining BA.5.1, BA.5.2 and BA.5.2.1
9/4/2022	99.36	15284.68	13304	BA.5.2.1	36 / 38	0.92	29 (0.91)	BA.5.2.1	High presence of BA.5 Detection of mutations defining BA.5.2 and BA.5.2.1
8/28/2022	98.11	10402.34	6659	BA.5.2.1	37 / 38	0.92	30 (0.91)	BA.5.2.1	High presence of BA.5 Detection of mutations defining BA.5.1, BA.5.2 and BA.5.2.1
8/21/2022	98.56	7493.1	3813	BA.5.2.1	36 / 38	0.89	29 (0.89)	BA.5.2.1	High presence of BA.5 Moderate presence of BA.4 Detection of mutations defining BA.5.1, BA.5.2, BA.5.2.1
8/14/2022	99.44	15792.56	8023	BA.5.2	37 / 38	0.92	30 (0.93)	BA.5.2	High presence of BA.5 Moderate presence of BA.4 Detection of mutations defining BA.5.1, BA.5.2 and BA.5.2.1
8/7/2022	98.94	17826.29	12224	BA.5.2	37 / 38	0.92	30 (0.93)	BA.5.2	High presence of BA.5 Moderate presence of BA.4 Trace presence of 2 BA.2.12.1-defining mutations Detection of mutations defining BA.5.1, BA.5.2 and BA.5.2.1
8/3/2022	99.42	7700.76	4204	BA.5.2.1	37 / 38	0.93	30 (0.93)	BA.5.2.1	High presence of BA.5 Moderate presence of BA.4 Detection of mutations defining BA.5.1, BA.5.2 and BA.5.2.1
7/24/2022	98.06	7806.52	4237	BA.5.2.1	34 / 38	0.83	28 (0.84)	BA.5.2.1	High presence of BA.5 Detection of mutations defining BA.5.2, BA.5.2.1
7/17/2022	98.09	10472.77	5479	BA.5	36 / 38	0.87	30 (0.91)	BA.5	Low presence of BA.2.12.1 Moderate presence of BA.4 and BA.5 Detection of mutations defining BA.5.1, BA.5.2/BA.5.2.1

Date									
7/10/2022	93.54	13102.82	7088	22B (Omicron)	37 / 38	0.89	30 (0.91)	Ambiguous	Moderate presence of BA.4 High presence of BA.5 Detection of mutations defining BA.5.1.1, BA.5.2/BA.5.2.1 and BA.5.5
7/3/2022	98.52	10978.16	6523	BA.5	38 / 38	0.88	31 (0.95)	BA.5	Moderate presence of BA.2.12.1, BA.4 and BA.5 Detection of mutations defining BA.5.1, BA.5.2 and BA.5.5
6/26/2022	97.72	8026.26	5574	BA.2	59 / 60	0.93	31 (0.96)	BA.2	Moderate presence of BA.4, BA.5 and BA.2.12.1 Detection of mutations defining BA.2.3, BA.4.1 and BA.5.2
6/19/2022	99.45	14759.13	9862	BA.2.12.1	59 / 60	0.96	31 (0.99)	BA.2.12.1	Trace the presence of BA.4 Low presence of BA.5 Moderate presence of BA.2.12.1 Detection of mutations defining BA.2.36 and BA.5.3
6/12/2022	98.63	10305.25	7217	BA.2.12.1	59 / 60	0.97	31 (0.99)	BA.2.12.1	High presence of BA.2.12.1 Trace presence of 3 BA.5 mutations
6/5/2022	98.92	11634.66	7107	BA.2	59 / 60	0.98	31 (0.995)	BA.2	Moderate presence of BA.2.12.1 Trace presence of 2 BA.5 mutations
5/29/2022	95.9	9426.08	5640	BA.2.3	57 / 60	0.94	30 (0.96)	BA.2.3	Moderate presence of BA.2.12.1 Detection of a mutation defining BA.2.3
5/22/2022	98.78	25415.08	17506	BA.2.12	59 / 60	0.97	31 (0.989)	BA.2.12	Moderate presence of BA.2.12.1 Detection of mutations defining BA.2.3
5/8/2022	98.75	12909.68	9673	BA.2	59 / 60	0.98	31 (0.997)	BA.2	Moderate presence of BA.2.12.1 Detection of a mutation defining BA.2.3
5/1/2022	97.83	8248.56	6152	BA.2	59 / 60	0.97	31 (0.996)	BA.2	Low presence of BA.2.12.1 Detection of mutations defining BA.2.3
4/22/2022	99.57	13648.27	9627	BA.2	59 / 60	0.98	31 (0.997)	BA.2	Trace the presence of BA.1 Detection of BA.2.12 mutations Detection of BA.2.3 mutations
5/15/2022	98.51	11016.26	4375	BA.2	59 / 60	0.97	31 (0.987)	BA.2	Trace the presence of BA.1 Moderate presence of BA.2.12.1 Detection of a mutation defining BA.2.3
4/13/2022	99.4	19083.36	12257	BA.2.3	59 \ 60	0.95		BA.2.3	Trace the presence of BA.1 Detection of BA.2.9 mutation Detection of BA.2.12/BA.2.12.1 mutations
4/6/2022	98.5	11074.71	5769	BA.2	57/60	0.92	30 (0.97)	BA.2	Trace the presence of BA.1 Detection of BA.2.3 mutation
3/20/2022	96.63	6949.01	2404	BA.2.3	54 / 60	0.78		BA.2.3	Low presence of BA.1
3/5/2022	98.01	10506.81	5116	BA.1	48 / 51	0.76		BA.1	Moderate presence of BA.2
2/28/2022	95.29	8057.34	1501	BA.1.1 (Omicron)	38 / 51	0.68		BA.1.1	Moderate presence of BA.2
2/23/2022	97.75	16031.41	5241	BA.1 (Omicron)	43 / 51	0.74		BA.1	Moderate presence of BA.2 (Omicron)
2/6/2022	97.6	11214.41	2982	BA.1 (Omicron)	48 / 51	0.88		BA.1	Low presence of BA.2 Trace the presence of Delta
1/30/2022	98.41	9983.54	4365	BA.1.1 (Omicron)	50/51	0.95		BA.1.1	Trace the presence of BA.2
1/23/2022	98.65	19700.52	10172	BA.1 (Omicron)	51 / 51	0.94		BA.1	Low presence of BA.2 Trace the presence of Delta
1/23/2022	98.65	98.65	98.65	BA.1 (Omicron)	51/51	0.94		BA.1	Low presence of BA.2 Trace the presence of Delta
1/9/2022	99.29	14499.08	5575	**BA.1 (Omicron)**	51/51	0.96		BA.1	Trace the presence of 4 Delta mutations
1/2/2022	99.57	13755.92	5132	**BA.1 (Omicron)**	48/51	0.64		BA.1	Moderate presence of 3 BA.2 mutations Low presence of Delta (AY.25/AY.25.1)

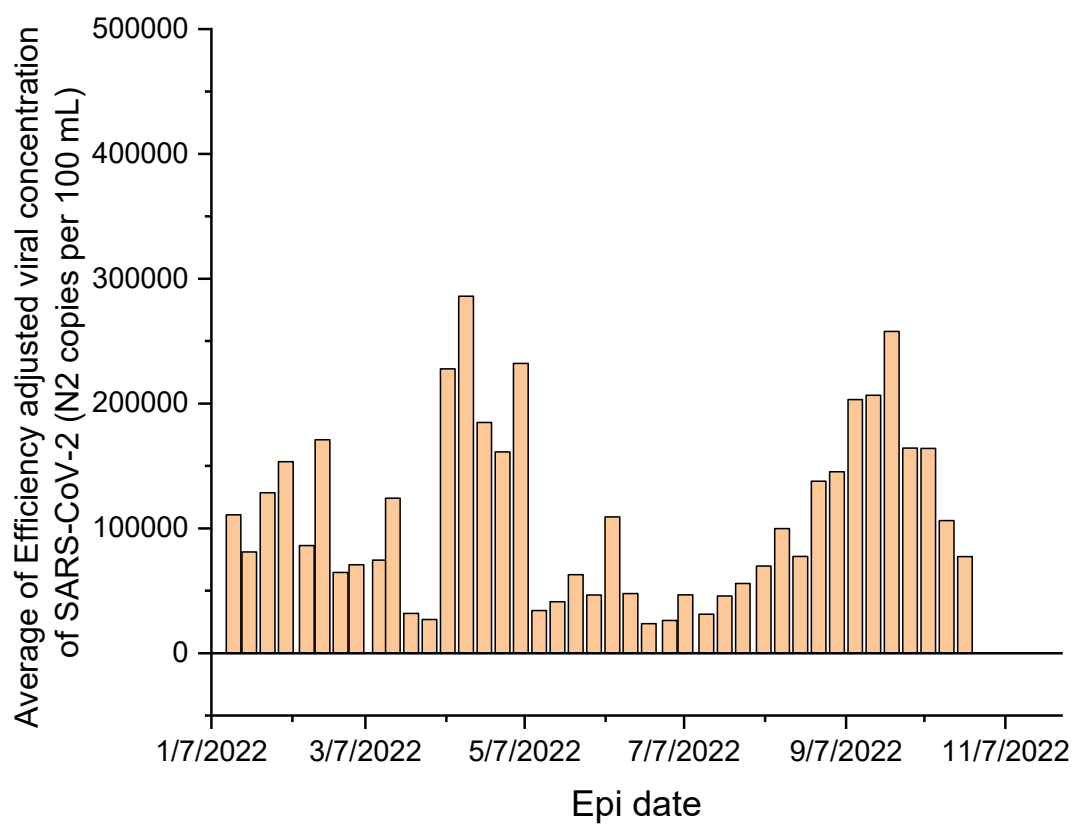**Figure S1**. Trend of wastewater SARS-COV-2 RNA between January and September 2022