

CORONASTEP Report 22 September 2020 SARS-CoV-2 Sewage Surveillance in Luxembourg

Summary


Monitoring of SARS-CoV-2 in wastewater has been established on a weekly basis in Luxembourg from 31st March 2020 for a total of **226 samples so far** (Table 3). For the wastewater treatment plants (WWTP) of Schifflange and Pétange, archived frozen samples have been analysed back to October 2019.

In general, since the beginning of the sampling carried out within the framework of the CORONASTEP project, the dynamics of SARS-CoV-2 RNA copies and fluxes in influents of WWTPs has followed the dynamics of active COVID-19 cases observed at the national level (Figure 1). Similar patterns are observed for each WWTP individually, especially for the four most impacted WWTP (Beggen, Schifflange, Bettembourg and Pétange). For the other WWTP, the detected SARS CoV-2 RNA is generally lower, and the dynamics may appear fuzzier, partly because many samples are close to the detection limit of the analytical method.

From the last samples analysed (week 38), the national level of contamination by SARS-CoV-2 was stable with results very similar to those of the previous week 37 (Table1). However, the re-emergence of the SARS-CoV-2 signal has been observed in three WWTP (Mersch, Uebersyren and Wiltz), with detection level very close to the limit of detection of the assay. The geographical differences persist with the same four most contaminated wastewater treatments plants (Pétange, Beggen, Schifflange and Bettembourg), while the others remained at a low level of contamination. The SARS-CoV-2 signal was no detectable in four out of thirteen wastewater treatment plants.

The detailed picture of each wastewater treatment plant situation is shown in Table 2 and graphs grouped in Figure 2.

Table 1 – National level of SARS-CoV-2 contamination of wastewaters in Luxembourg.

 Dark green: negative samples for SARS-CoV-2 gene E (-), Green to red: positive samples for SARS-CoV-2 gene E. The intensity of the color is related to the national SARS-CoV-2 flux (RNA copies / day / 100 000 equivalent inhabitants).

Year	2020																																					
Week	Week 41	Week 43	Week 46	Week 51	Week 3	Week 7	Week 9	Week 11	Week 14	Week 15	Week 16	Week 17	Week 18	Week 19	Week 20	Week 21	Week 22	Week 23	Week 24	Week 25	Week 26	Week 27	Week 28	Week 29	Week 30	Week 31	Week 32	Week 33	Week 34	Week 35	Week 36	Week 37	Week 38					
National Contamination Level	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			

Figure 1 – RT-qPCR quantification time-course monitoring of SARS-CoV-2 (E gene) in Luxembourgish wastewater samples from March to September 2020. Grey squares: daily-confirmed cases for Luxembourgish residents (<https://data.public.lu/fr/datasets/donnees-covid19/>), dots: cumulative SARS-CoV-2 flux (RNA copies / day / 100 000 equivalent inhabitants).

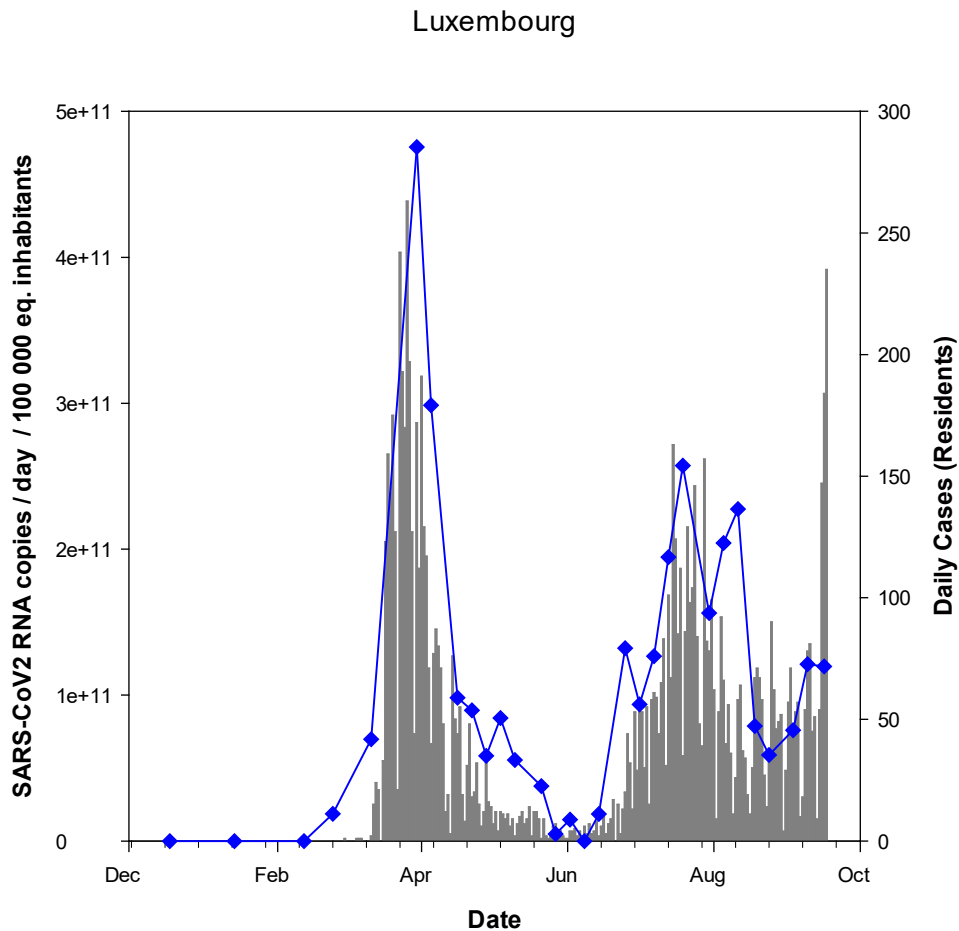
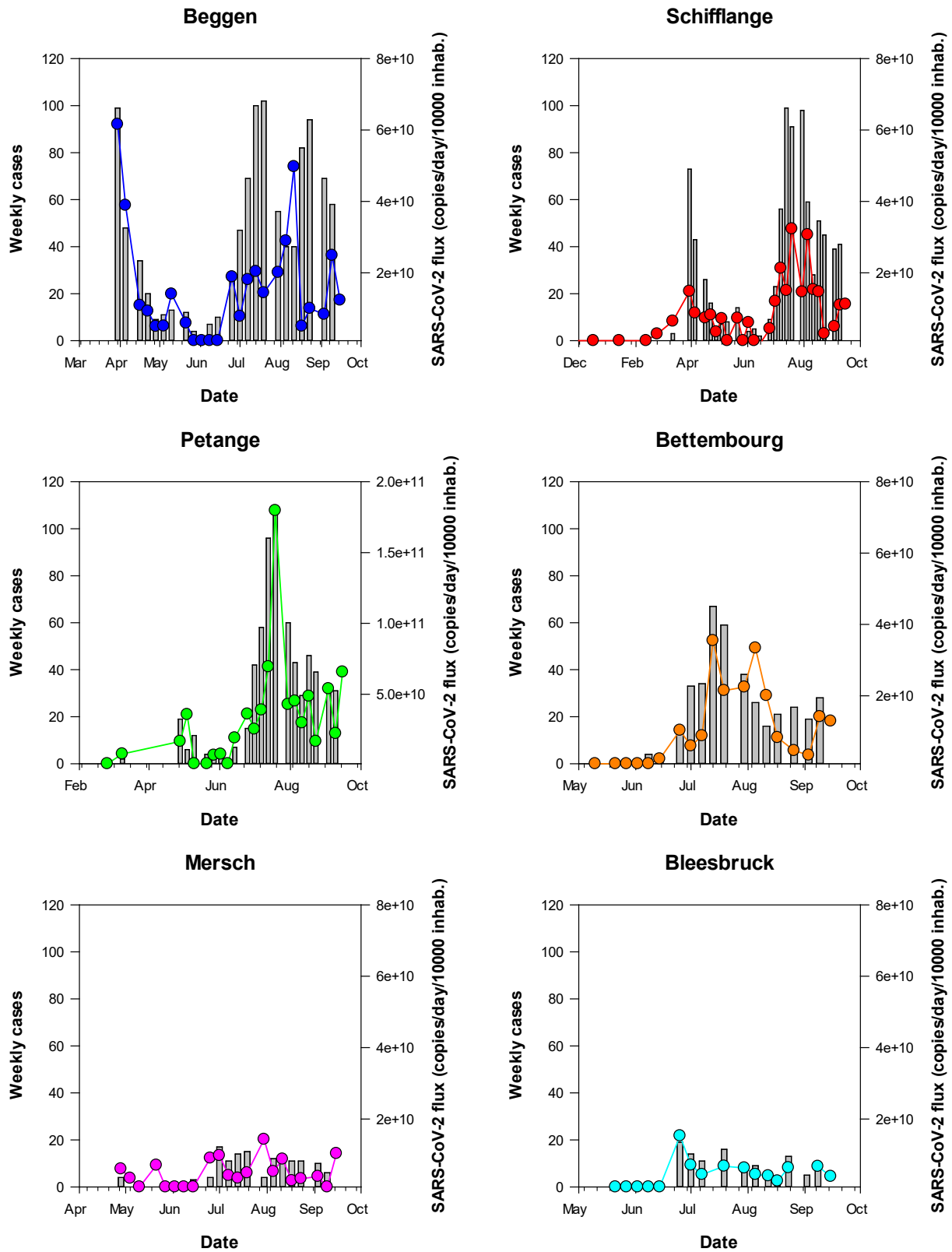


Table 2 - Level of SARS-CoV-2 contamination of each analyzed wastewater treatment plant in Luxembourg. BEG: Beggen, BET: Bettembourg, SCH: Schifflange, BLE: Blesbruck, MER: Mersch, PET: Pétange, HES: Hespérange, ECG: Echternach, UEB: Uebersyren, GRE: Grevenmacker, TRO: Troisvierges, BOE: Boevange sur Attert, WIL: Wiltz

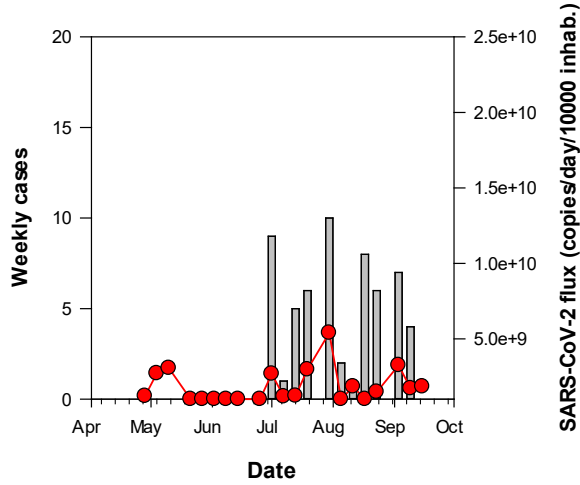
Dark green: negative samples for SARS-CoV-2 gene E (-), Green to red: positive samples for SARS-CoV-2 gene E. The intensity of the color is related to the RT-qPCR signal (Ct values)

WWTP	2019							2020																																	
	Before 1 st case			1 st wave									2 nd wave																												
	Week 41	Week 43	Week 46	Week 51	Week 3	Week 7	Week 9	Week 11	Week 14	Week 15	Week 16	Week 17	Week 18	Week 19	Week 20	Week 21	Week 22	Week 23	Week 24	Week 25	Week 26	Week 27	Week 28	Week 29	Week 30	Week 31	Week 32	Week 33	Week 34	Week 35	Week 36	Week 37	Week 38								
BEG									Red	Orange	Yellow	Light Green	Yellow			Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Yellow	Orange	Orange	Orange	Orange	Orange	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green				
BET														Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green		
SCH	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green		
BLE																Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	
MER														Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	
PET	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green						Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	
HES																Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	
ECH																Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	
UEB																Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	
GRE																Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	
TRO														Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	
BOE																																									
WIL																																									

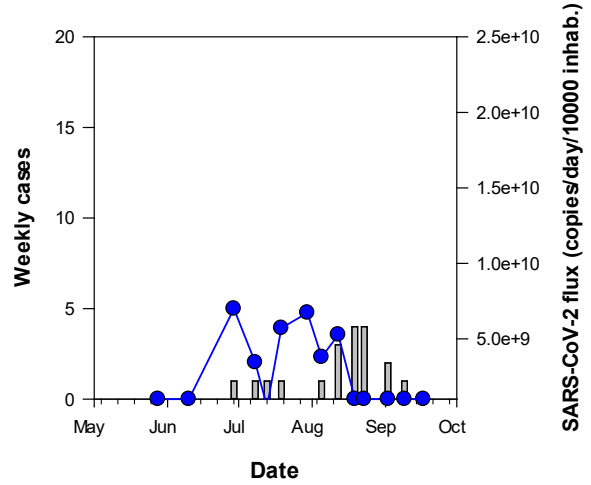
Figure 2 – RT-qPCR quantification time-course monitoring of SARS-CoV-2 (E gene) in wastewater samples from March to September 2020. Grey squares: daily-confirmed cases for the contributory area of each wastewater treatment plant, dots: SARS-CoV-2 flux (RNA copies / day / 10 000 equivalent inhabitants). The wastewater treatment plants of Boevange-sur-Attert and Wiltz are not presented.



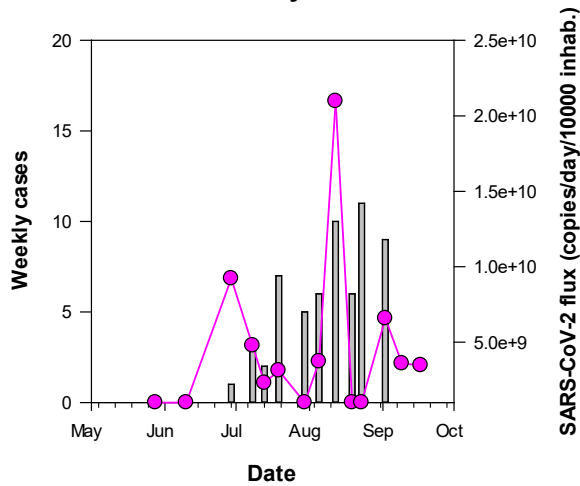
Hespérange



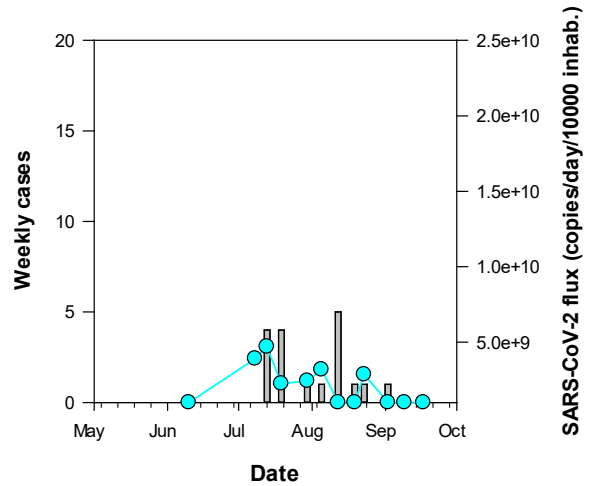
Grevenmacher



Uebersyren



Echternach



Troisvierges

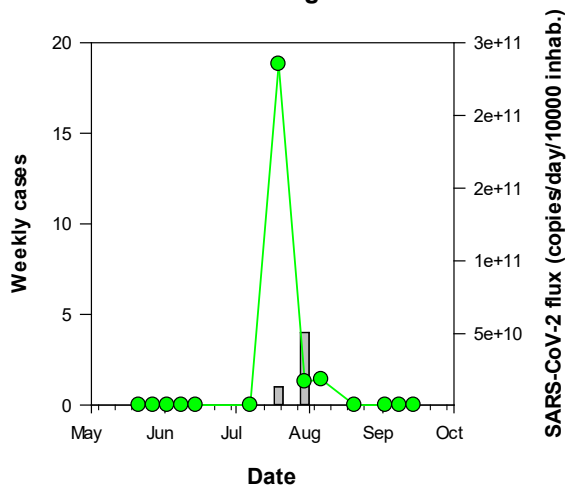


Table 3- Timing of sewage sampling

Wastewater Treatment Plant	Nominal capacity (eq. inhabitants)	Inhabitants connected	2019				2020																															Total samples
			Week 41	Week 43	Week 46	Week 51	Week 3	Week 7	Week 9	Week 11	Week 14	Week 15	Week 16	Week 17	Week 18	Week 19	Week 20	Week 21	Week 22	Week 23	Week 24	Week 25	Week 26	Week 27	Week 28	Week 29	Week 30	Week 31	Week 32	Week 33	Week 34	Week 35	Week 36	Week 37	Week 38			
Beggen	210000	139731									x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	25	
Bettembourg	95000	53606														x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	19	
Schifflange	90000	68143	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	33	
Bleesbrück	80000	30930														x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	17		
Mersch	70000	30473												x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	21		
Pétange	50000	59481	x	x	x	x	x	x	x					x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	29		
Hesperange	36000	15479												x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	21		
Echternach	36000	7499																		x						x	x	x	x	x	x	x	x	x	x	12		
Uebersyren	35000	18600																		x		x			x	x	x	x	x	x	x	x	x	x	14			
Grevenmacher	47000	9835																		x		x		x	x	x	x	x	x	x	x	x	x	x	15			
Troisvierges	5000	3411																		x	x	x	x	x								x	x	x	13			
Boevange / Attert	15000	1170																														x	x	x	5			
Wiltz	16500	6944																														x	x	x	3			
Total	785500	445302	2	2	2	2	2	2	2	2	2	2	2	2	5	5	6	8	10	8	11	8	9	9	7	11	9	11	11	11	11	10	12	12	12	13	13	226

Materials and Methods

Sewage samples

From March to September 2020, up to thirteen WWTPs were sampled at the inlet of the plant according to the planning presented in Table 2. The operators of the WWTPs sampled a 24-h composite sample of 96 samples according to your own sampling procedure. Composite sample was stored at 4°C until sample processing.

Sample processing

The samples were transported to the laboratory at 4°C and viral RNA was isolated on the day of sampling. Larger particles (debris, bacteria) were removed from the samples by pelleting using centrifugation at 2,400 x g for 20 min at 4°C. A volume of 120 mL of supernatant was filtered through Amicon® Plus-15 centrifugal ultrafilter with a cut-off of 10 kDa (Millipore) by centrifugation at 3,220 x g for 25 min at 4°C. The resulting concentrate was collected and 140 µL of each concentrate was then processed to extract viral RNA using the QIAamp Viral RNA mini kit (Qiagen) according to the manufacturer's protocol. Elution of RNA was done in 60 µL of elution buffer.

Real-time One-Step RT-PCR

Samples are screened for the presence of *Sarbecovirus* (*Coronaviridae*, *Betacoronaviruses*) and/or SARS-CoV-2 virus RNA by two distinct real-time one-step RT-PCR, one on the E gene (Envelope small membrane protein) and the second on the N gene (nucleoprotein). The E gene real-time RT-PCR can detect *Sarbecoviruses*, i.e. SARS-CoV, SARS-CoV-2 and closely related bat viruses. In the context of the COVID19 pandemic, it can be assumed that only SARS-CoV-2 strains will be detected by this assay given that SARS-CoV virus has been eradicated and other bat viruses do not commonly circulate in the human population. The E gene assay is adapted from Corman et al. [17]. The N gene real-time RT-PCR assay (N1 assay) specifically detects SARS-CoV-2 virus. It is adapted from the CDC protocol¹. The two primers/probe sets are presented in Table 3. The RT-qPCR protocols and reagents were all provided by the LIH.

Table 4 – RT-qPCR primer-probe sets

Target	Primer name	Primer sequence (5' to 3')	References
E gene	E_Sarbeco_F1	5-ACAGGTACGTTAATAGTTAATAGCGT-3	Corman et al., 2020
	E_Sarbeco_R2	5-ATATTGCAGCAGTACGCACACA-3	
	E_Sarbeco_P1	5'-FAM-ACACTAGCCATCCTTACTGCGCTTCG-BHQ1	
N gene	2019-nCoV_N1_Fw	5'-GAC CCC AAA ATC AGC GAA AT-3'	CDC
	2019-nCoV_N1_Rv	5'-TCT GGT TAC TGC CAG TTG AAT CTG-3'	
	2019-nCoV_N1 Probe	5'-FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1-3'	

Each reaction contained 5 µL of RNA template, 5 µL of TaqPath 1-step RT-qPCR MasterMix (A15299, Life Technologies), 0.5 µL of each primer (20 µM) and probe (5 µM) and the reaction volume was adjusted to a final volume of 20 µL with molecular biology grade water. Thermal cycling reactions were carried out at 50 °C for 15 min, followed by 95 °C for 2 min and 45 cycles of 95 °C for 3 sec and 58°C (E gene) or 53°C (N gene) for 30 sec using a Viia7 Real-Time PCR Detection System (Life Technologies). Reactions were considered positive (limit of detection – LOD) if the cycle threshold (Ct value) was below 40 cycles.

¹ <https://www.cdc.gov/coronavirus/2019-ncov/downloads/rt-pcr-panel-primer-probes.pdf>

Controls

A non-target RNA fragment commercially available (VetMAX™ Xeno™ IPC and VetMAX™ Xeno™ IPC Assay, ThermoFischer Scientific) was added to the viral RNA extract from sewage concentrates as an internal positive control (IPC). This IPC-RNA is used to control the performance of the RT-qPCR (E gene) and to detect the presence of RT-qPCR inhibitors.

Viral RNA copies quantification of both targeting genes in wastewater samples was performed using RT-qPCR standard curves generated using EDX SARS-CoV-2 Standard (Biorad). This standard is manufactured with synthetic RNA transcripts containing 5 targets (E, N, S, ORF1a, and RdRP genes of SARS-CoV-2, 200,000 copies/mL each). Using such a standard, the limits of quantification (LOQ) of both RT-qPCR assays were estimated to 1 RNA copy per reaction (Figure 3).

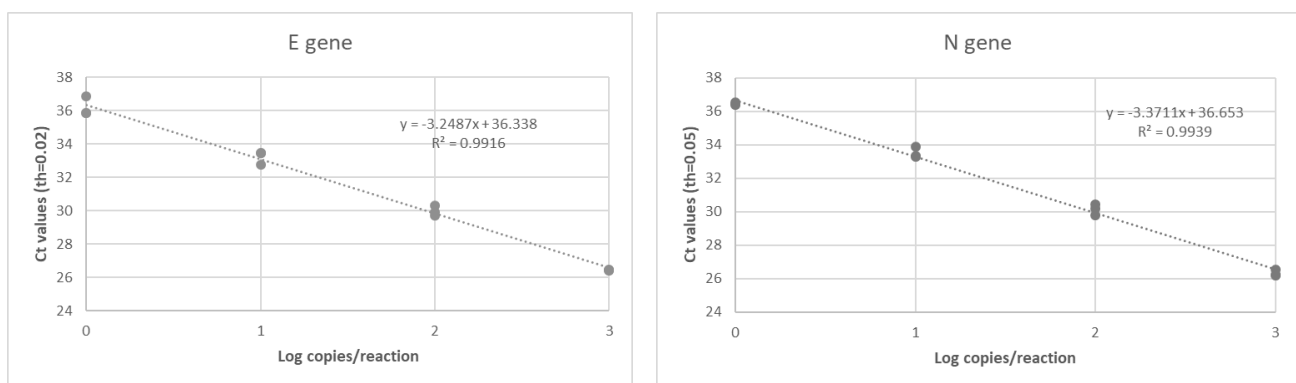


Figure 3 – RT-qPCR standard curves established for both targeting genes (E gene and N gene) of SARS-CoV-2 using a commercially available standard (Biorad).

Data interpretation

A sample is declared positive for the presence of SARS-CoV-2 if both targets (E and N gene) are detected with Ct values less than or equal to the LOQ. If only one target is detected or if target genes are detected with Ct values between the LOD and the LOQ, samples are reported as presumptive positive (+/-). A sample is declared negative when no target genes are detected (Ct values superior to the LOD).

In case of presumptive positive, sample is tested again using another RT-qPCR detection assay (Allplex 2019-nCoV Assay, Seegene). This commercially available detection kit is a multiplex real-time RT-PCR assay for simultaneous detection of three target genes of SARS-CoV-2 in a single tube. The assay is designed to detect RdRP and N genes specific for SARS-CoV-2, and E gene specific for all *Sarbecovirus* including SARS-CoV-2.

As shown in Figure 4, a good linear relationship (R^2 : 0.92) was obtained between the SARS-CoV-2 RNA concentrations estimated using the E gene and the N gene, respectively. Therefore, only the E gene results were presented in this report.

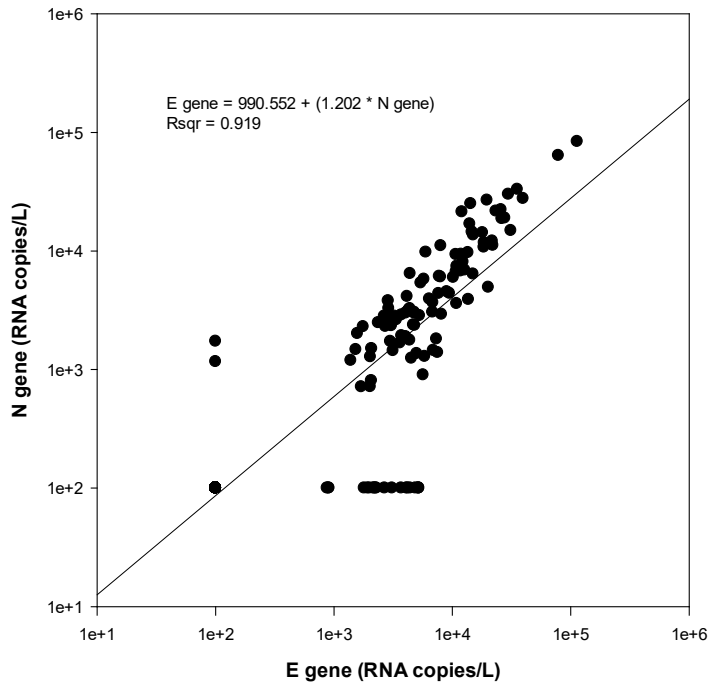


Figure 4 - Relationship between the SARS-CoV-2 RNA concentration (RNA copies / L of wastewater) estimated by the both distinct RT-qPCR systems targeting the E and N gene, respectively