The Catalan Surveillance Network of SARS-CoV-2 in Sewage: design, implementation, and performance

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Supplementary Material

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**Supplementary Table S1.** Details of sample collection for the 56 WWTP monitored by SARSAIGUA. BCN: Barcelona; GIR: Girona; LLE: Lleida, TAR: Tarragona.

| WWTP code | WWTP name | Province | Sampling frequency | Collection before grit chamber | 24h samples | Flow proportional | 20 min freq. | Refrigerated |
|-----------|-----------|----------|--------------------|---------------------------------|-------------|------------------|-------------|--------------|
| 1         | DABR      | Abrera   | Weekly             | YES                             | YES         | NO               | YES         | YES          |
| 2         | DAMP      | Amposta  | Weekly             | YES                             | YES         | YES              | YES         | NO           |
| 3         | DBAL      | Balaguer | Weekly             | YES                             | YES         | NO               | YES         | YES*         |
| 4         | DBAY      | Banyoles | Weekly             | YES                             | YES         | YES              | YES*        |
| 5         | DBBL      | Les Borges Blanques | Biweekly | NO                   | YES         | YES*             |
| 6         | DBER      | Berga    | Weekly             | NO                              | YES         | YES              | YES         |
| 7         | DBLN      | Blanes   | Weekly             | YES                             | NO          | YES              | YES         |
| 8         | DBSS      | Besòs    | Weekly             | NO                              | YES         | YES              | YES         |
| 9         | DCER      | Cervera  | Biweekly           | YES                             | YES         | NO               | YES         |
| 10        | DCPA      | Castell-Platja d’Aro | Biweekly (summer) | YES                             | YES         | YES              | YES         |
| 11        | DFAL      | Falset   | Weekly (winter)    | NO                              | YES         | YES              | YES         |
| 12        | DFIG      | Figueres | Weekly             | NO                              | YES         | YES              | YES         |
| 13        | DFON      | Fondarella | Biweekly         | YES                             | YES         | YES              | YES         |
| 14        | DGAN      | Gandesa  | Biweekly           | YES                             | YES         | YES              | YES*        |
| 15        | DGIR      | Girona   | Weekly             | YES                             | YES         | YES              | YES         |
| 16        | DGRA      | Granollers | Weekly        | YES                             | YES         | YES              | YES         |
| 17        | DGVC      | Gavà/Viladecans | Weekly   | YES                             | YES         | YES              | YES*        |
| 18        | DIGU      | Igualada | Weekly             | YES                             | YES         | YES              | YES         |
| 19        | DLDM      | Lloret de Mar | Biweekly      | YES                             | YES         | YES              | YES         |
| 20        | DLLE      | Lleida   | Biweekly           | YES                             | YES         | YES              | YES         |
| 21        | DLLL      | La Llagosta | Biweekly      | YES                             | YES         | YES              | YES         |
| 22        | DMAN      | Manlleu  | Weekly             | NO                              | YES         | YES              | YES         |
| 23        | DMAS      | Manresa  | Weekly             | YES                             | YES         | YES              | YES         |
| 24        | DMAT      | Mataró   | Weekly             | YES                             | YES         | YES              | YES*        |
| 25        | DMDV      | Montornès del Vallès | Biweekly | YES                             | YES         | YES              | YES*        |
| 26        | DMIR      | Montcada | Weekly             | YES                             | YES         | NO               | YES         |
| 27        | DMLN      | Móra d'Ebre/ Móra La Nova | Biweekly | NO                   | YES         | YES              | YES         |
| 28        | DMOB      | Montblanc | Biweekly        | YES                             | YES         | YES              | YES         |
| 29        | DMOF      | Montferrer | Weekly         | NO                              | NO          | NO               | YES         |
| 30        | DMRT      | Martorell | Weekly            | YES                             | YES         | YES              | YES*        |
| 31        | DOLO      | Olot     | Weekly             | YES                             | YES         | YES              | YES         |
| 32        | DPAM      | Palamós  | Weekly             | YES                             | YES         | YES              | YES*        |
|   | Code | Location                  | Code | Code | Frequency | Yes | No | Yes | No | Yes | Yes |
|---|------|---------------------------|------|------|-----------|-----|----|-----|----|-----|-----|
| 33 | DPDL | El Prat de Llobregat     | BCN  | Weekly | YES       | YES | YES | YES | YES | YES*|
| 34 | DPSU | El Pont de Suert         | LLE  | Biweekly | YES | YES | YES | YES | YES | YES*|
| 35 | DPUI | Puigcerdà                | GIR  | Weekly  | YES | YES | YES | YES | YES | YES*|
| 36 | DRIP | Ripoll                   | GIR  | Weekly  | NO  | YES | YES | NO  | YES*|
| 37 | DRSS | Roses                    | GIR  | Biweekly (summer) | NO | YES | YES | YES | YES |
| 38 | DRUB | Rubí                      | BCN  | Weekly  | YES | YES | YES | NO  | YES |
| 39 | DRUS | Reus                      | TAR  | Weekly  | YES | YES | YES | NO  | YES |
| 40 | DSFL | Sant Feliu de Llobregat  | BCN  | Weekly  | YES | YES | YES | NO  | YES |
| 41 | DSSL | Salent/Artés             | BCN  | Weekly  | YES | YES | YES | YES | YES |
| 42 | DSOL | Solsona                  | LLE  | Weekly  | NO  | YES | YES | YES | YES*|
| 43 | DSOR | Sort                      | LLE  | Fortnightly | YES | YES | YES | YES | YES |
| 44 | DSRS | Sabadell/Riu Sec         | BCN  | Weekly  | YES | YES | YES | NO  | YES |
| 45 | DTAR | Tarragona                | TAR  | Weekly  | NO  | YES | YES | YES | YES |
| 46 | DTOT | Tortosa-Roquetes         | TAR  | Weekly  | NO  | YES | YES | YES | YES*|
| 47 | DTRG | Tàrrega                  | LLE  | Biweekly | YES | YES | YES | YES | YES |
| 48 | DTRP | Tremp                     | LLE  | Biweekly | NO  | YES | YES | YES | YES*|
| 49 | DTRS | Terrassa                 | BCN  | Weekly  | NO  | YES | YES | YES | YES*|
| 50 | DVAL | Valls Poble              | TAR  | Biweekly | YES | YES | YES | YES | YES |
| 51 | DVDP | Vilafranca del Penedès   | BCN  | Weekly  | YES | YES | YES | YES | YES |
| 52 | DVEN | Riera de la Bisbal       | TAR  | Weekly  | YES | YES | YES | NO  | NO  |
| 53 | DVIC | Vic                       | BCN  | Weekly  | NO  | YES | YES | YES | YES |
| 54 | DVIE | Vielha e Mijaran         | LLE  | Biweekly | YES | YES | YES | YES | YES*|
| 55 | DVLC | Vila-Seca/Salou          | TAR  | Biweekly (summer) | YES | YES | YES | YES | YES*|
| 56 | DVLG | Vilanova i la Geltrú     | BCN  | Weekly  | YES | YES | YES | YES | YES |

*The autosampler is not refrigerated but we used an alternative cooling system in this case.
**Supplementary Table S2.** Details about RT-qPCR analysis performed by laboratories of SARSAIGUA.

| **Experimental Design** |  |
|-------------------------|---|
| Definition of experimental and control groups | Each lab analyses 15/16 samples per week including a process control in each batch |

| **Sample** |  |
|-------------|---|
| Description | 24h composite wastewater sample from the inlet of a WWTP |
| Processing procedure | Concentration based on precipitation (Aluminium hydroxide method) or ultrafiltration (Centricon-Millipore Corp. or CP-Select-InnovaPrep LLC) |
| Sample storage conditions and duration | Samples are kept at 4º until processed within the 12h after sampling |

| **Nucleic Acid Extraction** |  |
|-----------------------------|---|
| Procedure and/or instrumentation | Magnetic extraction/Silica columns |
| Name of kit and details of any modifications | Promega Maxwell® Viral Total Nucleic Acid Purification Kit, Total RNA Purification 96-Well Kit-Norgen, QIAamp® Viral RNA Kit-Qiagen |
| Inhibition testing (C<sub>q</sub> dilutions, spike or other) | Each sample is analyzed 1/10 dilution per duplicate to evaluate inhibition |

| **RT-qPCR Target Information** | N1 (CDC, 2019) | N2 (CDC, 2019) | IP4 (Pasteur, 2020) |
|-----------------------------|----------------|----------------|-------------------|
| Sequence accession number   | LC528233.1     | LC528233.1     | NC_004718         |
| Amplicon length (bases)     | 72             | 67             | 107               |

| **RT-qPCR Oligonucleotides** |  |
|-------------------------------|---|
| Primer sequences (5'→3') F   | GACCCCCAAATCCGCAAT |
|                              | TTACAAATTTGGCCGAAA |
|                              | GGTAACTGGTATGATTTCG |
| Primer sequences (5'→3') R   | TCTGGTTACTGGCAGTGTAATCGT |
|                              | GCACGACATTCCGAAAGA |
|                              | CTGTCAGATGGTTAATAAGG |
| Probe                        | ACCCGCATTACGTTTGTTGACC |
|                              | ACAATTGCCCCACGGCTTCAG |
|                              | TCATAAAACACGGCAGG |

| **RT-qPCR Protocol** | N1 | N2 | IP4 |
|----------------------|----|----|-----|
| Complete thermocycling parameters | 25ºC 2 min, 50ºC 15 min, 95ºC 2 min, 45 cycles (95ºC 3 sec, 55ºC 30 sec) | 25ºC 2 min, 50ºC 15 min, 95ºC 2 min, 45 cycles (95ºC 3 sec, 55ºC 30 sec) | 55ºC 20 min, 95ºC 3 min, 50 cycles (95ºC 15 sec, 58ºC 30 sec) |
| Reaction volume and amount of cDNA/DNA | 25µl reaction volume (5µl sample) | 25µl reaction volume (5µl sample) | 25µl reaction volume (5µl sample) |
| Primer, (probe), Mg++ and dNTP concentrations | 0,4µM primers, 0,2 µM probe, 0,8 mM Mg++, 3mM dNTPs | 0,4µM primers, 0,2 µM probe, 0,8 mM Mg++, 3mM dNTPs | 0,4µM primers, 0,2 µM probe, 0,8 mM Mg++, 3mM dNTPs |
| Polymerase identity and concentration | Following Kit specifications | Following Kit specifications | Following Kit specifications |
| Buffer/kit identity and manufacturer | RNA UltraSense™ One-Step Quantitative RT-PCR, Luna Universal Probe One-Step, Takara RR64A | RNA UltraSense™ One-Step Quantitative RT-PCR, Luna Universal Probe One-Step, Takara RR64A | RNA UltraSense™ One-Step Quantitative RT-PCR, Luna Universal Probe One-Step, Takara RR64A |
| Manufacturer of qPCR instrument | QuantStudio 1/3 Real-Time PCR System, 7900HT ABI, Stratagene Mx3005P, BioradCFX96B | QuantStudio 1/3 Real-Time PCR System, 7900HT ABI, Stratagene Mx3005P, BioradCFX96B | QuantStudio 1/3 Real-Time PCR System, 7900HT ABI, Stratagene Mx3005P, BioradCFX96B |

| **RT-qPCR Validation*** | N1 | N2 | IP4 |
|-------------------------|----|----|-----|
| Parameters of standard curves | lab #1 | lab #2 | lab #3 | lab #1 | lab #2 | lab #3 | lab #2 |
| Slope                   | –3.38 | –3.44 | –3.43 | –3.48 | –3.63 | –3.52 |
| y-intercept             | ± 0.12 | ± 0.21 | ± 0.19 | ± 0.18 | ± 0.27 | ± 0.16 |
| Efficiency              | 41.20 | 40.55 | 40.44 | 40.00 | 41.57 | 42.18 |
| Efficiency              | ± 1.11 | ± 1.40 | ± 0.90 | ± 1.55 | ± 1.37 | ± 1.81 |
| Efficiency              | 98.06 | 96.21 | 95.24 | 93.64 | 89.31 | 91.67 |
| Efficiency              | ± 4.83 | ± 7.71 | ± 7.59 | ± 10.54 | ± 9.83 | ± 10.18 |
| Efficiency              | 0.9887 | 0.9876 | 0.9928 | 0.9832 | 0.9915 | 0.9902 |
| Efficiency              | ± 0.015 | ± 0.009 | ± 0.008 | ± 0.034 | ± 0.010 | ± 0.001 |

| Standard controls       | Twist Biosciences RNA Synthetic Controls |

* Average values of the main RT-qPCR parameters for the three gene targets analyzed. Values show the mean ± standard deviation (n = 108).
Supplementary Figure S1. Relation between surveilled WWTPs, percentage of served population and territorial equilibrium measured as the average distance of any raster cell in Catalonia to the closest WWTP included in the network. A, B and C are the thresholds in the stepwise selection procedure (A: selection of WWTPs serving > 150,000 inhabitants; B: A plus including the largest WWTPs per county; C: B plus WWTPs to reach a population coverage = 80%).