

# WASTEWATER-BASED EPIDEMIOLOGICAL SURVEILLANCE

Methodological appendix

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# TABLE OF CONTENTS

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|  |          |
|--|----------|
| <b>ABBREVIATIONS</b> .....                   | <b>6</b> |
| <b>INTRODUCTION</b> .....                    | <b>7</b> |
| <b>METHODOLOGY</b> .....                     | <b>8</b> |
| 1. Samples collection.....                   | 8        |
| 2. Laboratory method .....                   | 10       |
| 3. Viral load.....                           | 11       |
| 4. Normalization using PMMoV .....           | 12       |
| 5. Wastewater-based alerting indicators..... | 13       |
| 6. Population coverage.....                  | 14       |
| 7. Data management and analysis.....         | 16       |

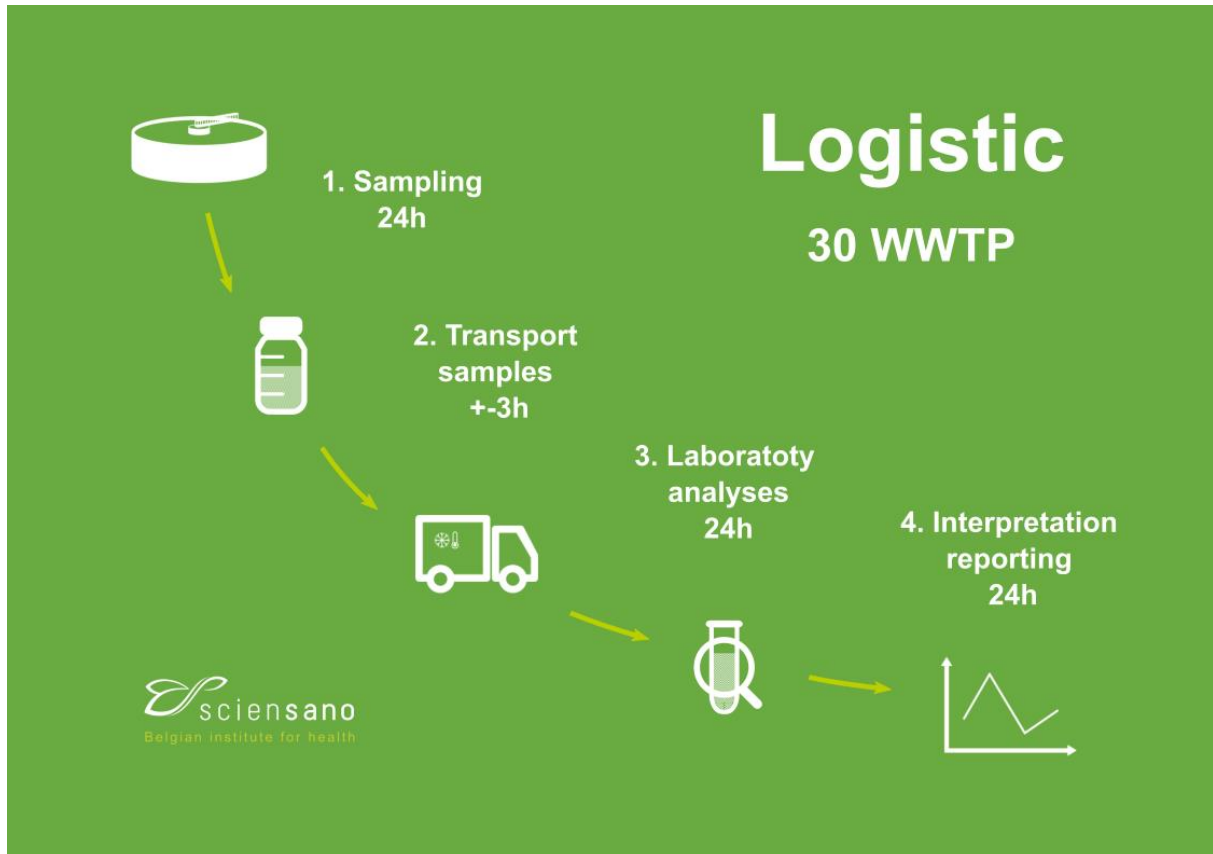
# ABBREVIATIONS



|             |                            |
|-------------|----------------------------|
| <b>WWTP</b> | Wastewater treatment plant |
| <b>IE</b>   | Inhabitant equivalent      |

# INTRODUCTION

The present report provides a detailed overview of the methodological steps needed for the surveillance from the sampling to the reporting of the results.



**Figure 1 • Overview of the logistical and methodological steps of the surveillance, and their respective allocated time.**

The wastewater situation can be followed on a weekly basis on:

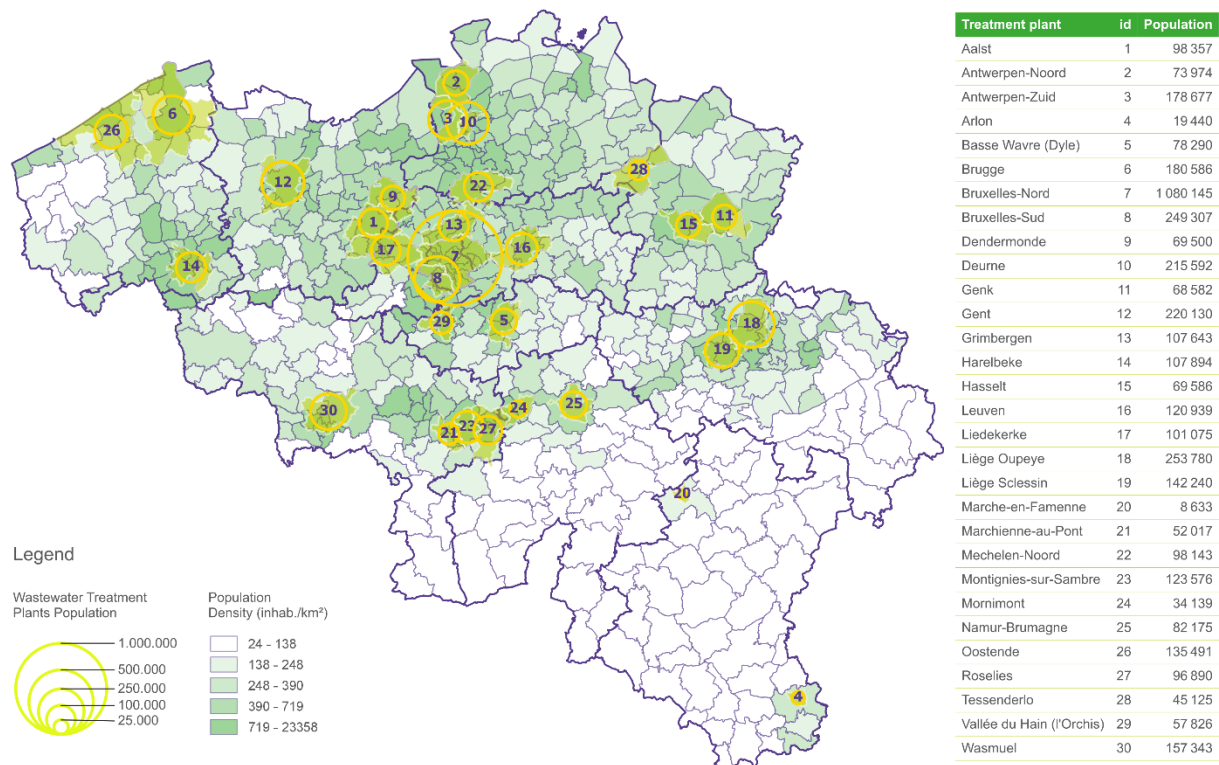
- the graphics available on the public [COVID-19 dashboard](#)
- the wastewater-based epidemiology weekly [report](#) of the SARS-CoV-2 surveillance
- the epidemiological update published on Fridays by the Risk Assessment Group (RAG) after validation
- by the Risk Management Group (RMG) in [French](#) and [Dutch](#).

# METHODOLOGY

## 1. Samples collection

The 30 wastewater treatment plants (WWTPs) and their corresponding catchment area (the area for which the wastewaters of the sewage network reach the WWTP) included in the wastewater-based surveillance can be seen in Figure 2.

The surveillance covers 38% of the Belgian population (cfr tables 4-6 for more details).



**Figure 2 • Wastewater treatment plants (WWTPs) and their catchment areas (yellow-green) included in the wastewater-based surveillance. The catchment areas of each WWTP are provided by the Vlaamse Milieumaatschappij (VMM) in Flanders, by the Société Publique de Gestion de l'Eau (SPGE) in Wallonia and by Leefmilieu Brussel/Bruxelles Environnement in Brussels. The catchment areas were redrawn in Wallonia and Brussels for a harmonized visualisation.**

The WWTPs were selected based on the following criteria:

- plants (WWTPs) covering areas with a high population density (epidemiology-efficiency approach)
- plants covering a large population, of more than 50.000 inhabitants (cost-efficiency approach)
- at least two plants should be present in each province
- at least 30% of the population must be covered in each region



24-hour composite samples are collected once a week on Mondays from the influents of the 30 wastewater treatment plants (WWTPs). The collection is done through either volume proportional or time proportional auto-samplers. In exceptional circumstances, because of sampler failure or works (maintenance, etc.) at the treatment plant, grab samples were taken.

Once collected, the samples are stored and transported to the respective analytical laboratory, the same day (cfr figure 1), at 4°C. The samples are analysed within 24 hours. Table 1 presents the list of WWTPs with the two distinct laboratories performing the analyses.

**Table 1 • List of the 30 covered WWTPs, and corresponding laboratories performing the analyses.**

| WWTP                         | Laboratory            |
|------------------------------|-----------------------|
| Aalst                        | Sciensano             |
| Antwerpen-Noord              | University of Antwerp |
| Antwerpen-Zuid               | University of Antwerp |
| Arlon                        | Sciensano             |
| Basse Wavre (Dyle)           | Sciensano             |
| Brugge                       | Sciensano             |
| Bruxelles Nord/Brussel Noord | Sciensano             |
| Bruxelles Sud/Brussel Zuid   | Sciensano             |
| Dendermonde                  | University of Antwerp |
| Deurne                       | University of Antwerp |
| Genk                         | University of Antwerp |
| Gent                         | Sciensano             |
| Grimbergen                   | Sciensano             |
| Harelbeke                    | Sciensano             |
| Hasselt                      | University of Antwerp |
| Leuven                       | Sciensano             |
| Liedekerke                   | Sciensano             |
| Liège Oupeye                 | Sciensano             |
| Liège Sclessin               | Sciensano             |
| Marche-en-Famenne            | Sciensano             |
| Marchienne-au-Pont           | Sciensano             |
| Mechelen-Noord               | University of Antwerp |
| Montignies-sur-Sambre        | Sciensano             |
| Mornimont                    | Sciensano             |
| Namur-Brumagne               | Sciensano             |
| Oostende                     | Sciensano             |
| Roselies                     | Sciensano             |
| Tessenderlo                  | University of Antwerp |
| Vallée du Hain (l'Orchis)    | Sciensano             |
| Wasmuel                      | Sciensano             |

## 2. Laboratory method

The targeted sequences located in two genes of SARS-CoV-2 were selected to maximize the specificity of our analytical detection methods (specificity for all SARS-CoV-2 lineages). The N2 sequence targets the N gene encoding for the nucleoprotein, and the E sequence targets the E gene encoding for the viral envelope protein. In addition to SARS-CoV-2, the Pepper Mild Mottle virus (PMMoV) fecal indicator is also quantified. The PMMoV is used as an indicator of the population effectively covered by the sample (through an estimation of the fecal load). See section 5 of the present report for more info on the PMMoV strategy and methodology. Details about the respective sequences can be found in table 2.

Samples are first ultrafiltrated, concentrated, and their RNA is then extracted. Analyses are done by RT-dPCR using Qiacuity (QIAGEN GmbH, Germany). The viral concentrations (copies/mL) are computed as the mean of the viral concentrations measured on the N2 and E genes. The limit of quantification (LOQ) is fixed at 10 gene copies/mL.

**Table 2 • Overview of primers/probes sequences used for the RT-dPCR assays (cc. = concentration).**

| Targeted gene            | Primer/probe | Final cc. | Sequence (5' – 3')          |
|--------------------------|--------------|-----------|-----------------------------|
| Nucleo-capsid N2         | nCoV_N2-F    | 800 nM    | TTACAAACATTGGCCGCAAA        |
|                          | nCoV_N2-R    | 800 nM    | GCGCGACATTCCGAAGAA          |
|                          | nCoV_N2-P    | 250 nM    | ACAATTTGCCCCAGCGCTTCAG*     |
| Envelop E                | E_Sarbeco-F  | 800 nM    | ACAGGTACGTTAATAGTTAATAGCGT  |
|                          | E_Sarbeco-R  | 800 nM    | ATATTGCAGCAGTACGCACACA      |
|                          | E_Sarbeco-P  | 250 nM    | ACACTAGCCATCCTTACTGCGCTTCG* |
| Pepper Mild Mottle Virus | PMMV-R       | 400 nM    | TTGTCGGTTGCAATGCAAGT        |
|                          | PMMV-P       | 200 nM    | CCTACCGAAGCAAATG*           |

### 3. Viral load

In order to account for the dilution caused by rain events, quantified viral concentrations (copies/mL) are expressed as viral load per capita (SARS-CoV-2 RNA copies/day/100.000 inhabitants) by multiplying the quantified viral concentration (SARS-CoV-2 RNA gene copies/mL) by the flow (m<sup>3</sup>) of the 24-hour sampling period and normalized with the domestic inhabitant-equivalent (IE) of the corresponding treatment plant.

When expressing the wastewater results at the national, regional, or provincial level, the means ( $\mu$ ) of the viral load ( $cc_i$ ) measured in the corresponding catchment areas ( $i$ ) were weighted with the domestic inhabitant equivalent (IE). Therefore, the viral loads measured in the catchment areas covering a larger population account for a larger share in the mean load computed. The following expression was used to compute the weighted mean:

$$\mu = \frac{\sum_{i=1}^n (cc_i * IE_i)}{\sum_{i=1}^n (IE_i)}$$

## 4. Normalization using PMMoV

The normalization of the wastewater results using a faecal indicator is used in parallel of the viral load as a secondary approach to interpret the results of the surveillance. The aims of this approach are to correct SARS-CoV-2 viral concentration for:

- a possible dilution effect of concentrations measured in samples (rain events)
- the population covered in each catchment area, as well as its effective mobility. Therefore, faecal ratios are computed dividing the SARS-CoV-2 viral concentrations by the PMMoV concentrations measured in samples:  $(\text{SARS-CoV-2 RNA copies/mL}) / (\text{PMMoV RNA copies/mL})$ . The PMMoV virus is a well-known indicator of human faecal contamination used to estimate the population represented by a wastewater sample. As the PMMoV and the SARS-CoV-2 are analyzed simultaneously, their ratios correct for rain dilution and population's mobility

PMMoV measures are used as internal controls for inhibitors and dilution effects. The faecal indicator allows to highlight high dilution events leading to a viral concentration being below the LOQ, reported as a 'negative result'.

## 5. Wastewater-based alerting indicators

Three alerting indicators were developed to assess the wastewater-based epidemiological situation: the High Circulation, the Fast Increase, and the Increasing Trend. Each of the three indicators is computed for each covered area, on the viral loads (cfr Section 4).

The High Circulation indicator provides information on the level of SARS-CoV-2 concentration. The Fast Increase indicator highlights the areas where the viral concentrations are increasing quickly in a short term. The areas where the concentrations show a rising trend for a longer term are indicated by the Increasing Trend indicator.

In further details, before computing the indicators, a normalization step is applied on the viral loads. The normalization allows for a comparison of the viral loads with the ones of a reference period. This way of normalizing removes the analytical bias resulting from the distinct laboratories. The normalization is performed by expressing viral loads in percentage of the maximum value recorded during a previous viral wave for each area. It is worth mentioning that the maximum value recorded in a specific area depends on the epidemiological context, and thus varies according to the reference period considered.

In case the analytical method is different between the reference period and the moment of computing the indicators, correction factors were applied. The correction factors were computed by gene and laboratory using linear regression between the viral concentrations measured with the old and new analytical method. The correction factors are thus applied to ensure continuity and comparability of the indicators.

The format of the indicators is Boolean. Therefore, if the normalized viral load exceeds half (50%) of the highest value recorded during a virus wave of reference, the value of the High Circulation indicator is set to 1. Else it is set to 0. The value of the Fast Increase is set to 1 if the moving average on the past 7 days of the normalized viral load has increased more than 70% over the week. Else it is set to 0. Additionally, the Increasing Trend is set to 1 if the moving average on the past 14 days of the normalized viral load has increased for 14 days or more. Otherwise, it is set to 0.

## 6. Population coverage

The domestic equivalent inhabitants (EI) are used to estimate the population connected to the sewage system of a WWTP. The domestic EI are based on several data sources (population, building type, network connections, and WWTPs geographical coverage areas). These domestic inhabitants equivalent are available by municipality and wastewater treatment plant.

It is worth mentioning that some municipalities are covered by several treatment plants and a treatment plant catchment area can cover more than one province or region.

The inhabitant equivalent data are provided by the Société Publique de Gestion de l'Eau (SPGE) in Wallonia and by the Vlaamse Milieumaatschappij (VMM) in Flanders. In Brussels, the population is estimated based on the population by statistical sector data provided by STATBEL and the global connection percentage to the sewage network in the region. Table 3 presents the estimated population covered in each wastewater catchment area. Tables 4, 5 and 6 present the domestic inhabitant equivalent and the resulting population coverage at provincial, regional and country levels.

**Table 3 • Location of the WWTPs covered by the surveillance with their respective inhabitant equivalents (Data 2020).**

| WWTP                         | Inhabitant equivalent | Province        | Region   |
|------------------------------|-----------------------|-----------------|----------|
| Aalst                        | 98 357                | Oost-Vlaanderen | Flanders |
| Antwerpen-Noord              | 73 974                | Antwerpen       | Flanders |
| Antwerpen-Zuid               | 178 677               | Antwerpen       | Flanders |
| Arlon                        | 19 440                | Luxembourg      | Wallonia |
| Basse Wavre (Dyle)           | 78 290                | Brabant Wallon  | Wallonia |
| Brugge                       | 180 586               | West-Vlaanderen | Flanders |
| Bruxelles Nord/Brussel Noord | 1 080 145             | Brussels        | Brussels |
| Bruxelles Sud/Brussel Zuid   | 249 307               | Brussels        | Brussels |
| Dendermonde                  | 69 500                | Oost-Vlaanderen | Flanders |
| Deurne                       | 215 592               | Antwerpen       | Flanders |
| Genk                         | 68 582                | Limburg         | Flanders |
| Gent                         | 220 130               | Oost-Vlaanderen | Flanders |
| Grimbergen                   | 107 643               | Vlaams-Brabant  | Flanders |
| Harelbeke                    | 107 894               | West-Vlaanderen | Flanders |
| Hasselt                      | 69 586                | Limburg         | Flanders |
| Leuven                       | 120 939               | Vlaams-Brabant  | Flanders |
| Liedekerke                   | 101 075               | Vlaams-Brabant  | Flanders |
| Liège Oupeye                 | 253 780               | Liège           | Wallonia |
| Liège Sclessin               | 142 240               | Liège           | Wallonia |
| Marche-en-Famenne            | 8 633                 | Luxembourg      | Wallonia |
| Marchienne-au-Pont           | 52 017                | Hainaut         | Wallonia |
| Mechelen-Noord               | 98 143                | Antwerpen       | Flanders |
| Montignies-sur-Sambre        | 123 576               | Hainaut         | Wallonia |
| Mornimont                    | 34 139                | Namur           | Wallonia |

|                           |         |                 |          |
|---------------------------|---------|-----------------|----------|
| Namur-Brumagne            | 82 175  | Namur           | Wallonia |
| Oostende                  | 135 491 | West-Vlaanderen | Flanders |
| Roselies                  | 96 890  | Hainaut         | Wallonia |
| Tessengerlo               | 45 125  | Limburg         | Flanders |
| Vallée du Hain (l'Orchis) | 57 826  | Brabant Wallon  | Wallonia |
| Wasmuel                   | 157 343 | Hainaut         | Wallonia |

**Table 4 • Inhabitant equivalent and population coverage (%) at the provincial level (Data 2020).**

| Province        | Inhabitant equivalent | Population coverage |
|-----------------|-----------------------|---------------------|
| Antwerpen       | 564 854               | 30.2%               |
| Brabant Wallon  | 135 340               | 33.3%               |
| Brussels        | 1 212 163             | 99.5%               |
| Hainaut         | 420 555               | 31.2%               |
| Liège           | 396 020               | 35.7%               |
| Limburg         | 182 851               | 20.8%               |
| Luxembourg      | 28 073                | 9.8%                |
| Namur           | 125 585               | 25.3%               |
| Oost-Vlaanderen | 409 093               | 26.8%               |
| Vlaams-Brabant  | 428 590               | 37.1%               |
| West-Vlaanderen | 423 971               | 35.3%               |

**Table 5 • Inhabitant equivalent and population coverage (%) at the regional level (Data 2020).**

| Region   | Inhabitant equivalent | Population coverage |
|----------|-----------------------|---------------------|
| Brussels | 1 212 163             | 99.5%               |
| Flanders | 2 009 359             | 30.3%               |
| Wallonia | 1 105 573             | 30.3%               |

**Table 6 • Inhabitant equivalent and population coverage (%) at the country level (Data 2020).**

| Country | Inhabitant equivalent | Population coverage |
|---------|-----------------------|---------------------|
| Belgium | 4 327 095             | 37.7%               |

## 7. Data management and analysis

Data management was performed using SAS (SAS 7.15, NC, USA) while statistical analysis and visualization were carried out with the R software (version 4.2.3).



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## MORE INFORMATION

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