

METHODOLOGICAL APPENDIX TO THE WEEKLY REPORT

“WASTEWATER-BASED EPIDEMIOLOGICAL SURVEILLANCE OF SARS-COV-2”

Authors: R. Janssens¹, H. Maloux¹, S. Hanoteaux¹, B. Verhaegen², K. Van Hoorde²,
K. Dierick², S. Quoilin¹, M. Lesenfans*¹

¹ Sciensano, Epidemiology of infectious diseases Service

² Sciensano, Foodborne pathogens Service

*Project responsible and contact: marie.lesenfans@sciensano.be

In collaboration with:



Methodological appendix

ORGANIZATIONAL ASPECTS

The SARS-CoV-2 national wastewater surveillance was launched in Belgium in mid-September 2020. Sciensano coordinates this project with 5 direct partners: the University of Antwerp, Ghent University, the University of Namur, e-Biom (spin-off of the University of Namur) and the SPGE (Société Publique de Gestion de l'Eau). Indirect partners are also involved: the SBGE (Société Bruxelloise de Gestion de l'Eau), Aquiris (Veolia) and Aquafin. The regional public health agencies are members of the project's Support Committee. The project also collaborates with the Dutch Institute for Public Health and the Environment (RIVM) and the Luxembourg Institute of Science and Technology (LIST) to develop the wastewater surveillance program. Additionally, contacts with other European countries that have developed similar surveillance were made, notably through the European efforts to connect and foster the different Member states' initiatives on the topic, and also more largely within the World Health Institute (WHO) network.

SAMPLE COLLECTION AND ANALYSIS

Once collected, the samples are stored and transported at 4°C and analyzed upon arrival within 24 hours. After sample concentration and RNA extraction the samples are analyzed by RT-qPCR. Three target sequences located in two regions of the SARS-Cov-2 genome are analyzed in duplicate in Flanders and Brussels and triplicates in Wallonia: the N1- and N2- sequence, targeting the N-gene encoding for the nucleoprotein, and the E-gene encoding for the viral envelope protein. The 24 hours-representative inflow rates of each wastewater treatment plant are provided by the regional wastewater agencies (SPGE, SBGE, Veolia, Aquafin) as well as the inhabitant-equivalents (IE) connected to the sewage system of each treatment plant.

POPULATION COVERAGE

As the population connected to a treatment plan may live in different municipalities, and a municipality may be only partially covered by a treatment plant, the total population represented in a wastewater sample was calculated by summing the shares of the population from all the municipalities connected to it.

The population covered in the catchment area of a particular treatment plant was computed as follows:

- The number of domestic Inhabitant Equivalent (IE)¹ (as an estimation of the population connected to the treatment plant) of a particular municipality connected to the treatment plant was divided by the total number of domestic IE present in the municipality. This coverage ratio represents thus the proportion of the population from a specific municipality connected to the treatment plant.
- The population from a municipality which is actually covered by the plant was estimated by multiplying the here above coverage ratio with the total population registered in the municipality.
- The total population covered by the treatment plant was estimated by summing the estimated municipalities populations connected to the sewage system covered by the plant.

This methodology was applied for the 42 treatment plants included in the project. The IE data were provided by the regional wastewater agencies, while the population data were given by the national institute of Statistics (Statbel).

¹ The Inhabitant Equivalent (IE) unit is used in the wastewater treatment field to refer to a particular pollution load (volume and concentration). In the case of the domestic wastewater, 1 IE is equal to 1 inhabitant. In the present work, 1 IE is assumed to be equal to 1 inhabitant located in the catchment area covered by the wastewater treatment plant.

Table A 1 presents the estimated population covered in each catchment area, as well as the laboratory analyzing the corresponding wastewater samples.

A 1: Catchment areas covered by the surveillance with the corresponding laboratories, location and the estimated population covered. Some catchment areas are located on several provinces.

Catchment area	Estimated covered population	Province	Region	Laboratory
Aalst	104 654	Oost-Vlaanderen	Flanders	Sciensano
		Vlaams-Brabant	Flanders	Sciensano
Aartselaar	68 565	Antwerpen	Flanders	UAntwerpen
Amay	53 984	Liege	Wallonia	e-Biom
Antwerpen-Noord	77 442	Antwerpen	Flanders	UAntwerpen
		Antwerpen	Flanders	UAntwerpen
Arlon	20 594	Luxembourg	Wallonia	e-Biom
Basse Wavre (Dyle)	81 537	Brabant Wallon	Wallonia	e-Biom
Beersel	80 328	Vlaams-Brabant	Flanders	Sciensano
Brugge	200 926	Oost-Vlaanderen	Flanders	Sciensano
		West-Vlaanderen	Flanders	Sciensano
Bruxelles-Nord / Brussel-Noord	1 047 684	Brussels	Brussels	Sciensano
		Vlaams-Brabant	Flanders	Sciensano
Bruxelles Sud / Brussel-Zuid	312 532	Brussels	Brussels	Sciensano
		Vlaams-Brabant	Flanders	Sciensano
Dendermonde	88 484	Antwerpen	Flanders	UAntwerpen
		Oost-Vlaanderen	Flanders	UAntwerpen
		Vlaams-Brabant	Flanders	UAntwerpen
Destelbergen	64 421	Oost-Vlaanderen	Flanders	Sciensano
Deurne	214 436	Antwerpen	Flanders	UAntwerpen
Froyennes	37 664	Hainaut	Wallonia	e-Biom
Genk	73 812	Antwerpen	Flanders	UAntwerpen
		Limburg	Flanders	UAntwerpen
Gent	249 092	Oost-Vlaanderen	Flanders	Sciensano
Grimbergen	123 016	Vlaams-Brabant	Flanders	Sciensano
Harelbeke	126 614	West-Vlaanderen	Flanders	Sciensano
Hasselt	82 754	Limburg	Flanders	UAntwerpen
Houthalen-Centrum	22 244	Limburg	Flanders	UAntwerpen
Leuven	137 368	Vlaams-Brabant	Flanders	Sciensano
Liedekerke	114 874	Oost-Vlaanderen	Flanders	Sciensano
		Vlaams-Brabant	Flanders	Sciensano
Liège (Grosses Battes)	28 808	Liege	Wallonia	e-Biom
Liège Oupeye	257 131	Liege	Wallonia	e-Biom
Liège Sclessin	143 369	Liege	Wallonia	e-Biom
Marche-en-Famenne	10 359	Luxembourg	Wallonia	e-Biom
Marchienne-au-Pont	53 394	Hainaut	Wallonia	e-Biom
Mechelen-Noord	117 253	Antwerpen	Flanders	UAntwerpen
		Vlaams-Brabant	Flanders	UAntwerpen
Menen	70 518	West-Vlaanderen	Flanders	Sciensano
Montignies-sur-Sambre	123 961	Hainaut	Wallonia	e-Biom
Mornimont	35 212	Hainaut	Wallonia	e-Biom
		Namur	Wallonia	e-Biom
Mouscron versant Espierres	21 616	Hainaut	Wallonia	e-Biom
Namur-Brumagne	89 232	Namur	Wallonia	e-Biom
Oostende	154 313	West-Vlaanderen	Flanders	Sciensano
Roeselare	83 031	West-Vlaanderen	Flanders	Sciensano
Sint-Niklaas	56 226	Oost-Vlaanderen	Flanders	UAntwerpen

Tessenderlo	56 377	Antwerpen	Flanders	UAntwerpen
		Limburg	Flanders	UAntwerpen
		Vlaams-Brabant	Flanders	UAntwerpen
Turnhout	45 732	Antwerpen	Flanders	UAntwerpen
Vallée du Hain (L'Orchis)	60 433	Brabant Wallon	Wallonia	e-Biom
Wasmuel	161 105	Hainaut	Wallonia	e-Biom
Wegnez	82 753	Liege	Wallonia	e-Biom

WASTEWATER RESULT EXPRESSION

Graphic visualization

The mean of the concentrations obtained for the three genes fragments is linearly interpolated and averaged on a centered moving window of 14 days. This reduces the daily variations.

Alerting indicators assessment

The alerting indicators are assessed on the non-interpolated mean concentrations obtained for the three genes fragments and the moving averages on the past 7 and 14 days for the “Fast increase” and the “Increasing trend” indicators, respectively.

Viral concentration uncertainties

The wastewater results obtained before mid-February 2021 present a higher level of uncertainty:

- Before mid-February 2021, except for Wallonia, the viral concentrations (SARS-CoV-2 RNA copies/ml) were estimated based on the Ct values measured by the laboratories. The Ct values were transformed in estimated quantitative RNA copies/ml using the mean parameters of 10 calibration curves provided by each corresponding laboratory. The mean values and standard deviation of the calibration curves obtained in mid-February 2021, at the start of the quantification procedure, were stable.
- From mid-February 2021 onwards, the laboratory protocol was adapted to directly quantify the viral concentrations (SARS-CoV-2 RNA copies/ml).

Standardized wastewater results

Current work is performed to standardize the wastewater results: i) to take into account a possible dilution effect of the concentration measured in the samples (the dilution can be high when rainy events occur); ii) to report the concentration measured to a

standard population (e.g. 100k inhabitants) reducing the difference of concentrations observed in catchment areas of different sizes.

Even though the SARS-CoV-2 viral concentrations (RNA copies/ml) are not standardized, the normalization applied on the three alerting indicator removes their dependency towards the population size covered by the catchment areas. However, the alerting indicators have currently no correction for the sample dilution.

Up to date, the two following standardization methodologies are under investigation:

- The viral daily load per capita (SARS-CoV-2 RNA copies/day.100k inhabitants) is computed by multiplying the viral concentration with the 24h-representative inflow rate (m³/day) and reported to the number of inhabitants covered by the corresponding treatment plant. The viral daily load per capita accounts thus for the size of the population covered, as well as for the dilution (mainly caused by the rain).
- The viral to the faecal ratio (SARS-CoV-2 RNA copies/ml / PMMoV RNA copies/ml) is computed by dividing the SARS-CoV-2 viral concentration by the Pepper Mild Mottle Virus (PMMoV) concentration measured in the same sample. This virus is a well-known indicator of human faecal contamination (load). The objective of the PMMoV measurements is to estimate the population effectively tracked by the wastewater sample, through an estimation of the faecal matter concentration contained in the wastewater samples. As the PMMoV and the SARS-CoV-2 are analyzed from the same sample, computing the ratio corrects for an eventual dilution caused by rainy events.

Only the SARS-CoV-2 viral concentrations (see section “Viral concentration uncertainties” of this Appendix) are currently used in this report.

Results aggregation by Province and Region

When expressing the wastewater results by Province or Region, the mean (μ) of the concentrations (cc_i) measured in the corresponding catchment areas were weighted with the estimated size of the population respectively covered (Pop_i). Therefore, the concentrations measured in the catchment areas covering a large population (see A 1) account for a larger share in the mean concentration computed for a province or region. The following expression was used to compute the weighted mean:

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

CORRELATION STUDY

A positive significant mean correlation ($R^2 > 0.7$) is observed between the wastewater viral concentrations and the cases numbers over the entire wastewater project duration. The correlation was observed to be higher during the main outbreak virus phases than for the periods in between. For the outbreak phase associated with the 2nd wave (15/09/2020 - 01/11/2020), all the treatment plants have a correlation coefficient higher than 0.5, except for the Antwerpen-North area, with a mean value of 0.85. As for the 3rd wave (15/02/2021 – 01/03/2021), all the treatment plants have a correlation coefficient higher than 0.5, except for Dendermonde, with a mean value of 0.82. Further studies are currently being performed to understand deeper the variation in the correlations observed as the correlations vary according to the catchment area considered.

Regarding the predictive character of the wastewater results, preliminary results indicate that the wastewater concentrations of the best-correlated areas have a predictive character on the cases number of 2 to 3 days.

In conclusion, the wastewater results should be interpreted as complementary and independent information from the cases number results.