

NATIONAL SURVEILLANCE OF SARS-COV-2 IN WASTEWATER

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In collaboration with

The Partners listed here below, with a closer contribution of **e-Biom**, **UNamur**, **SPGE**, **UGent** and **UAntwerpen**

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SUMMARY

The present report is dedicated to the SARS-CoV-2 wastewater national surveillance in Belgium and aims to communicate about the project and the resulting epidemiological information which is available.

The national surveillance of SARS-CoV-2 in wastewater covers the three regions of Belgium and is effective since mid-September 2020. Over 40% of the Belgian population is included in the surveillance through 24h-representative wastewater samples taken at the inlet of 42 wastewater treatment plants, twice a week (see Figure 1). Three laboratories, spread over the country, are analyzing SARS-CoV-2 in these wastewater samples.

The principle of the present surveillance is to have a global view of the SARS-CoV-2 circulation in the population through a non-invasive method, meaning the analysis of the viral load in the wastewater. Given that a large part of infected people, even if asymptomatic, are excreting viral particles in their faeces, the viral load of SARS-CoV-2 in wastewater can be used as an indicator of its circulation in the population.

The objectives of the national wastewater surveillance are (i) to early-detect any significant increase of the SARS-CoV-2 circulation and, more largely, (ii) to provide information about the SARS-CoV-2 circulation among populations, which complements other existing epidemiological indicators (i.e. laboratory-based confirmed cases, positivity ratio of tested persons, hospital admissions, seroprevalence, mortality, ...).

The main added value of the wastewater surveillance is triple:

1. Wastewater can track the part of the population which is infected but may not be tested, for example, due to the absence of symptoms.
2. It can early detect a significant surge of the sanitary situation days before a surge in the laboratory-based confirmed cases, the hospital admissions, and on mortality rate.
3. Wastewater surveillance captures all persons physically present in a certain geographic catchment area, no matter their administrative status, health care seeking behavior, or compliance with COVID-19 testing recommendations. Besides, the results are independent of possible changes occurring in the testing strategy, motivations for testing, and barriers for testing.

The present report provides the study set-up, the analysis methodology, and additional background information which supports the interpretation of the SARS-CoV-2 wastewater surveillance results. Based on the surveillance results, a predictive early-warning model is under development. The preliminary analysis of the predictive performance of the model, as well as the first results of the tool, are also presented in the present report. The model highlights the areas of concern, among the areas covered by the surveillance (see Figure 1), and classifies them in 3 categories: "High circulation of SARS-CoV-2", "Fast increase in wastewater viral load" and "Increasing trend in wastewater viral load", (see the "Results and discussion" section for more details).

This early warning information will also become available for real-time risk assessment of the epidemiological situation in Belgium and is a promising tool for decision-makers in the current COVID-19 crisis management.

The results of SARS-CoV-2 wastewater surveillance can support crisis management and decision-making. They should be considered complementary to the other available surveillance indicators (laboratory-based positive cases, hospitalizations, changes in testing policy, positivity ratio, mobility, the situation in schools, etc.) in the light of the emergence of SARS-CoV-2 variants of concern.

In conclusion, although further developments are still ongoing to better define the predictive performance of the SARS-CoV-2 wastewater-based surveillance, it appears as a valuable tool for the follow-up of the epidemiological situation in Belgium.

INTRODUCTION

The SARS-CoV-2 national wastewater surveillance has been launched in Belgium since 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. We also collaborate with the Dutch Institute for Public Health and the Environment (RIVM). Besides, we are in contact with other European countries which have developed similar surveillance program, 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.

The preliminary results of the wastewater surveillance were communicated in December 2020 in agreement with the stakeholders (cfr. Sciensano press release¹). At that time, too many factors were still missing in the model to validate the detection tool for SARS-CoV-2 resurgence.

From mid of February 2021, some of the uncertainty factors were tackled by performing extra lab analyzes on the wastewater samples: the quantification of SARS-CoV-2 genomic traces in RNA copies/ml, and a faecal load estimation by quantifying the PMMoV RNA (Pepper mild mottle virus, or "red pepper virus"). Thanks to these extra lab results, the interpretability of the wastewater surveillance was improved.

Precautions must still be taken in the interpretation of the wastewater surveillance results as an increase in SARS-CoV-2 concentrations in wastewater does not systematically correlate with an increase of the COVID-19 laboratory-based confirmed cases. A perfect correlation of the two metrics is however not expected. For instance, unlike case-based surveillance, wastewater surveillance monitors the whole population and includes untested asymptomatic cases. Also, wastewater is a complex matrix that is more unstable compared to clinical samples and has a fluctuating physical and chemical composition, which has an impact on the quantification of SARS-CoV-2 RNA concentration. Also, several additional factors are expected to influence the correlation between SARS-CoV-2 concentrations in wastewater and the laboratory-based confirmed cases (see the "Uncertainties and future developments" section). The changes in the clinical testing and/or in the virus shedding dynamic due to the emergence of variants () may also impact the correlation. While some of these factors are measurable and the impact of these factors can be included in the qualitative analysis, the impact of other factors remains unknown. The identification of the interfering factors causing non-significant or weak correlations is currently performed intending to correct their effect.

¹ <https://www.sciensano.be/fr/coin-presse/covid-19-surveillance-des-eaux-usees-resultats-preliminaires>, <https://www.sciensano.be/nl/pershoek/covid-19-surveillance-van-het-afvalwater-voorlopige-resultaten>

METHODOLOGY

Wastewater sample collection and SARS-CoV-2 analysis

The project covers over 40% of the Belgian population. The choice of the population included in the present surveillance is based on the following epidemiologic hypothesis: high-density areas are more likely to lead to a more rapid increase in the circulation of the virus in that population (in comparison to areas of less density). Regarding the early-warning objective of the tool, this hypothesis was used as the main criterion to select the treatment plants to investigate in this project (see Figure 1). The map below shows the population density of each municipality, the location of the selected stations, and with the yellow circles, the population equivalents (EI) monitored by the wastewater surveillance program.

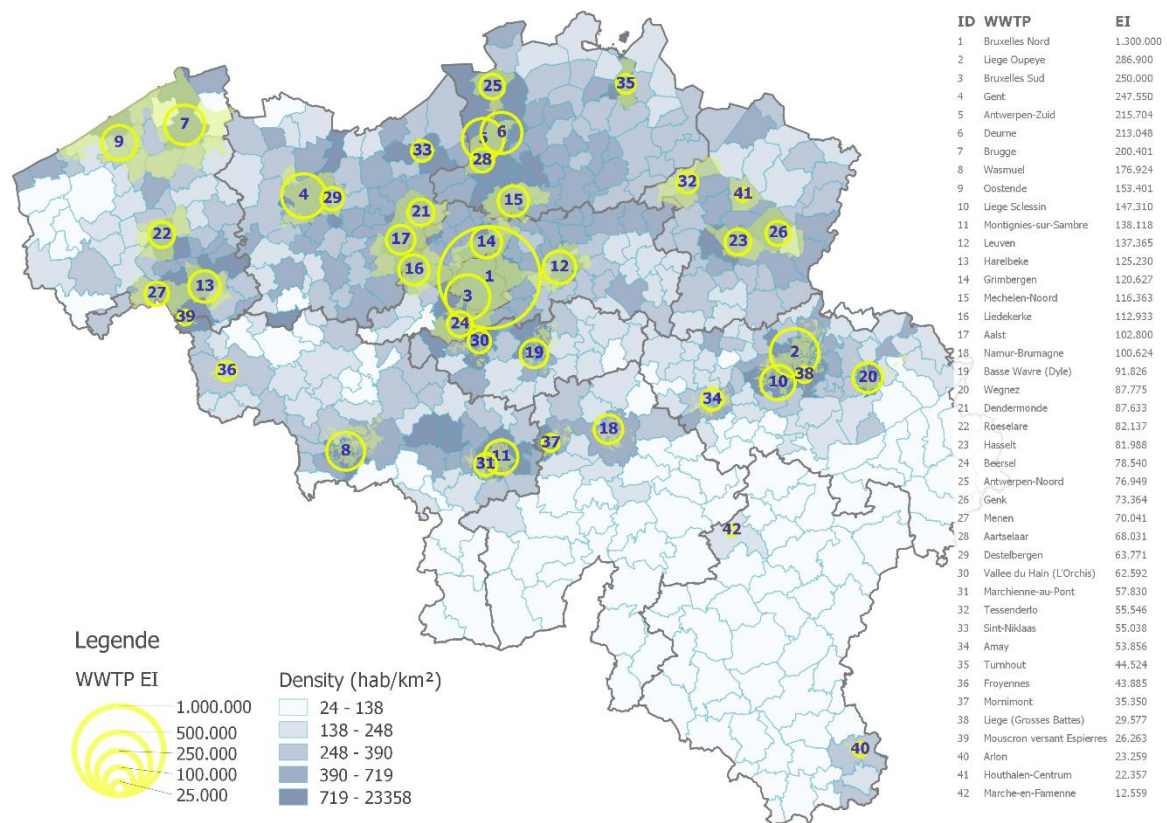


Figure 1: wastewater sampling plan coverage

Practically, 24-hour composite samples are collected twice a week on Mondays and Wednesdays from the 42 wastewater treatment plants (WWTPs) and analyzed by three different laboratories on Tuesdays and Thursdays. Once collected, the samples are stored at 4°C and analyzed by RT-qPCR within 24 hours. Three target sequences located in two genes of the SARS-CoV-2 genome are analyzed in duplicate: the N1- and N2-sequence, targeting the N-gene encoding for the nucleoprotein, and the E-gene encoding for the viral envelope protein.

Influent flow rates of each WWTPs, as well as the estimated population connected to the sewage system of each WWTP, are provided by the regional water agencies.

Laboratory-based confirmed cases data

COVID-19 laboratory tests from clinical samples (negative and positive results) are reported at the municipality level. From the laboratory-based confirmed cases at the municipality level, we estimate the number of confirmed cases related to each wastewater catchment area using a population-based

proportional rule, intending to compare wastewater- and confirmed case indicators for a similar geographical zone.

Towards SARS-CoV-2 quantification

From mid-September 2020 until mid-February 2021, an approach called **semi-quantitative** was developed to report the results in a semi-quantitative way by expressing analytical measures in Cycle threshold values (Ct). Avoiding performing quantification at the early stage of the project allowed to reduce the analysis costs while starting to develop the surveillance tool on observed trends of the viral circulation in the areas covered by the surveillance. In a second time, **from mid-February 2021 onwards**, a **quantitative** approach was used expressing the viral concentrations in RNA copies/ml. This requires an additional calibration step to the analytical procedure. The quantitative approach allows to reduce the measurement uncertainty related to the difference of analytical methods existing between laboratories, and thus reach better comparability between regions. Moreover, the quantification facilitates the normalization of SARS-CoV-2 concentrations with respect to dilution by precipitation and faecal load parameters.

The stepwise approach was used to optimize the accuracy of the project outcomes with the limited resources available.

During the semi-quantitative approach period, we chose to convert the Ct values into RNA relative amount (RNA/RNA_{min}), which allows a better comparison of the trends between that first period and the second one (quantitative). The transposition of Ct values in relative RNA follows a specific methodology that was improved over time and whose current state is summarized hereafter.

Each of the 3 laboratories performed multiple calibration curves, on each of the 3 genomic targets. These calibration curves present the relation between the Ct and RNA values as follows: $Ct = a * \ln(RNA) + b$. From ten calibration curves distributed over time, we estimate statistically the parameter “a” of the relation, for each lab, to finally be able to express RNA in function of Ct, using the following expression:

$$RNA/RNA_{min} = \exp\left(\frac{Ct/Ct_{min}}{a}\right)$$

The second approach, called quantitative, was launched in mid-February when the laboratories started to quantify the number of copies of the three targets present in each sample (copies of viral genomic RNA/ml). For each of the three targets, calibration curves were produced, and Ct values were enumerated towards copies RNA/ml according to the above formula. The sources of uncertainties related to each approach are discussed later in this report.

A preliminary study evaluated the limits of detection (LOD - 95% confidence interval) of the three targeted genes to be around 1000 copies/ml. Further analyzes will be performed to define the limits of quantification and detections accurately.

PMMoV quantification

From mid-February 2021, the Pepper mild mottle virus (PMMoV) viral load concentration was measured in the samples (copies/ml) in addition to the SARS-Cov-2 quantification. This virus is a well-known indicator of human faecal contamination (load). The objective of those extra PMMoV measurements is to estimate the population effectively represented by the area covered by each WWTP, through an estimation of the fecal matter concentration contained in the wastewater samples.

RESULTS AND DISCUSSION

Semi-quantitative approach

The results of the semi-quantitative approach are presented in Figure 2, for the Brussels North wastewater treatment plant (WWTP). The highest signal was observed at the beginning of November 2020, corresponding to the second wave of the SARS-CoV-2 outbreak in Belgium when high levels of laboratory-based confirmed cases were recorded. The correlation between the wastewater surveillance results and the laboratory-based confirmed cases is high for the second wave period (14/09/2020 to 01/12/2020) for all the 42 treatment plants studied with a mean correlation coefficient of 0.86, and one plant having a correlation coefficient below 0.5. However from the first of December 2020 to the latest results, 5th May 2021, the correlation is weaker with a mean correlation coefficient of 0.53, and 16 plants having a correlation coefficient below 0.5. The lower mean correlation observed after the first December can be explained by the variation in time of the analytical methods and the viral shedding dynamics, as discussed later in the section “Uncertainties and future developments”.

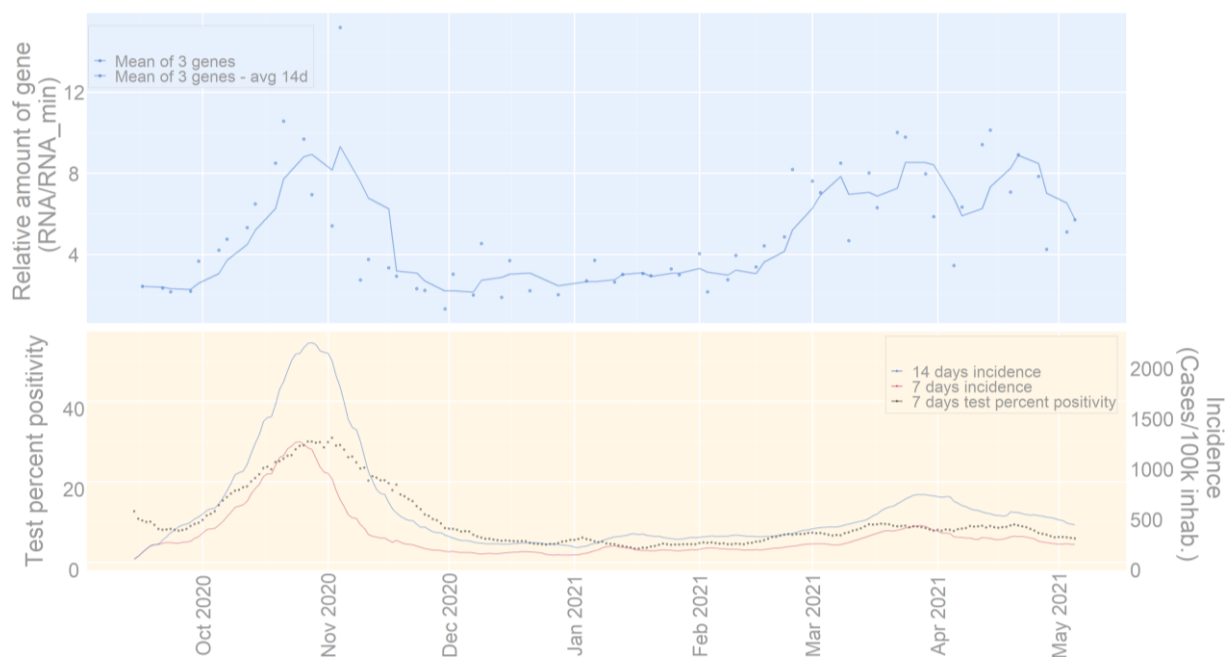


Figure 2: Results for the catchment area of Brussels North WWTP. Blue background: Wastewater surveillance results with the relative amount of mean gene copies, and the 14 days rolling average. Orange background: Laboratory-based positive tests dataset, test percent positivity, 7 and 14 days incidence filtered on the wastewater area of concern

Quantitative approach

Since mid-February 2021, the results are expressed for each of the three targeted genes in RNA copies per milliliter of wastewater sample. As presented in Figure 3 for the Brussels North WWTP, the rolling average on the past 14 days of the mean viral concentration of the three targeted genes shows a peak with a maximum value recorded at the end of March. In this particular case, the wastewater signal is correlated with the confirmed clinical cases.

A recent correlation study conducted on the quantitative results shows that, for the period of mid-February to the 5th May 2021, the wastewater signal correlates well with the laboratory-based positive results with a mean correlation coefficient of 0.55 considering all the 42 treatment plants covered by the surveillance, and 14 treatment plants having a coefficient below 0.5 (considered period: 08/02/2021 – 05/05/2021).

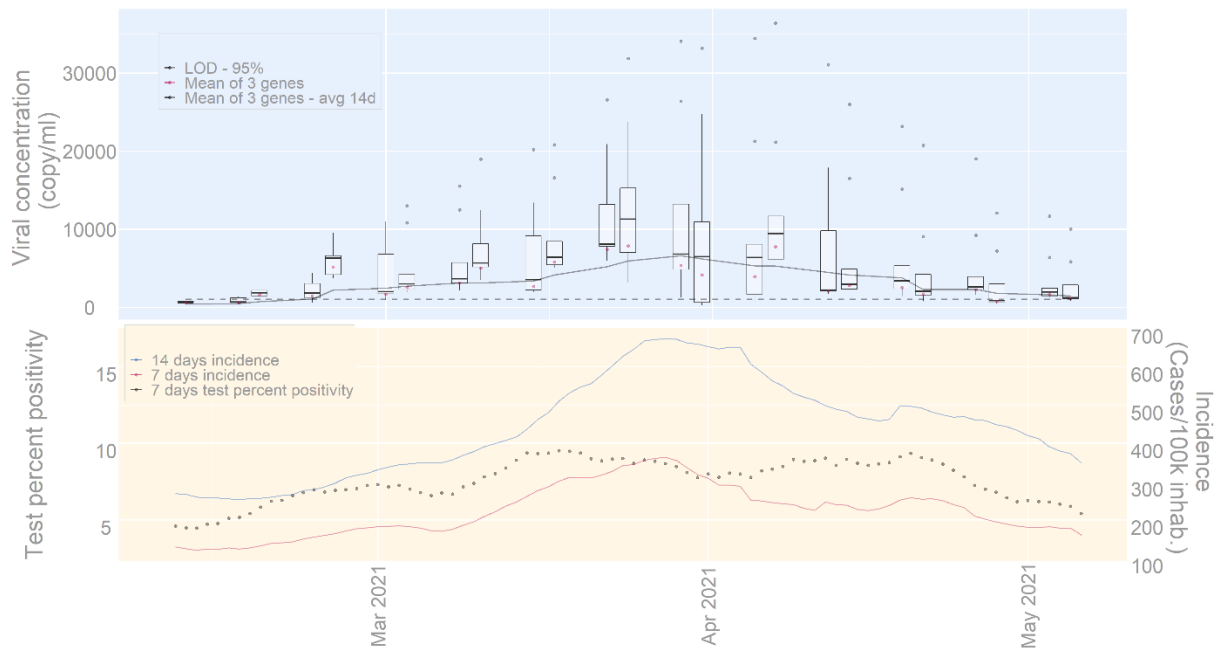


Figure 3: Viral concentration (the maximum and minimum values indicated as vertical lines, the first and third quartiles as box sides and median as horizontal lines), its rolling average on the past 14 days, the estimated limit of detection (LOD-95%), test percent positivity, and incidence for the catchment area of the Brussels North WWTP

Alerting indicators

To highlight the treatment plants of possible concerns, three types of alerting indicators are assessed twice a week based on the wastewater surveillance results. For example, Table 1 shows the indicators obtained on the quantitative approach results of the 5th May 2021:

1. WWTPs where the virus circulation has quickly increased, are classified as **“Fast increase”**. One treatment plant fulfilled the following condition: rolling average of the 3 genes mean concentrations² on the past 7 days evolving faster than 20% per day.
2. WWTPs where the virus circulation is higher than half of the highest value reported since the beginning of the quantification approach (mid-February) for the corresponding treatment plant, are classified as **“High circulation”**. Three treatment plants fulfilled to this condition (Mean cc./max > 50%).
3. WWTPs where the circulation is of concern because increasing for 7 days or more, are classified as **“Increasing trend”**. Six treatment plants fulfilled the following condition: rolling average of the 3 genes mean concentrations on the past 14 days is increasing for 7 days or more.

² Mean of six concentrations for each sample: two duplicate for each three targeted genes

Table 1: WWTPs selected by the early warning tool

WWTP	Mean cc/max (%)	Mean cc (copies/ml)	Cc avg. past 7 d.	Evolution (%/day)	Cc avg. past 14 d.	Incr. days	High circ.	Fast incr.	Incr. trend
Mouscron versant Espierres	73	127	83	26.88	94	2	Yes	Yes	No
Wasmuel	37	66	123	4.15	85	7	No	No	Yes
Montignies-sur-Sambre	66	165	138	0.55	163	7	Yes	No	Yes
Wegnez	16	40	79	-7.06	72	21	No	No	Yes
Liege Oupeye	52	118	123	11.10	90	7	Yes	No	Yes
Houthalen-Centrum	8	24	55	-28.12	58	7	No	No	Yes
Marche-en-Famenne	34	65	65	-48.17	101	14	No	No	Yes

UNCERTAINTIES AND FUTURE DEVELOPMENTS

Several future improvements are planned to reduce the uncertainties related to the semi-quantitative and quantitative approaches. This will support the use of the wastewater indicator in the decision-making of crisis management.

Results inter-comparability: The temporal and spatial comparison of the results is associated with several sources of uncertainties. Regarding the temporal aspect, the semi-quantitative results on the period before mid-February 2021 are not easily comparable to the quantitative results starting afterward. Regarding the spatial comparison³, the transition to the quantitative approach was evaluated to reduce the related uncertainties. However, the extent to which the results measured by different laboratories on different treatment plants can be compared to each other remains unknown. This uncertainty will be mitigated by performing cross-check analyzes between the 3 laboratories, and by assessing the limit of quantification and detection of the analytical methods. Also, the quantitative approach allows for future standardization of the results using (i) the dilution factor accounting mainly for the rain flow, and (ii) the faecal concentrations (PMMoV) adding information on the population tracked in the wastewater sample. These future improvements are expected to improve the comparison between the areas covered by the different laboratories, as well as between the treatment plants.

Hospitals and nursing homes: Different major point sources of SARS-CoV-2 RNA shedding, such as hospitals and nursing homes, are currently listed and will be considered as influencing factors for the risk assessment.

Mobility: The mobility of the persons in the areas covered by the surveillance is a key factor that could partially explain the variability in the correlation between the wastewater curves and the curves corresponding to the confirmed cases (expressed through incidence and positivity ratio indicators). UNamur is currently studying the mobility influence on the correlation results, which results will then be implemented in the national model.

³ To note that the spatial comparability does not only concern the regional level as Flanders is analyzed by two different labs

Parameters influencing the shedding dynamic: The individual viral shedding dynamic is influenced by several factors: not all infected persons (~60%) are effectively shedding the virus in their faeces, the variability in individual viral load, the variability of the duration of shedding, etc. The relation between shedding and the severity of symptoms could also be impacted by the vaccination, which would impact the correlation between the signal measured in wastewater compared to the positive tests. Besides, the measured viral concentration may also depend on environmental factors, such as the temperature of the water, the residence time⁴, the chemical composition (presence of industries discharging detergents in the area), etc. The temperature effect is currently studied by UGent as a sub-research and, in case of any promising conclusions, we foresee valorizing them in the national model.

Early detection: The predictive character of the alerting indicators still requires further analysis to understand the correlation and the possible temporal association between the wastewater signal and the positive cases.

Several hypotheses could explain the weak correlation observed on the semi-quantitative results from December onwards:

- Before mid-February 2021, most of the wastewater treatment plants were below the estimated limit of detection of the analytical method, which would lead to low accuracy measurements;
- Another hypothesis could be the larger uncertainties behind semi-quantitative results compared to quantitative ones.

Several reasons can be stated to explain why some treatment plants have a correlation coefficient below 0.5: i) a lack of correspondence between the population tracked by wastewater compared to the clinical tests in these specific areas, ii) higher mobility of the persons living in the area (which would not be tracked by wastewater while being counted as laboratory-based confirmed cases, based on their residential postcode), iii) the presence of hospitals or collectivities in the area, iv) the presence and activity of industries, etc.

In terms of future development to support the predictive character of the tool, we will also study the temporal lag between the wastewater and the laboratory-based confirmed case signals. Our partner e-Biom, together with UNamur and SPGE, will further support Sciensano with the ongoing correlation studies.

⁴ Time for the wastewater to reach the treatment plant

CONCLUSIONS

Since the start of the wastewater surveillance, we significantly improved our mastery of the tool. Nevertheless, developments are still ongoing. Among those, two major developments are:

- 1) The reduction of the impact of several major parameters of influence
- 2) The further assessment of the predictive character of the model

The standardization of the SARS-CoV-2 viral concentrations will contribute to the first development, as it will take into account the real population covered by the wastewater treatment plants. Regarding the second one, we will continue improving the alerting tool and related indicators, in parallel of improving our understanding of the background defining the correlation quality between the wastewater indicators with the other available epidemiological indicators, such as the incidence and positivity ratio, obtained by the laboratory-based confirmed cases surveillance.

The 3 categories of indicators developed aim to identify significant changes in wastewater surveillance signals which should be highlighted and communicated for COVID-19 risk assessment. More precisely, the wastewater alerting indicators are complementary to the other available indicators (hospitalizations, positivity rate, mobility, situation in schools, etc., i.e.), and together with additional contextual elements (e.g. testing policy, measures in place) enable policymakers and risk assessors to appraise the epidemiological situation in its evolving context.

Once those indicators are further stabilized, they will be the core of the wastewater surveillance outputs by supporting the weekly appraisal of the COVID-19 epidemiological situation and its risk assessment in Belgium.

To conclude, wastewater molecular surveillance is an innovative and new technology, which needs to be carefully developed to validate its interpretation tools for public health purposes. The quality of the reported information, the profound scientific grounding of context in which results are presented and the reliability of the resulting health recommendations are shared values among all participating partners of the project.

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