# WEEKLY REPORT – WASTEWATER-BASED EPIDEMIOLOGICAL SURVEILLANCE OF THE SARS-COV-2

# **RESULTS OF 12/07/2023**

Authors: R. Janssens<sup>1</sup>, H. Maloux<sup>1</sup>, S. Hanoteaux<sup>1</sup>, V. Hutse<sup>1</sup>, L. Van Poelvoorde<sup>3</sup>, N. Roosens<sup>3</sup>, B. Verhaegen<sup>2</sup>, K. Van Hoorde<sup>2</sup>, K. Dierick<sup>2</sup>, S. Quoilin<sup>1</sup>, K. Blot<sup>1</sup>, M. Lesenfants<sup>1\*</sup>

<sup>1</sup> Sciensano, Service Epidemiology of infectious diseases
 <sup>2</sup> Sciensano, Service Foodborne pathogens
 <sup>3</sup> Sciensano, Transversal activities in Applied Genomics
 \*Project leader and contact: <u>marie.lesenfants@sciensano.be</u>

In collaboration with:















# TABLE OF CONTENTS

TABLE OF CONTENTS	2
1. Summary	3
2. Introduction	
3. Methodology	
3.1. SAMPLE COLLECTION AND ANALYSIS	
3.2. WASTEWATER RESULTS	5
3.3. ALERTING INDICATORS	
3.4. CASES RESULTS	6
3.5. CAUTION POINTS FOR THE RESULTS INTERPRETATION	6
4. Results	7
4.1. NATIONAL LEVEL	7
4.2. REGIONAL LEVEL	
4.3. PROVINCIAL LEVEL	
4.4. INDIVIDUAL CATCHMENT AREAS LEVEL	Error! Bookmark not defined.
5. Appendix – Areas classified by alerting indicator	13

## 1. Summary

In the present work, the circulation of the SARS-CoV-2 virus in the environment is assessed based on three indicators. The analysis of wastewater samples collected in 42 wastewater treatment plants covers 45% of the Belgian population, with increased accuracy in the urban areas. The results of the wastewater surveillance are a source of complementary information to the infection cases number. Indeed, the wastewater results do notably include all asymptomatic persons, and are independent of the testing strategy.

Here are the conclusions based on the latest results of July 12<sup>th</sup> 2023<sup>1</sup>:

- Methodological note: On 1<sup>st</sup> July, the analytical method was modified from qPCR to dPCR. This modification has a negligible impact on the indicators.
- At the national level: The number of areas in High Circulation and Increasing Trend is low. Overall, the viral loads are at a low level compared to the 9th wave.
- At the regional level: The situation is similar in Wallonia and Flanders. However, a slight increase of viral loads is observed in Brussels.
- At the covered areas level: among the 25 areas covered, the number of treatment plants positive to the different indicators are 11 for the Increasing Trend indicator, 4 for the Fast Increase indicator, and 0 for the High Circulation indicator.

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

- The graphics available on the public <u>COVID-19 dashboard</u>
- The Risk Assessment Group (RAG) updating the weekly epidemiological situation through a report validated by the Risk Management Group (RMG) and published every Friday in <u>French</u> and <u>Dutch</u>.
- Further details on the methodology applied for the wastewater surveillance can be found in <u>the</u> <u>Appendix Methodology document (access available online)</u>. The methodology was updated on June 20th 2022.

<sup>&</sup>lt;sup>1</sup> Due to logistic issues, the areas in Wallonia are not sample this week.

## 2. Introduction

In mid-September 2020 started the SARS-CoV-2 national wastewater surveillance project. The present report is an outcome of this project aiming to assess weekly the wastewater-based epidemiological situation of Belgium.

The surveillance is based on the analysis of water samples collected twice per week from the influent of 42 WasteWater Treatment Plants (WWTPs) spread over Belgium. The evolution of the SARS-CoV-2 wastewater concentrations measured over time in the different treatment plants is analysed at different levels: regional, provincial, and the catchment area covered by the individual treatment plants. Also, three alerting indicators were developed to highlight areas of concern regarding the high circulation, the fast evolution, and the increasing trend of the observed wastewater concentrations.

In this report, the weekly wastewater-based epidemiological situation is presented and discussed at the above-mentioned levels based on the three categories of alerting indicators. Moreover, the remaining sources of uncertainties are discussed together with their expected impacts on the wastewater results interpretation.

## 3. Methodology

#### **3.1. SAMPLE COLLECTION AND ANALYSIS**

The surveillance project, which started in mid-September 2020 covers around 45% of the Belgian population. The population covered is 40% in the Flemish region, 35% in the Walloon region, and nearly 100% in the Brussels region. Figure 1 shows the catchment areas covered by the 42 WWTPs included in the project, which are located in high population density areas. A catchment area is defined by the area delimiting the population covered by a specific wastewater sample, collected at the inlet of the WWTPs. Further coverage details can be found in Table 2 by province (see also Table A1 in the Appendix Methodology document (access available online)) and on the Sciensano public dashboard. In practice, 24-hour composite samples are collected twice a week on Mondays and Wednesdays from the influent of WWTP and are analysed for the presence of SARS-CoV-2 RNA by three different laboratories. The resulting SARS-CoV-2 concentrations (3 targeted genes) are delivered to Sciensano within 2 days for data analysis purpose. Concretely, the results which are representative of Mondays and Wednesdays are respectively available on Wednesdays and Fridays.

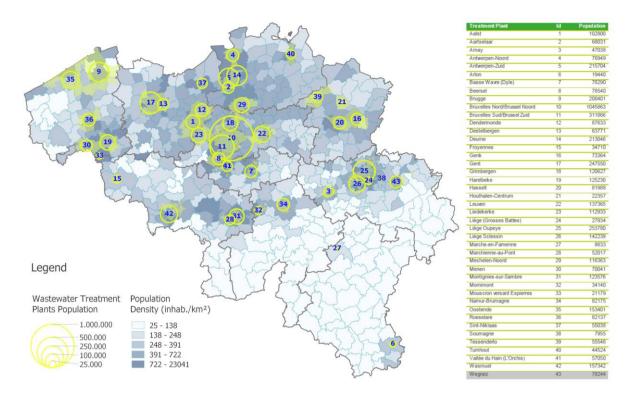


Figure 1: The population located in the areas covered by the wastewater treatment plants (highlighted in yellow) and the population density for each municipality (indicated by the blue scale). Note that due to the recent flooding, the treatment plants of Wegnez is out of order.

#### **3.2. WASTEWATER RESULTS**

Between mid-September 2020 and mid-February 2021, the concentration were not quantified for all treatment plant and were expressed in Ct values. In mid-February 2021, the quantification of SARS-CoV-2 viral concentration (RNA copies/ml) was generalized to all the treatment plants investigated. Until 10th June 2022 the NIBSC standard was used to quantify the SARS-CoV-2 and after this date, the EURM standard was used.

In order to account for possible dilution by rainy events and for the number of people persons living in the catchment of each WWTP, a correction is applied on the viral concentration: the concentration is multiplied by the inlet flow and divided by the number of inhabitant equivalent of its respective WWTP and expressed by 100k inhabitant. The units of viral loads are copy/day/100k inhabitants equivalent.

The limit of quantification of the analytical method was estimated at 10 copies/ml .

#### **3.3. ALERTING INDICATORS**

To highlight the areas of possible concern, the three alerting indicators are assessed twice a week, based on viral loads (RNA copies/day/100k inhabitants) measured for the three targeted gene fragments (E, N1, and N2):

- 1. The **Increasing Trend** indicator highlights the catchment areas where the viral loads have been increasing for more than 6 days. The indicator is computed based on the moving average on the past two weeks of the viral load.
- 2. The Fast Increase indicator highlights the catchment areas where the viral loads have rapidly increased for the last week. It corresponds to a situation where the moving average on the past 7 days of the viral load has increased faster than 70% per week if being above the estimated limit of quantification. The increasing slope is normalized for each treatment plant.

3. The **High Circulation** indicator highlights the catchment areas where the viral loads are high. It corresponds to a situation where the viral loads exceed half of the highest value recorded during the ninth wave (i.e. from 21th of November 2022 till first of January 2023).

The indicators were developed in order to be able to track the different phases of an outbreak. Typically, when the viral loads in wastewaters in an area start to increase, the indicator Increasing Trend will be the first fulfilled. If the viral loads increase quickly, the Fast Increase indicator will be fulfilled. Finally, after the initial increasing phase, the viral loads in an area may be sufficiently high to result in the High Circulation to be fulfilled.

#### 3.4. CASES RESULTS

The cases number data presented in this report come from the COVID-19 laboratory-confirmed cases database centralized by Sciensano. The cases number, used to compute the 14 days-incidence, only accounts for the physical areas covered by the wastewater project (see Figure 1).

#### 3.5. CAUTION POINTS FOR THE RESULTS INTERPRETATION

Only the trends, observed through the alerting indicators, should be assessed for the comparison of different areas. As the viral load values differ from a WWTP to another, notably due to the differences in population sizes covered, absolute values should not be compared. Additionally, the situation comparison between the regions should be considered with caution. The degree of comparability is not yet known and depends on the comparability of the results between the different laboratories performing the analyses (see <u>the Appendix Methodology document (access online)</u>).

Moreover, the wastewater viral loads and the cases numbers presented in this report do not originate from the same population, even though the positive cases are selected only for the municipalities covered by the wastewater surveillance. For instance, the wastewater results account for all infected persons (whatever age or symptomatic condition) while the cases numbers include only the persons clinically tested positive. Likewise, an infected person covered by the wastewater results could be associated with another area in the clinical surveillance as the person's postal code is used for clinical statistics, i.e. mobility bias. Therefore, the correlation between the wastewater viral load and the cases number varies according to the area considered. The wastewater results are thus complementary and independent information to the results of the cases.

The correlation between the wastewater viral loads and the cases numbers could also be influenced by the vaccination campaign and the circulation of variants.

### 4. Results

#### **4.1. NATIONAL LEVEL**

Table 1 shows the national results allowing to track the changes between the situation as of today (July 12<sup>th</sup> 2023) and the situation as of last week (July 05<sup>th</sup> 2023). In this table, any change in indicator fulfilment (i.e. if the value for any indicator has changed from 0 to 1 or from 1 to 0) is indicated in **coloured bold text**.

A the national level, only the Fast Increase indicator is positive.

Table 1: Alerting indicators fulfilled (1) or not (0) on September 28th 2022. Columns represent the population coverage of Belgium (Pop. coverage) and the three alerting indicators High Circulation (High), Fast Increase (Fast) and Increasing Trend (Incr.). The specifications of the four last columns are explained in the footnotes 1-4 below the table. Missing data is indicated with a "/".

Country	Pop. coverage	High	Fast	Incr.	Norm. viral load (%) <sup>1</sup>	Mean viral load²	Norm. evol. (%/wee k) <sup>3</sup>	lncr. days⁴
Belgium	44%	0	1	0	6	0.57	81	6

<sup>1</sup>: the viral load normalized with the maximum viral load measured in the corresponding catchment area during the ninth wave (i.e. from 21th of November 2022 till the first of January 2023).

<sup>2</sup>: the viral load computed on the mean of the replicate of the three targeted gene fragments as explained in section "3.2 Wastewater results". The mean viral load is expressed in 10^12 copies/day/100k inhabitants.

<sup>3</sup>: the slope (%/week) of the past 7 days moving average of the viral load if the corresponding concentration is above the estimated limit of quantification.

<sup>4</sup>: the cumulative number of days of increase of the past 14 days moving average of the viral load.

Figure 2 was developed to show the localization of the covered areas by the wastewater surveillance in Belgium and the state of the three alerting indicators in each of them. Figure 3 was developed to offer a dynamic view of the three alerting indicators over time. For further insights on the dynamic of the different indicators, see Section 3.3.

The number of areas in High Circulation and Increasing Trend is low, with less than 5 and 10 areas positive respectively. Overall, the viral loads are at a low level compared to the 9th wave.

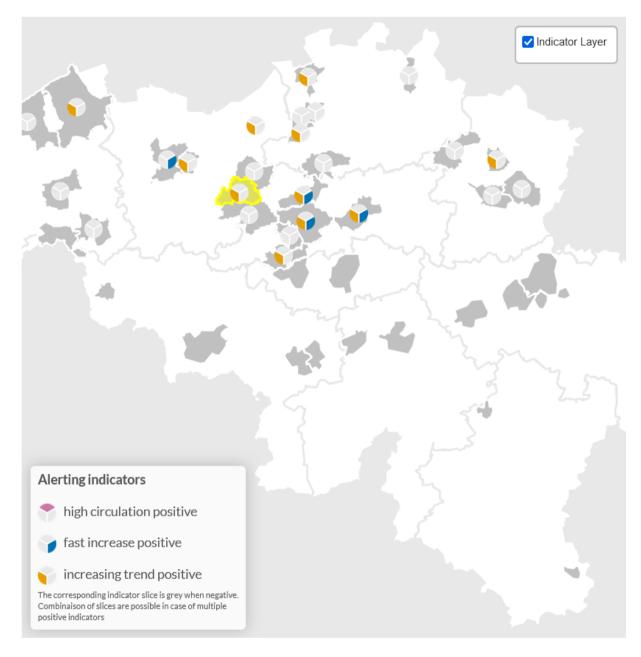
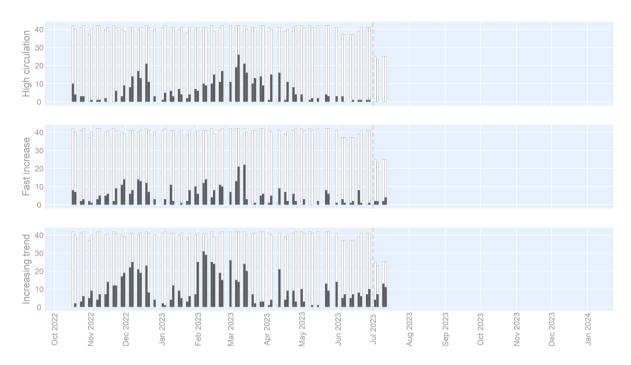
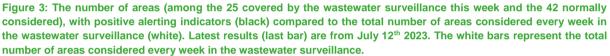


Figure 2: Localisation of the covered areas (grey polygons) by the wastewater surveillance in Belgium. In each area, the fulfillment of the High Circulation, Fast Increase and Increasing Trend indicator is indicated by a pink, blue and yellow slice, respectively. The names of the covered areas with respect of their localization can be found in Figure 1.



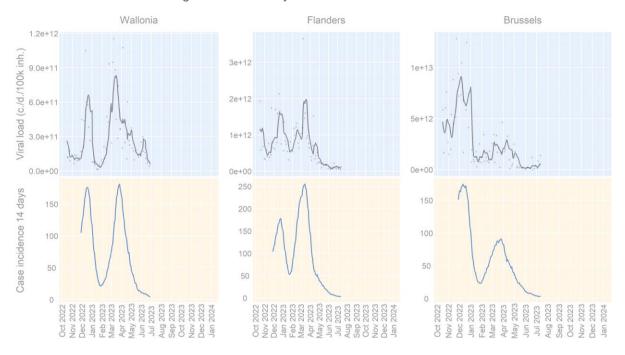


#### **4.2. REGIONAL LEVEL**

Figure 4 represents the quantitative SARS-CoV-2 RNA viral loads in the wastewaters and the 14 days incidence for each region, compiling the incidence data of the area covered by the wastewater surveillance.

Two waves can be seen in Figure 4:

- The 9th wave starting on 21st November 2022.
- The 10th wave starting on 23rd January 2023.



# Figure 4: The SARS-CoV-2 RNA viral loads expressed as copies/days/100k habitants (based on the past two weeks moving average applied on the linear interpolation) (Top), and the 14 days incidence in the population covered by the wastewater surveillance since mid-February 2021 (bottom).

Table 2 shows, for each region, the results associated with the samples of Wednesday July 12<sup>th</sup> 2023. It allows to track the changes between the situation as of today (July 12<sup>th</sup> 2023) and the situation as of last week (July 05<sup>th</sup> 2023). Hereby, two distinct cases are taken into account:

- 1. If a region has at least one indicator fulfilled this week and it was not the case last week, its **name is displayed in bold** in the table.
- 2. If a region has at least one indicator fulfilled this week and if it also was the case last week, any change in indicator fulfilment (i.e. if the value for any indicator has changed from 0 to 1 or from 1 to 0) is indicated in **coloured bold text**.

At the regional level, none of the three indicators is positive, except the Fast Increase and the Increasing Trend in Brussels.

Table 2: Alerting indicators fulfilled (1) or not (0) on July 12<sup>th</sup> 2023. Columns represent the population coverage of the regions (Pop. coverage) and the three alerting indicators High Circulation (High), Fast Increase (Fast) and Increasing Trend (Incr.). The specifications of the four last columns are explained in the footnotes 1-4 below the table. Missing data is indicated with a "/".

Region	Pop. coverage	High	Fast	Incr.	Norm. viral load (%) <sup>1</sup>	Mean viral load²	Norm. evol. (%/wee k) <sup>3</sup>	Incr. days⁴
Brussels	100%	0	1	1	9	1.39	83	7
Flanders	41%	0	0	0	4	0.12	0	5
Wallonia	31%	/	/	/	/	/	/	/

<sup>1</sup>: the viral load normalized with the maximum viral load measured in the corresponding catchment area during the ninth wave (i.e. from 21th of November 2022 till the first of January 2023).

<sup>2</sup>: the viral load computed on the mean of the replicate of the three targeted gene fragments as explained in section "3.2 Wastewater results". The mean viral load is expressed in 10^12 copies/day/100k inhabitants.

<sup>3</sup>: the slope (%/week) of the past 7 days moving average of the viral load if the corresponding concentration is above the estimated limit of quantification.

<sup>4</sup>: the cumulative number of days of increase of the past 14 days moving average of the viral load.

#### 4.3. PROVINCIAL LEVEL

Table 3 shows, for each Province, the results associated with the samples of Wednesday July 12<sup>th</sup> 2023. It allows to track the changes between the situation as of today (July 12<sup>th</sup> 2023) and the situation as of last week (July 05<sup>th</sup> 2023). Hereby, two distinct cases are taken into account:

- 1. If a province has at least one indicator fulfilled this week and it was not the case last week, its **name is displayed in bold** in the table.
- 2. If a province has at least one indicator fulfilled this week and if it also was the case last week, any change in indicator fulfilment (i.e. if the value for any indicator has changed from 0 to 1 or from 1 to 0) is indicated in **coloured bold text**.

Table 3 shows, for each Province, the results associated with the samples of Wednesday July 12<sup>th</sup> 2023, for the three alerting indicators:

- The Increasing Trend indicator is positive in the province of Brussels and Vlaams-Brabant, and was in none last week.
- The Fast Increase indicator is only positive in the province of Brussels, and was in none last week.
- The High Circulation indicator is not positive in any province, and was in none last week.

Table 3: Alerting indicators fulfilled (1) or not (0) on July 12<sup>th</sup> 2023. Columns represent the population coverage of the WWTPs within the Province (Pop. coverage) and the three alerting indicators High Circulation (High), Fast Increase (Fast) and Increasing Trend (Incr.). The specifications of the four last columns are explained in the footnotes 1-4 below the table. Missing data is indicated with a "*I*".

Province	Pop. coverage	High	Fast	Incr.	Norm. viral load (%) <sup>1</sup>	Mean viral load²	Norm. evol. (%/w) <sup>3</sup>	Incr. days⁴
Antwerpen	39%	0	0	0	3	0.016	0	3
Brabant Wallon	33%	/	/	/	/	/	/	/
Brussels	100%	0	1	1	9	1.392	83	7
Hainaut	29%	/	/	/	/	/	/	/
Liège	43%	/	/	/	/	/	/	/
Limburg	26%	0	0	0	3	0.026	0	2
Luxembourg	10%	/	/	/	/	/	/	/
Namur	23	/	/	/	/	/	/	/
Oost-Vlaanderen	38%	0	0	0	7	0.197	0	5
Vlaams-Brabant	49%	0	0	1	4	0.289	0	8
West-Vlaanderen	52%	0	0	0	2	0.099	0	6

<sup>1</sup>: the viral load normalized with the maximum viral load measured in the corresponding catchment area during the ninth wave (i.e. from 21th of November 2022 till the first of January 2023).

<sup>2</sup>: the viral load computed on the mean of the replicates of the three targeted gene fragments as explained in section
"3.2 Wastewater results". The mean viral load is expressed in 10^12 copies/day/100k inhabitants.

<sup>3</sup>: the slope (%/week) of the past 7 days moving average of the viral load if the corresponding concentration is above the estimated limit of quantification.

<sup>4</sup>: the cumulative number of days of increase of the past 14 days moving average of the viral load.

# 5. Appendix – Areas classified by alerting indicator

Table A1: Areas for which the data are missing on July 12<sup>th</sup> 2023.

Province	WWTP
Antwerpen	Aartselaar
Liège	Amay
Luxembourg	Arlon
Brabant Wallon	Basse Wavre (Dyle)
Hainaut	Froyennes
Liège	Liege (Grosses Battes)
Liège	Liege Oupeye
Liège	Liege Sclessin
Luxembourg	Marche-en-Famenne
Hainaut	Marchienne-au-Pont
Hainaut	Montignies-sur-Sambre
Namur	Mornimont
Hainaut	Mouscron versant Espierres
Namur	Namur-Brumagne
Liège	Soumagne
Limburg	Tessenderlo
Brabant Wallon	Vallee du Hain (L'Orchis)
Hainaut	Wasmuel