Wastewater Surveillance Dashboard Technical Notes

Last updated: November 29, 2023

Toronto Public Health (TPH) monitors COVID-19, influenza A, and influenza B activity in the community using a various data sources, one of which is wastewater surveillance. Ontario's wastewater surveillance initiative is coordinated by the Ministry of the Environment, Conservation and Parks (MECP). Toronto Metropolitan University (TMU) and the University of Toronto (U of T) are our laboratory partners that complete analyses on wastewater samples collected by the City of Toronto.

What is wastewater surveillance?

Wastewater surveillance involves testing for a virus in wastewater, looking at changes over time, and using the data to establish Toronto-specific trends. It is particularly useful when access to clinical testing is limited, and captures people both with and without symptoms. Thus, wastewater surveillance can help us understand the true presence of viral activity in a community regardless of clinical testing strategies, access to testing, and test seeking behaviour

Wastewater surveillance does not replace traditional clinical testing, but can provide a broader understanding of disease activity and local trends. This was especially helpful for COVID-19 with the end of universal confirmatory polymerase chain reaction (PCR) testing in early January 2022.

It can help us understand viral trends (increasing, stable, or decreasing) and can help us understand how much viral activity (very high, high, medium, and low) we have in the city.

Where do we sample our wastewater?

The majority of Toronto's wastewater is treated at four wastewater treatment plants (WWTPs) located across the city: Ashbridges Bay (covering approximately 50% of Toronto's population), Humber (23%), Highland Creek (18%), and North Toronto (6%). Peel's Lakeview treatment plant covers the remaining approximately 3% of Toronto's population. Toronto's wastewater surveillance program does not include samples from North Toronto or Peel's Lakeview treatment plants as they only cover a small fraction of Toronto's population (Table 1). Wastewater sampling occurs in the remaining three wastewater treatment plants in Toronto 3-5 times per week and covers 91% of Toronto's population.

Figure 1. Catchment areas of wastewater treatment plants in Toronto

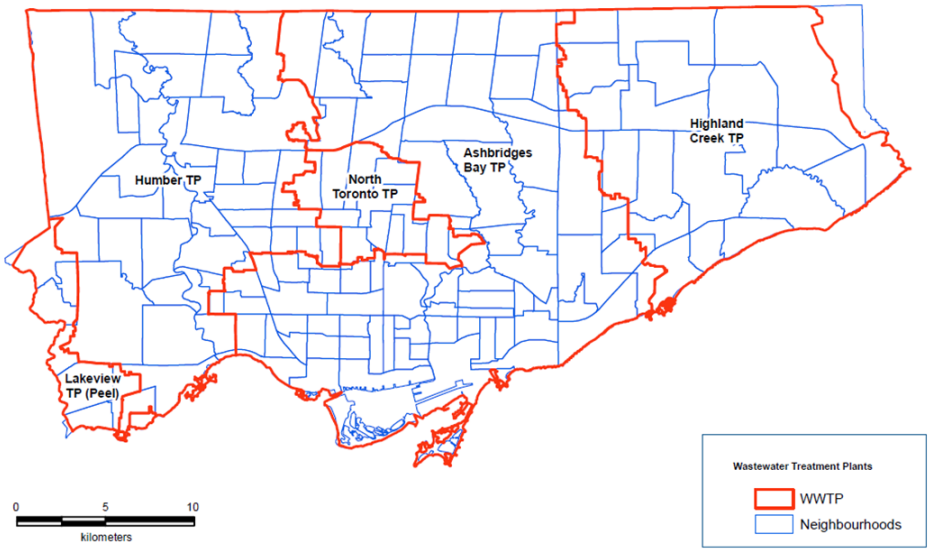

Table 1. Proportions of Toronto's population by wastewater treatment plant.

Wastewater Treatment Plant	n	%
Toronto	**3,020,796**	**100%**
Ashbridges Bay	1,506,692	49.9%
Humber	701,639	23.2%
Highland Creek	536,103	17.7%
North Toronto	193,958	6.4%
Lakeview (Peel)	82,404	2.7%

Source: Environics Analytics sewershed derived population 2021 data.

Note: The northeast region of Toronto that is not covered by an identified treatment plant does not have a sewer network digitized, and mainly includes the Toronto Zoo and other open area.

When are our wastewater treatment plants (WWTP) sampled?

Toronto Water collects samples of raw wastewater from Ashbridges Bay WWTP and Highland creek WWTP and sends these to a U of T laboratory. Toronto Water also collects samples of raw wastewater from Humber WWTP and sends this to a TMU laboratory. Ashbridges Bay and Humber WWTPs are sampled 5 times per week, while Highland Creek WWTP is sampled 3 times per week for both SARS-CoV-2 and Influenza.

Samples are collected using a 24-hour sampler at similar times in each WWTP, which collects a set amount of raw wastewater every hour creating a 24hour sample. The collection could start one day and end the next, so the sample date is the day the majority of the sample was taken.

How do we measure SARS-CoV-2 (COVID-19) wastewater viral signal?

To measure SARS-CoV-2 viral signals, laboratory assays have been developed to detect and quantify concentrations of two viral regions (N1 and N2) of the SARS-CoV-2 virus in raw wastewater. To adjust for non-human inputs (such as snow melt, rainfall, and non-human fecal matter) in wastewater, the N1 and N2 viral signals are normalized using measured levels of Pepper Mild Mottle virus (PMMoV), a human fecal matter biomarker found in wastewater. To analyze the wastewater monitoring indicator, N1 and N2 concentrations are averaged together to generate a mean concentration of N1N2 viral RNA (copies/mL). This viral signal is then normalized by dividing by the concentration of PMMoV (copies/mL) to standardize against human fecal content. Additionally, we applied a 100,000 multiplier to the normalized data to make the number easier to read.

How do we measure influenza A and influenza B wastewater viral signal?

To measure Influenza wastewater viral signals, two laboratory assays have been developed to detect and quantify concentrations of (1) a viral region specific to the Influenza A virus and (2) a viral region specific to the Influenza B virus in raw wastewater. To adjust for non-human inputs (such as snow melt, rainfall, and non-human fecal matter) in wastewater, these Influenza A and Influenza B viral signals are normalized using measured levels of Pepper Mild Mottle virus (PMMoV), a human fecal matter biomarker found in wastewater. This is done by taking the influenza A and Influenza B viral RNA (copies/mL) and dividing by the concentration of PMMoV (copies/mL). Additionally, we applied a 100,000 multiplier to the normalized data to make the number easier to read.

How does Toronto Public Health obtain Toronto wastewater data?

TMU and the U of T analyse our wastewater data and upload the data into the Ministry of the Environment, Conservation and Parks (MECP) Ontario's Wastewater Surveillance Hub. Toronto Public Health downloads

wastewater data weekly on Tuesday morning and includes data for samples collected up to the previous week (ending on Wednesday) in the wastewater signal strength & trend analysis described below. Please note that occasionally there may be a lag in uploading wastewater data into the Hub for some WWTPs and data for the previous week may be incomplete at the time of download.

How do we calculate viral signal trend?

Using wastewater samples collected in three Toronto WWTPs (Ashbridges Bay, Humber, and Highland Creek) in the 17 days prior to and including the most recent Wednesday (where available), the trend for each WWTP is determined on a log-linear regression[1], where the slope alongside the p-value (with alpha=0.10) describe the trends of normalized viral signal concentrations in wastewater. A minimum of three samples over the past 17 days are required to generate a trend.

- Increasing (**upward red arrow**) if slope is positive and the p-value is less than 0.1
- Present/stable (**horizontal yellow arrow**) with no trend if p-value is equal to or greater than 0.1
- Decreasing (**downward green arrow**) if slope is negative and the p-value is less than 0.1.

How do we assess viral signal strength?

Use the last available 7-day moving average prior to and including the most recent Wednesday in each WWTP and compare this to the WWTP-specific thresholds to assign signal strength.

Threshold cut-offs based on quartiles (25^{th}, 50^{th}, 75^{th} percentiles) are used to determine signal strength. Separate thresholds are calculated per disease (SARS-CoV-2, Influenza A, and Influenza B) and per WWTP (Ashbridges Bay, Highland Creek, and Humber). WWTP-specific quartiles are required as the viral signal concentration varies in each WWTP due to their size and flow differences. SARS-CoV-2 thresholds were calculated using post-omicron data (Dec 1, 2021, and later) as the omicron viral signal was much stronger in concentration than the previous SARS-CoV-2 variants. Influenza thresholds were calculated using interim 2022-2023 influenza A and B wastewater data, provided to Toronto Public Health by TMU and U of T pilot projects. Influenza A and B data was limited to the dates each virus was active due to the seasonality patterns in influenza. Thresholds quartile cut-offs were calculated using the 7-day moving average.

Limitations of the data

As with any source of data, there are some limitations to consider. There can be variation in the wastewater data due to environmental factors (for example rain or snow) which can affect the wastewater samples. There is also a large variation in the proportion of Toronto's population each wastewater treatment plant serves. Combined with environmental factors, the population size may dilute or concentrate the amount of virus that is detected.

Additionally, the interim 2022-2023 influenza A and B wastewater data used to calculate influenza thresholds was only sampled once per week and includes just one influenza respiratory season. Humber WWTP was not sampled for influenza in September 2022. Thus, the influenza threshold cut-offs may not be as reflective as the current data which is sampled more frequently per WWTP and for the whole respiratory season. However, it is the best available data for our threshold cut-offs, and will be modified in future seasons as we gather more data.

1. Analysis is based on the US CDC recommendations for trend reporting. A number of models were assessed for linear regression assumptions and were validated against historical trends. The model which using a 17-day period, $\log_{10}$ (PMMoV normalized N1N2+1), and alpha=0.10 met all the assumptions and was the most robust among all tested models. A 17-day period, $\log_{10}$ (PMMoV normalized Flu A/Flu B +1), and alpha=0.10 was used for Influenza A and B respectively, however model fit could not be assessed as we only have aggregate level Influenza counts.

Indicator	Definition:
Sample Date	The date the day the majority of the 24-h raw wastewater sample was taken.
SARS-Cov-2 normalized viral signal (copies/ mL)	Measured N1N2 SARS-CoV-2 wastewater RNA viral signal concentration, normalized to the Pepper Mild Mottle virus (PMMoV) per sample date. Additionally, we applied a 100,000 multiplier to the normalized data to make the number easier to read. For more information, see the section above on 'How do we measure SARS-CoV-2 (COVID-19) wastewater viral signal?'. Since trends and changes over time for each site are the best way to assess activity, and comparisons of units or scale between sites are not meaningful, the y-axis scale is not shown. For those interested in this detail, it can be downloaded from the excel file associated with the dashboard.
Avg SARS-Cov-2 normalized viral signal (copies/ mL)	7-day moving average of the "SARS-Cov-2 normalized viral signal concentration" mentioned above
COVID-19 Signal Strength	Use the last available "Avg SARS-Cov-2 normalized viral signal" prior to and including the most recent Wednesday in each WWTP and compare this to the WWTP-specific thresholds to assign signal strength. Thresholds are used to determine the signal strength of SARS-CoV-2 and are based on post-omicron SARS-CoV-2 N1N2 PMMoV viral signal. It was limited to post-omicron data (Dec 1, 2021, and later), as the viral signal concentration is much higher with the omicron variants. Quartiles cut-offs (25^{th}, 50^{th}, 75^{th} percentiles) were calculated on the "Avg SARS-Cov-2 normalized viral signal" in each WWTP using post omicron data as of October 17, 2023. For more information, see the section above on 'How do we assess viral signal strength?'. The following cut-offs are applied: <u>Ashbridges Bay WWTP:</u> 0 copies/ml to 32.257 copies/ml = Low (Green) 32.257 copies/ml to 63.350 copies/ml = Medium (Yellow) 63.350 copies/ml to 111.368 copies/ml = High (Orange) 111.368 copies/ml or more = Very High (Red) <u>Highland Creek WWTP:</u> 0 copies/ml to 76.932 copies/ml = Low (Green) 72.932 copies/ml to 144.981 copies/ml = Medium (Yellow) 144.981 copies/ml to 234.840 copies/ml = High (Orange) 234.840 copies/ml or more = Very High (Red) <u>Humber WWTP:</u> 0 copies/ml to 94.657 copies/ml = Low (Green) 94.657 copies/ml to 201.237 copies/ml = Medium (Yellow) 201.237 copies/ml to 333.596 copies/ml = High (Orange) 333.596 copies/ml or more = Very High (Red)
COVID-19 Trend	Please see the 'How do we calculate viral signal trend?' section for more information.
Influenza A normalized viral signal (copies/ mL)	Measured Influenza A wastewater RNA viral signal concentration, normalized to the Pepper Mild Mottle virus (PMMoV) per sample date. Additionally, we applied a 100,000 multiplier to the normalized data to make the number easier to read. For more information, see the section above on 'How do we measure Influenza A and Influenza B wastewater viral signal?'. Since trends and changes over time for each site are the best way to assess activity, and comparisons of units or scale between

Indicator	Definition:
	sites are not meaningful, the y-axis scale is not shown. For those interested in this detail, it can be downloaded from the excel file associated with the dashboard.
Avg Influenza A normalized viral signal (copies/ mL)	7-day moving average of the "Influenza A normalized viral signal" mentioned above
Influenza A Signal Strength	Use the last available "Avg Influenza A normalized viral signal" prior to and including the most recent Wednesday in each WWTP and compare this to the WWTP-specific thresholds to assign signal strength. Thresholds are used to determine the signal strength of Influenza A and are based on preliminary influenza A wastewater data provided to us by TMU and U of T laboratory partners for the 2022-2023 influenza A season. 2022-2023 data was limited to the active Influenza A season which was September 2022 – April 2023 in Ashbridges Bay and Highland creek WWTPs, and October 2022 – April 2023 for Humber WWTP. Quartiles cut-offs (25^{th}, 50^{th}, 75^{th} percentiles) were calculated on the "Avg Influenza A normalized viral signal" in each WWTP. For more information, see the section above on 'How do we assess viral signal strength?'. The following cut-offs are applied: <u>Ashbridges Bay WWTP:</u> 0 copies/ml ≥ to ≤ 0.818 copies/ml = Low (Green) 0.818 copies/ml > to ≤ 2.029 copies/ml = Medium (Yellow) 2.029 copies/ml > to ≤ 13.436 copies/ml = High (Orange) 13.436 copies/ml or more = Very High (Red) <u>Highland Creek WWTP:</u> 0 copies/ml ≥ to ≤ 0.882 copies/ml = Low (Green) 0.882 copies/ml > to ≤ 6.425 copies/ml = Medium (Yellow) 6.425 copies/ml > to ≤ 32.768 copies/ml = High (Orange) 32.768 copies/ml or more = Very High (Red) <u>Humber WWTP:</u> 0 copies/ml ≥ to ≤ 0.141 copies/ml = Low (Green) 0.141 copies/ml > to ≤ 9.804 copies/ml = Medium (Yellow) 9.804 copies/ml > to ≤ 31.445 copies/ml = High (Orange) 31.445 copies/ml or more = Very High (Red)
Influenza A Trend	Please see the 'How do we calculate viral signal trend?' section for more information.
Influenza B normalized viral signal (copies/ mL)	Measured influenza B wastewater viral signal concentration, normalized to the Pepper Mild Mottle virus (PMMoV) per sample date. Additionally, we applied a 100,000 multiplier to the normalized data to make the number easier to read. For more information, see the section above on 'How do we measure influenza A and influenza B wastewater viral signal?'. Since trends and changes over time for each site are the best way to assess activity, and comparisons of units or scale between sites are not meaningful, the y-axis scale is not shown. For those interested in this detail, it can be downloaded from the excel file associated with the dashboard.
Avg Influenza B normalized viral signal (copies/ mL)	7-day moving average of the "Influenza B normalized viral signal" mentioned above.
Influenza B Signal Strength	Use the last available "Avg Influenza B normalized viral signal" prior to and including the most recent Wednesday in each WWTP and compare this to the WWTP-specific thresholds to assign signal

Indicator	Definition:
	strength. Thresholds are used to determine the signal strength of SARS-CoV-2 and are based on preliminary influenza B wastewater data provided to us by TMU and U of T laboratory partners for the 2022-2023 influenza B season. 2022-2023 data was limited to the active Influenza B season which was December 2022 – July 2023 in Ashbridges Bay and Highland creek WWTPs, and February 2023 – May 2023 for Humber WWTP. Quartiles cut-offs (25^{th}, 50^{th}, 75^{th} percentiles) were calculated on the "Avg Influenza B normalized viral signal" in each WWTP. For more information, see the section above on 'How do we assess viral signal strength?'. The following cut-offs are applied: <u>Ashbridges Bay WWTP:</u> 0 copies/ml ≥ to ≤ 0.527 copies/ml = Low (Green) 0.527 copies/ml > to ≤ 1.848 copies/ml = Medium (Yellow) 1.848 copies/ml > to ≤ 5.135 copies/ml = High (Red) 5.135 copies/ml or more = Very High (Dark Red) <u>Highland Creek WWTP:</u> 0 copies/ml ≥ to ≤ 1.547 copies/ml = Low (Green) 1.547 copies/ml > to ≤ 5.730 copies/ml = Medium (Yellow) 5.730 copies/ml > to ≤ 12.088 copies/ml = High (Orange) 12.088 copies/ml or more = Very High (Red) <u>Humber WWTP:</u> 0 copies/ml ≥ to ≤ 1.184 copies/ml = Low (Green) 1.184 copies/ml > to ≤ 5.240 copies/ml = Medium (Yellow) 5.240 copies/ml > to ≤ 21.149 copies/ml = High (Orange) 21.149 copies/ml or more = Very High (Red)
Influenza B Trend	Please see the 'How do we calculate viral signal trend?' section for more information.