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Method Article

Keywords: wastewater surveillance, wastewater metrics, wastewater-based epidemiology

Posted Date: November 19th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11625409/v1

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Additional Declarations: No competing interests reported.

Validation of Centers for Disease Control and Prevention National Wastewater Surveillance System's Wastewater Viral Activity Level for Respiratory Viruses, 2025 –2026 Respiratory Season

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Abstract

Background: The Centers for Disease Control and Prevention (CDC) developed the Wastewater Viral Activity Level metric (WVAL) for SARS-CoV-2 in 2023 to meet the need for a public metric that aggregates wastewater viral RNA concentration data to provide jurisdictional, regional, and national surveillance system estimates for the United States. The WVAL was expanded in August 2024 to include virus-specific metrics for influenza A and respiratory syncytial virus (RSV). This paper describes results

the 2025 validation of the updated WVAL methodology. **Methods:** During validation conducted from March through July 2025, candidate WVAL methods were evaluated using wastewater surveillance data collected through March 22, 2025, from SARS-CoV-2, influenza A, and RSV sampling sites. Evaluated components included the baseline calculation and stabilization period, the frequency of baseline and standard deviation updates, the sample look-back period used to calculate the baseline, and the cut points used to assign WVAL categories. **Results:** Data included 4,447 SARS-CoV-2, 1,592 influenza A, and 1,454 RSV data submitter level sampling sites. A z-score threshold of 4 was selected for outlier removal, and non-normalized wastewater concentrations with a 24-month look-back period were selected for baseline and standard deviation calculations across pathogens. Baseline and standard deviation updates were scheduled for April 1 and October 1 for SARS-CoV-2 and August 1 for influenza A and RSV. An eight-week stabilization period was selected. Median-of-medians aggregation was selected for regional and national estimates, and pathogen-specific cut-points were selected to align WVAL categories with clinical surveillance data. National WVALs were moderately to strongly correlated with emergency department surveillance data for SARS-CoV-2 (Kendall's tau = 0.78), influenza A (0.81), and RSV (0.74). **Conclusions:** Annual validation supported updates to the WVAL methodology that reduced extreme values, improved comparability across pathogens and geographic levels, and maintained alignment with clinical surveillance. The updated methods provide a more consistent framework for public wastewater surveillance of SARS-CoV-2, influenza A, and RSV.

Keywords: wastewater surveillance, wastewater metrics, wastewater-based epidemiology

Contributions to the literature

- Improves transparency and reproducibility by detailing the CDC NWSS data processing methods in Appendix 1 of Additional files 1.
- Demonstrates an approach for validating and updating public wastewater surveillance metrics as data, methods, and respiratory virus seasonality evolve.

- Supports accessible jurisdictional, regional, and national estimates of respiratory virus activity using wastewater data.
- Provides a consistent framework for monitoring SARS-CoV-2, influenza A, and RSV through wastewater surveillance.

Background

Wastewater surveillance has been used globally and in the United States for both routine monitoring and outbreak response to detect pathogens and inform timely public health action. It has supported public health responses to a wide range of pathogens, including monkeypox virus, highly pathogenic avian influenza A virus (H5N1), and measles morbillivirus, as well as routine surveillance for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A virus (influenza A), and respiratory syncytial virus (RSV).(1-8) Notably, wastewater surveillance has been a critical tool in global polio eradication efforts, enabling the detection of poliovirus circulation even in the absence of reported clinical cases.(9) Once genetic material from a pathogen is detected in wastewater, public health authorities can take action to slow or prevent further transmission. For example, local health departments in New York have used wastewater surveillance data to track SARS-CoV-2 trends and inform planning for the respiratory virus season, including resource allocation and staffing decisions.(10) Similarly, school communities in Houston, Texas have used wastewater surveillance alerts for SARS-CoV-2, influenza A and B, and RSV to inform community members about protective measures, such as getting vaccinated, staying home when sick, and practicing regular hand hygiene.(11)

In response to the COVID-19 pandemic, the Centers for Disease Control and Prevention (CDC) established the National Wastewater Surveillance System (NWSS) in 2020.(1) CDC NWSS provides a national public health infrastructure to monitor pathogens through wastewater, supporting public health agencies at the state, tribal, local, territorial (STLT), and national levels in understanding disease burden and informing public health decision-making.(12) CDC's COVID Data Tracker, the first webpage

to display national SARS-CoV-2 wastewater data, calculated and displayed four SARS-CoV-2 wastewater concentration metrics using data reported by partners: 15-day rolling detection proportion, percent change, trends, and percentiles.(1) However, variability in wastewater sampling and laboratory testing practices limited the comparability of these metrics across geographic areas and over time, which, in turn, made them challenging for the public to interpret.

CDC NWSS developed the wastewater viral activity level (WVAL) metric in 2023 to standardize and aggregate wastewater viral RNA concentration (wastewater concentration) data, enabling comparisons across state/territorial, regional, and national levels, and over time. WVALs are publicly available on the CDC NWSS website.(13) In the spring of 2024, the WVAL was further adapted and initially validated for influenza A and RSV. At that time, approximately 600 wastewater surveillance sites were testing for influenza A; however, only about 70 of these sites had data available from before October 1, 2022. Similarly, approximately 400 sites were testing for RSV, with only five sites having data prior to October 1, 2022. Because influenza A and RSV differ from SARS-CoV-2 in their seasonality and frequency of detection in wastewater, modifications to the WVAL methodology were required. These metrics were added to CDC webpages in August 2024 ahead of the 2024–2025 respiratory virus season. However, because relatively few sites were included in the initial validation, additional validation of WVALs for SARS-CoV-2, influenza A, and RSV is warranted. The objective of this paper is to describe the WVAL validation process for SARS-CoV-2, influenza A, and RSV in preparation for the 2025–2026 respiratory virus season, to ensure that WVALs perform as intended, and to evaluate and implement potential methodological improvements.

Methods

The validation of the WVAL for SARS-CoV-2, influenza A, and RSV was conducted from March through July of 2025 using wastewater concentration data from all samples collected through March 22, 2025. Updated methods were released on the CDC webpages on August 15, 2025. Here we describe

how data are ingested and processed and the WVAL validation methods used in preparation for the 2025–2026 respiratory season. Methods for data processing and calculating the 2024-2025 WVAL can be found in Appendix 1 of Additional files 1. In this paper, the 2024 –2025 WVAL method will be referred to as the 'current method'. Analyses were conducted in R 4.5.1 using RStudio Pro or Palantir Foundry Code Repositories using a Spark environment.

For the 2025–2026 validation, data included 4,447 data submitter level sampling sites for SARS-CoV-2, 1,592 for influenza A, 1,486 for influenza B, and 1,454 for RSV. It was determined in the early stages of validation that influenza B had too few detections to reliably calculate WVALs and was subsequently dropped from this validation.

Outliers

Three outlier-identification approaches were evaluated: z-score based removal, the interquartile range (IQR) method, and a no removal approach. A threshold was considered preferable if it resulted in a percent change of ≥15%, an author-derived criterion selected for this validation. To determine an appropriate z-score threshold for outlier removal, the absolute difference between the mean and median wastewater concentration for each data submitter level sampling site under a series of candidate thresholds (2.5, 3.0, 3.5, 4.0, and 4.5) were calculated and then averaged across all data submitter level sampling sites to produce a mean absolute difference. The threshold of 4 reflects the currently implemented approach and served as the reference. The percent change in the mean absolute difference relative to the reference threshold of 4 was subsequently calculated.

The IQR method defined outliers as observations falling above the 75th percentile plus 1.5 times the interquartile range or below the 25th percentile minus 1.5 times the interquartile range. This approach was applied at the data submitter level. For comparison, a no removal approach was included where all observed wastewater concentrations were retained. These analyses were conducted separately for normalized (natural logarithm transformed) and non-normalized wastewater

concentrations. For normalized wastewater concentrations, flow-population normalization was prioritized over human fecal normalization.

Baseline and standard deviation

This 2025-2026 validation of baselines and standard deviations focused on the update schedule for SARS-CoV-2, the evaluation of look-back periods, and the comparison of normalized versus non-normalized wastewater concentrations. The 10th percentile baseline and standard deviation methods remained fixed and were not re-evaluated.

To determine an appropriate schedule for updating SARS-CoV-2 baselines and standard deviations, the timing and recurrence of SARS-CoV-2 wastewater concentration peaks were assessed. Because SARS-CoV-2 wastewater concentrations in the United States commonly exhibit two seasonal peaks per year, the analysis focused on identifying the months in which peaks most consistently occur and selecting update periods that precede these anticipated increases. Normalized, spline-smoothed linear wastewater concentrations from October 2022 through March 2025 were used, along with a random sample of 50 site ID and data submitter combinations. For each combination, normalized spline curves were visually examined to identify increases in SARS-CoV-2 signal intensity. To align baseline recalculations with the observed bimodal seasonal pattern, April 1 and October 1 were selected as update dates for SARS-CoV-2 baselines and standard deviations so that recalculations precede anticipated increases in activity. For influenza A and RSV, baseline and standard deviation updates were set to August 1 to occur before the start of the clinical respiratory virus season, consistent with consensus from CDC clinical subject matter experts

Three look-back periods (12, 18, and 24 months) were evaluated for calculating the 10th percentile baseline and standard deviation. Only sites with a sample collected during the Morbidity Mortality Weekly Report (MMWR) epidemiologic week ending December 22, 2024, or later were included. Additionally, for each candidate look-back period, only sites with data spanning at least that

duration were eligible. These candidate look-back periods were used to generate WVALs separately for normalized and non-normalized wastewater concentrations. Medians were calculated at the site level and compared with the 2024–2025 WVAL method for each pathogen. To statistically compare WVALs generated using the candidate look-back periods with WVALs produced under the 2024–2025 method, paired nonparametric tests were conducted using site level WVALs. Wilcoxon signed rank tests (WSRT)(14) were used to evaluate two hypotheses:

1. Two-sided test
    a. Null hypothesis: The median difference between WVALs produced by the current method and those produced using a candidate look-back period equals 0.
    b. Alternative hypothesis: The median difference is not equal to 0.
2. One-sided test
    a. Null hypothesis: The median difference between WVALs produced by the current method and those produced using a candidate look-back period is ≤ 0.
    b. Alternative hypothesis: The median paired difference is > 0.

A Bonferroni correction was applied to adjust for multiple comparisons. The adjusted significance level was set at 0.025 or 0.017 depending on the number of candidate look-back periods tested. To complement statistical testing, site-level time series plots were generated to visually compare WVAL trajectories under the different candidate look-back periods. For each pathogen, 50 site IDs were randomly selected for review.

Baseline stabilization

Rolling 10th percentile baselines were calculated for each data submitter level sampling site, updating the baseline as new wastewater concentrations were added in chronological order. Each data submitter level sampling site's data were segmented into continuous sampling periods, with a new period initiated whenever the gap between consecutive sample collection dates exceeded 14 days.

Within each period, the baseline at each sample was defined as the 10th percentile of all concentrations observed up to and including that sample. The data were processed month by month from March 2022 through February 2025 for the 3-month analysis and from April 2022 through January 2024 for the 6-month analysis. For a given month, data submitter level sampling sites were included if they began sampling on or before the end of that given month, and only samples collected on or after the first day of that month were retained. Each data submitter level sampling site's first sample collection date was reassigned to the first day of the month if they began sampling prior to the given month, ensuring that time since method initiation was aligned to the start of the month for the analysis window.

To assess stabilization for each data submitter level sampling site and month, baselines were compared between consecutive samples within each continuous sampling period. Stabilization was defined as a run in which the absolute percent change in baseline between consecutive samples was less than or equal to 5%, and separately 1%, sustained for at least 90 days (3-month analysis) or at least 183 days (6-month analysis), with the stabilization sequence restarting whenever the data submitter level sampling site stopped sampling for more than two weeks. When a qualifying stable run was identified, the earliest sample meeting these criteria was flagged as the stabilization point, and the number of days from the most recent method-start date (or reset) to stabilization was recorded. For each month in each analysis (3-month and 6-month), summary statistics describing time to stabilization were calculated across all data submitter level sampling sites that had achieved stabilization by that month.

Aggregation

Population estimates were obtained from the U.S. Census Bureau and included all 50 states, the District of Columbia, Puerto Rico, Guam, U.S. Virgin Islands, American Samoa, and Northern Mariana Islands(15). State/territory level aggregation procedures were consistent across all methods, with WVALs calculated as the median of site level WVALs. Four aggregation methods were evaluated for U.S. Census Bureau regional and national level WVALs relative to the current method (median of all site

WVALs). The median of medians aggregation was calculated as the median of state/territory WVALs for each MMWR week, assigning equal weight to each jurisdiction regardless of population size or number of reporting sites. The hierarchical geometric weight decay aggregation applied weights following a geometric progression with a 20% decay per tier, normalized to sum to one, such that larger populated jurisdictions received greater influence with exponentially decreasing weights for smaller populated jurisdictions (Equation 4). Linear rank weighted aggregation assigned weights based on rank, with larger populated jurisdictions receiving proportionally larger weights using equal linear increments (Equation 5). Population weighted aggregation used jurisdictional population estimates to weight state level contributions proportionally.

Comparison of clinical and wastewater data

Using all available data through March 2025, WVALs were evaluated using both clinical surveillance datasets and wastewater concentration based smooth spline estimates. Associations over time with clinical surveillance data were assessed at the state/territory, regional, and national levels using Kendall's tau-b correlation coefficient. Influenza A WVALs were compared with data from the U.S. Outpatient Influenza-like Illness Surveillance Network (ILINet) (data available for 55 states and territories) and the National Syndromic Surveillance Program (NSSP) (data available for 49 states and DC). RSV WVALs were compared with unadjusted rates from the RSV Hospitalization Surveillance Network (RSV-NET) (data available for 13 states) and NSSP, and SARS-CoV-2 WVALs were compared with the Coronavirus Disease 2019 Hospitalization Surveillance Network (COVID-NET) (data available for 15 states) and NSSP. To compare clinical datasets at the regional level, the median percent positive was taken for each region.

Alignment between WVALs and smooth wastewater concentration splines were assessed visually. For this analysis, 50 sites were randomly selected, and WVAL time series were plotted alongside normalized smooth wastewater concentration splines to evaluate concordance in the timing of

increases and decreases. Because WVALs and smooth wastewater concentration splines are derived from the same underlying wastewater concentration data, formal statistical correlation between these metrics was not performed.

Categories

WVALs were separated into five categories using three methods for generating cut-points: k-means clustering, quintiles, and Jenks natural breaks. Cut-points were generated at three levels of aggregation: (1) using all WVALs nationally, (2) using all WVALs within each region, and (3) using the average of cut-points calculated for each region. For each method and aggregation, plots were created to visualize categories in relation to percent positivity or the proportion of patient visits reported by clinical surveillance systems (see *Comparison of clinical and wastewater data*). The methods that visually aligned most closely with the clinical datasets were selected for each pathogen.

To evaluate the performance of the updated categories, we compared WVALs with emergency room (ED) visit data from NSSP at the national level. Associations between WVAL categories and the corresponding NSSP threshold categories were assessed using Kendall's tau-b correlation coefficient. Analyses were conducted separately for each pathogen; regional data were not evaluated due to differences between regional definitions between NSSP and NWSS (Human Health Service (HHS) regions vs U.S. Census regions, respectively).

Results

Outliers

A z-score with a threshold of 4 was determined to be the optimal method for removing outliers for both normalized and non-normalized concentration data for SARS-CoV-2, influenza A and RSV, as none of the other candidate thresholds (2.5, 3, 3.5 and 4.5) resulted in a 15% or more change in the mean absolute difference when compared to a threshold of 4 for either normalized or non-normalized concentrations. Across all three pathogens, the z-score threshold of 2.5 resulted in the largest percent

change in the absolute difference (minimum -5.4%, maximum -9.7%), and a z-score threshold of 4.5 resulted in the smallest percent change in the absolute difference (minimum 0.4%, maximum 0.6%).

Not removing any outliers prior to WVAL calculation had mixed results depending on the pathogen. For SARS-CoV-2, some sites had little to no noticeable change when examining plots of the WVAL over time, while others had high peaks when no outliers were removed. Similarly for influenza A and RSV, not removing outliers resulted in some sites with high peaks in the WVAL, or shifted the timing of the peak of the WVAL by a couple of days.

The IQR method for identifying and removing outliers caused many more samples to be removed prior to WVAL calculation for all three pathogens and, therefore, resulted in longer gaps between sample collection dates for which WVALs could be calculated at the site level. For SARS-CoV-2, using a z-score threshold of 4 resulted in the removal of 427 samples from 258 sites, while the IQR method resulted in the removal of 7,424 samples from 1,287 sites. For influenza A, using a z-score threshold of 4 resulted in the removal of 226 samples from 177 sites, while the IQR method resulted in the removal of 6,974 samples from 646 sites. For RSV, using a z-score threshold of 4 resulted in the removal of 258 samples from 184 sites, while the IQR method resulted in the removal of 5,408 samples from 649 sites.

Baseline and standard deviation

For the 50 randomly selected site ID and data submitter combinations, the month and year of each identified SARS-CoV-2 wastewater visual peak were recorded (Table 1). Since data from multiple years were included, peak timing was summarized by month. Peaks occurred most frequently in January (57) and August (43), followed by December (28) and September (24). To align baseline recalculations with the observed bimodal seasonal pattern, April 1 and October 1 were selected as update dates for SARS-CoV-2 baselines and standard deviations so that recalculations precede anticipated increases in activity. For influenza A and RSV, baseline and standard deviation updates were set to August 1 to occur

before the start of the clinical respiratory virus season, consistent with consensus from CDC clinical subject matter experts.

Table 1. SARS-CoV-2 wastewater concentration peaks for 50 randomly selected sites

Winter peak 2022/2023 (n)	Summer peak 2023 (n)	Winter peak 2023/2024 (n)	Summer peak 2024 (n)	Winter peak 2024/2025 (n)
Dec 2022 (18)	Sept 2023 (19)	Jan 2024 (27)	Aug 2024 (36)	Jan 2025 (18)
Jan 2023 (12)	Aug 2023 (7)	Dec 2023 (7)	Sept 2024 (5)	Feb 2025 (14)
Feb/Mar 2023 (10)	Mar 2023 (4)	Feb 2024 (4)	July 2024 (4)	Dec 2024 (3)
Other month (4)	Other month (3)	Other month (6)	Other month (1)	Other month (2)
No peak (6)	No peak (17)	No peak (6)	No peak (4)	No peak (13)

Normalized, spline-smoothed linear SARS-CoV-2 wastewater concentration curves from October 2022 through March 2025 were visually assessed for a random sample of 50 site ID–data submitter combinations. For each seasonal period, n represents the number of combinations for which a concentration peak was visually identified in the indicated month or months; column totals equal 50. Entries spanning two months, such as February/March 2023, indicate that the peak extended across both months. "Other month" indicates a peak in a month not listed, and "No peak" indicates that no distinct peak was identified during the seasonal period.

The number of eligible sites decreased as the look-back period increased (Table 2). For SARS-CoV-2, 1,298 sites were included under the current method; candidate baselines included 699–1,182 sites depending on data type and period. Median WVALs ranged from 4.24–4.81 using normalized data and 4.35–4.88 using non-normalized data. For influenza A, 918 sites were included under the current method, with median WVALs ranging from 2.08–2.19 (normalized) and 1.35–1.77 (non-normalized). For RSV, 872 sites were included, with median WVALs ranging from 1.98–2.20 (normalized) and 1.21–1.39 (non-normalized).

Table 2. Summary statistics for wastewater viral activity levels by candidate look-back period, pathogen, and normalization method

	Look-back periods			
	Current	**12 months**	**18 months**	**24 months**
SARS-CoV-2				

Normalized data				
Number of sites	1298	1182	961	699
Median	4.36	4.81	4.24	4.70
Non-normalized data				
Number of sites	1298	1179	958	698
Median	4.36	4.88	4.35	4.75
Influenza A				
Normalized data				
Number of sites	918	652	-	243
Median	2.13	2.08	-	2.19
Non-normalized data				
Number of sites	918	649	375	243
Median	2.13	1.35	1.77	1.59
Respiratory Syncytial Virus				
Normalized data				
Number of sites	872	583	-	250
Median	2.14	1.98	-	2.2
Non-normalized data				
Number of sites	872	570	347	250
Median	2.14	1.21	1.39	1.25

WVALs were calculated using normalized and non-normalized wastewater concentrations and candidate baseline look-back periods of 12, 18, and 24 months. "Current" represents WVALs calculated using the method in use before validation. For each pathogen, normalization status, and look-back period, "Number of sites" indicates the number of sites with sufficient data to calculate a WVAL, and "Median" indicates the median WVAL across those sites. A dash (–) indicates that WVALs were not calculated for that combination because that is the current method. WVAL, wastewater viral activity level.

Two-sided Wilcoxon signed-rank tests (WSRTs) indicated that WVALs generated using candidate look-back periods frequently differed from those produced by the current method (Table 3). The rejection of the null hypothesis varied based on the normalization method and candidate look-back period, with 79.0–92.4% of SARS-CoV-2 sites, 47.9–93.0% of influenza A sites, and 34.0–94.4% of RSV sites rejecting the null hypothesis.

Table 3. Site counts and percentages from two-sided Wilcoxon tests comparing wastewater viral activity level look-back periods

	Look-back periods		
	12 months n (%)	**18 months n (%)**	**24 months n (%)**
SARS-CoV-2			
Normalized data			
Reject null	1069(90.4)	883(91.9)	646(92.4)
Fail to reject null	106(9.0)	78(8.1)	53(7.6)
Same values	7(0.6)	0(0.0)	0(0.0)
Non-normalized data			
Reject null	936(79.4)	757(79.0)	586(84.0)
Fail to reject null	243(20.6)	201(21.0)	112(16.0)
Same values	0(0.0)	0(0.0)	0(0.0)
Influenza A			
Normalized data			
Reject null	312(47.9)	-	138(56.8)
Fail to reject null	8(1.2)	-	7(2.9)
Same values	332(50.9)	-	98(40.3)
Non-normalized data			
Reject null	544(83.8)	302(80.5)	226(93)
Fail to reject null	103(15.9)	71(18.9)	16(6.6)
Same values	2(0.3)	2(0.5)	1(0.4)
Respiratory Syncytial Virus			
Normalized data			
Reject null	204(35.0)	-	85(34.0)
Fail to reject null	7(1.2)	-	2(0.8)
Same values	372(63.8)	-	163(65.2)
Non-normalized data			
Reject null	418(73.3)	319(91.9)	236(94.4)
Fail to reject null	61(10.7)	26(7.5)	13(5.2)
Same values	91(16.0)	2(0.6)	1(0.4)

WVALs were generated separately using normalized and non-normalized wastewater concentrations. Two-sided Wilcoxon signed-rank tests compared site-level WVALs generated using each candidate look-back period with WVALs generated using the 2024–2025 method. Values are presented as n (%) of eligible sites. "Reject null" indicates that the null hypothesis of a median paired difference of 0 was rejected after Bonferroni correction; "Fail to reject null" indicates that the null hypothesis was not rejected. "Same values" indicates identical WVALs under the candidate and 2024–2025 methods. A dash (–) indicates that no eligible sites were available for that pathogen, normalization status, and look-back period. WVAL, wastewater viral activity level; MMWR, Morbidity and Mortality Weekly Report.

One-sided WSRTs were used to determine whether the current method produced larger WVALs compared to candidate look-back periods (Table 4) and indicated that WVALs using 24-month non-normalized candidate look-back periods were significantly lower than those from the current method for the largest proportion of sites for influenza A (87.7%) and RSV (91.6%). For SARS-CoV-2, the 18- and 24-month candidate look-back periods showed lower WVALs for 54.2% and 50.9% of sites, respectively. The 24-month non-normalized candidate look-back period was selected for consistency across pathogens.

Table 4. Site counts and percentages from one-sided Wilcoxon tests comparing wastewater viral activity level look-back periods

	Look-back periods		
	12 months n (%)	**18 months n (%)**	**24 months n (%)**
SARS-CoV-2			
Normalized data			
Reject null	297(25.1)	617(64.2)	380(54.4)
Fail to reject null	878(74.3)	344(35.8)	319(45.6)
Same values	7(0.6)	0(0.0)	0(0.0)
Non-normalized data			
Reject null	288(24.4)	519(54.2)	355(50.9)
Fail to reject null	891(75.6)	439(45.8)	343(49.1)
Same values	0(0.0)	0(0.0)	0(0.0)
Influenza A			
Normalized data			
Reject null	202(31.0)	-	104(42.8)
Fail to reject null	118(18.1)	-	41(16.9)
Same values	332(50.9)	-	98(40.3)
Non-normalized data			
Reject null	451(69.5)	277(73.9)	213(87.7)
Fail to reject null	196(30.2)	96(25.6)	29(11.9)
Same values	2(0.3)	2(0.5)	1(0.4)
Respiratory Syncytial Virus			
Normalized data			
Reject null	192(32.9)	-	77(30.8)
Fail to reject null	19(3.3)	-	10(4.0)
Same values	372(63.8)	-	163(65.2)

Non-normalized data			
Reject null	408(71.6)	313(90.2)	229(91.6)
Fail to reject null	71(12.5)	32(9.2)	20(8.0)
Same values	91(16.0)	2(0.6)	1(0.4)

WVALs were generated separately using normalized and non-normalized wastewater concentrations. One-sided Wilcoxon signed-rank tests compared site-level WVALs generated using each candidate look-back period with WVALs generated using the 2024–2025 method under the prespecified directional hypothesis that the median paired difference was greater than zero. Values are presented as n (%) of eligible sites. "Reject null" indicates that the null hypothesis of a median paired difference less than or equal to zero was rejected after Bonferroni correction; "Fail to reject null" indicates that the null hypothesis was not rejected. "Same values" indicates identical WVALs under the candidate and 2024–2025 methods. A dash (–) indicates that no eligible sites were available for that pathogen, normalization status, and look-back period. WVAL, wastewater viral activity level; MMWR, Morbidity and Mortality Weekly Report.

Baseline stabilization

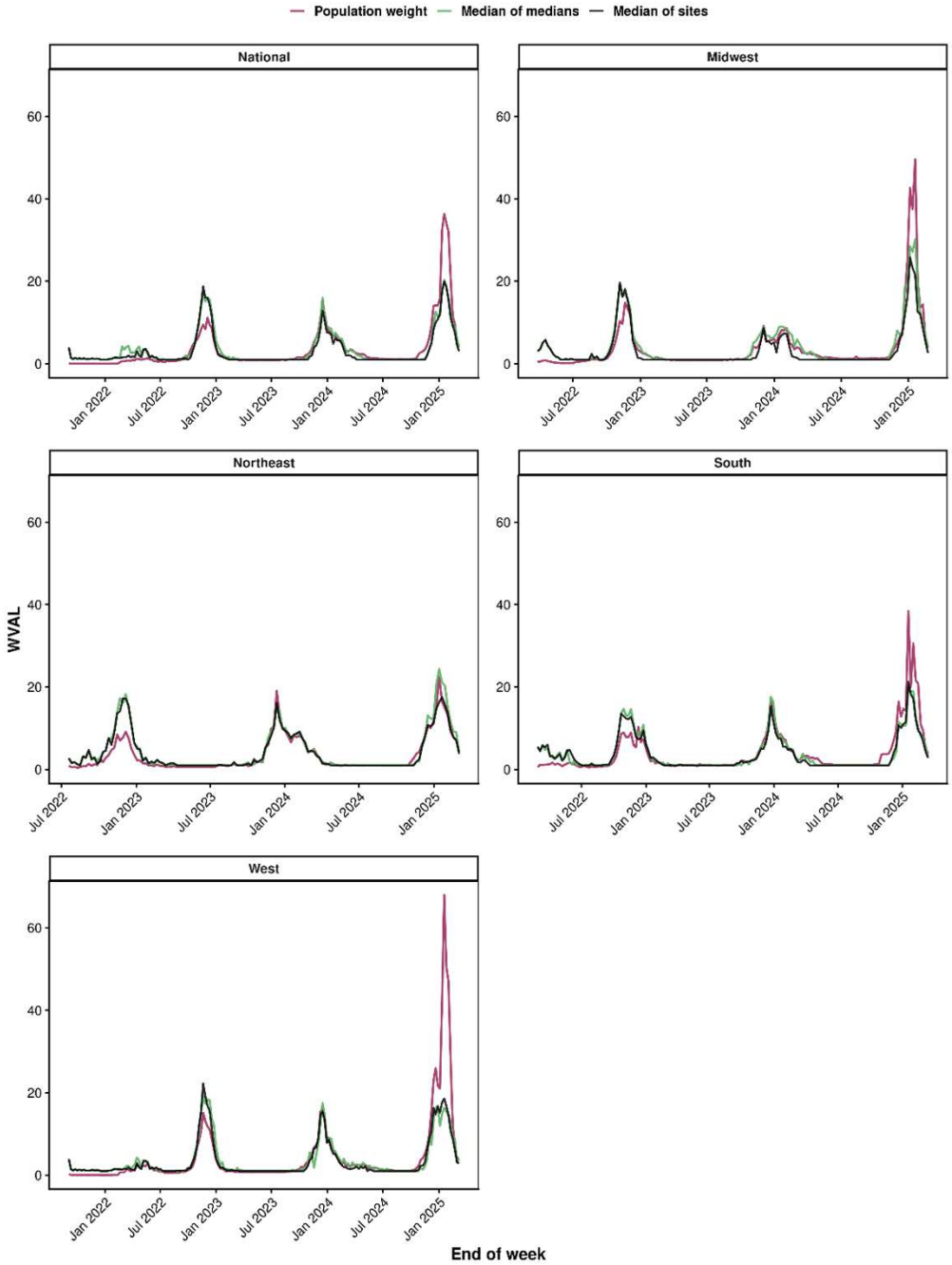
For SARS-CoV-2, the mean time for baselines to change by ≤1% between consecutive samples were 16.3 weeks (3 months) and 17.6 weeks (6 months). For a ≤5% change, it was 11.1 and 17.5 weeks, respectively. Influenza A stabilized faster, with a ≤1% change achieved in 3.8 weeks (3 months) and 4.5 weeks (6 months), and a ≤5% change in 3.7 and 4.2 weeks. RSV required 5.1 weeks (3 months) and 6.2 weeks (6 months) for a ≤1% change, and 4.5 and 6.1 weeks for a ≤5% change. Across all pathogens, the mean stabilization times were 8.4 weeks (≤1%, 3 months), 9.4 weeks (≤1%, 6 months), 6.3 weeks (≤5%, 3 months), and 9.3 weeks (≤5%, 6 months). These estimates produced an average of 8.4 weeks which was rounded to 8 weeks for display on the CDC NWSS website.

Aggregation

The hierarchical geometric weight decay and the linear rank weight approaches were stopped early in the analysis for all three pathogens because they produced similar values to the population weighted aggregation, which is a simpler approach. Additionally, these methods produced extreme WVALs at higher geographic levels, ranging from approximately 70 to 100. The median of medians

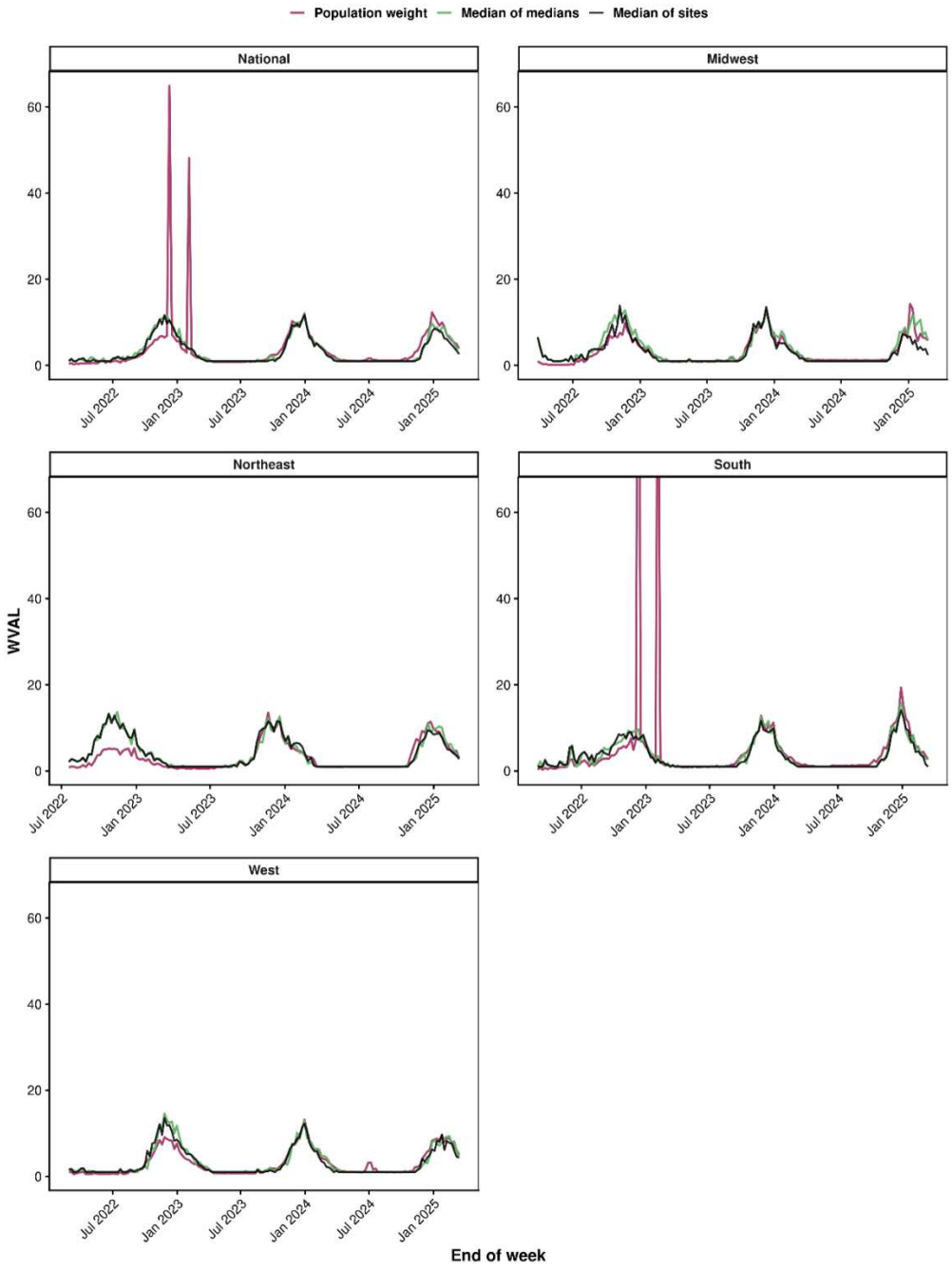
approach was selected because it produced aggregate WVALs similar to the method used previously, did not produce extreme WVALs, and gave all jurisdictions within a region equal weight (Figure 1-3).

Figure 1. Wastewater viral activity level aggregation methods for influenza A by region and national



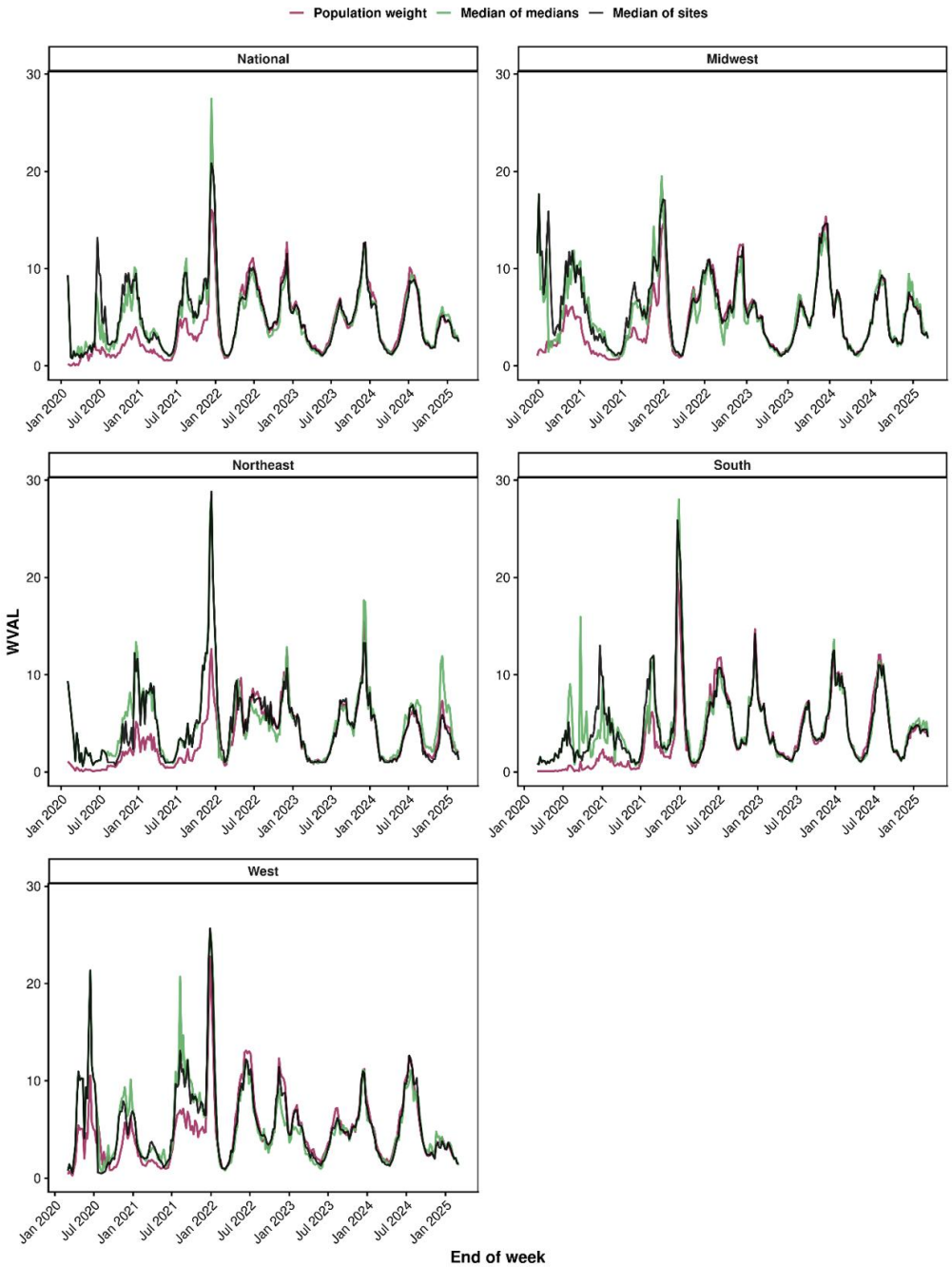
Weekly influenza A wastewater viral activity levels (WWAL) are shown for the national aggregate and the Midwest, Northeast, South, and West. Lines compare population-weighted estimates, the median of medians, and the median of sites.

Figure 2. Wastewater viral activity level aggregation methods for Respiratory Syncytial Virus by region and national



Weekly RSV wastewater viral activity levels (WWAL) are shown for the national aggregate and the Midwest, Northeast, South, and West. Lines compare population-weighted estimates, the median of medians, and the median of sites.

Figure 3. Wastewater viral activity level aggregation methods for SARS-CoV-2 by region and national



Weekly SARS-CoV-2 wastewater viral activity levels (WWAL) are shown for the national aggregate and the Midwest, Northeast, South, and West. Lines compare population-weighted estimates, the median of medians, and the median of sites.

Comparison of clinical and wastewater data

The strength of the correlation between influenza A WVALs and NSSP data for influenza at the jurisdiction-level ranged 0.16–0.84 (Kendall's tau), with 48 out of 49 values having a p-value less than 0.05. At the regional level, the strength of the correlations were 0.80 in the Northeast ($p<0.001$), 0.83 in the South ($p<0.001$), 0.78 in the Midwest ($p<0.001$), 0.75 in the West ($p<0.001$), and 0.83 nationally ($p<0.001$). The strength of the correlations between influenza A WVALs and ILINet data at the jurisdiction level ranged 0.42– 0.75 (Kendall's tau), with all 49 comparisons having a p-value less than 0.05. At the regional level, the strength of the correlations were 0.73 in the Northeast ($p<0.001$), 0.69 in the South ($p<0.001$), 0.67 in the Midwest ($p<0.001$), 0.49 in the West ($p<0.001$), and 0.59 nationally ($p<0.001$).

The strength of the correlation between the RSV WVALs and NSSP data for RSV at the jurisdiction-level ranged 0.20–0.79 (Kendall's tau), with all 49 comparisons having a p-value less than 0.05. At the regional level, the strength of the correlations were 0.76 in the Northeast (p-value <0.001), 0.77 in the South (p-value <0.001), 0.77 in the Midwest ($p<0.001$), 0.83 in the West ($p<0.001$), and 0.78 nationally ($p<0.001$). The strength of the correlations between RSV WVALs and RSV-NET data at the jurisdiction level ranged 0.15–0.84 (Kendall's tau), with all 13 comparisons having a p-value less than 0.05. At the regional level, the strength of the correlations were 0.73 in the Northeast ($p<0.001$), 0.72 in the South ($p<0.001$), 0.73 in the Midwest ($p<0.001$), 0.78 in the West ($p<0.001$), and 0.80 nationally ($p<0.001$).

Categories

Aggregating WVALs to the regional level before applying k-means clustering, Jenks, and quintiles led to the optimal options for determining category cut-points (Table 5). The quintiles method at the

national geographic level led to the optimal alignment between SARS-CoV-2 WVAL activity levels and NSSP categories for COVID-19 when determining activity level cut points. This resulted in the activity levels for SARS-CoV-2 WVALs to be assigned as follows: WVALs up to 2, very low; WVALs greater than 2 and up to 3.4, low; WVALs greater than 3.4 and up to 5.3, moderate; WVALs greater than 5.3 and up to 7.8, high; WVALs greater than 7.8, very high (Table 6).

Table 5. Comparison of methods used to generate possible 2025–2026 category cut points

	SARS-CoV-2			Influenza A			Respiratory Syncytial Virus		
	Jenks	**K-means**	**Quintiles**	**Jenks**	**K-means**	**Quintiles**	**Jenks**	**K-means**	**Quintiles**
Very low	Up to 4	Up to 4	Up to 2	Up to 2.7	Up to 2.7	Up to 1	Up to 2.5	Up to 2.2	Up to 1
Low	>4 and <=8.2	>4 and <=8.2	>2 and <=3.4	>2.7 and <=6.2	>2.7 and <=6.1	>1 and <=1.1	>2.5 and <=5.2	>2.2 and <=4.6	>1 and <=1.1
Moderate	>8.2 and <=19.3	>8.2 and <=19.3	>3.4 and <=5.3	>6.2 and <=11.2	>6.1 and <=11.2	>1.1 and <=2.2	>5.2 and <=8	>4.6 and <=7.6	>1.1 and <=2.7
High	>19.3 and <=42.5	>19.3 and <=42.5	>5.3 and <=7.8	>11.2 and <=17.6	>11.2 and <=17.6	>2.2 and <=6.5	>8 and <=11	>7.6 and <=10.8	>2.7 and <=6.9
Very high	>42.5	>42.5	>7.8	>17.6	>17.6	>6.5	>11	>10.8	>6.9

Candidate cut-points for five WVAL categories were generated using k-means clustering, quintiles, and Jenks natural breaks at three aggregation levels: all national WVALs, WVALs within each region, and the average of region-specific cut-points. Candidate category ranges were compared visually with percent positivity or the proportion of patient visits reported by clinical surveillance systems. The method and aggregation level with the closest visual alignment to clinical surveillance data were selected for each pathogen. WVAL, wastewater viral activity level.

Table 6. Comparison of 2024–2025 and 2025–2026 category cut points

	SARS-CoV-2		Influenza A		Respiratory Syncytial Virus	
	2024-2025 cut points	**2025-2026 cut points**	**2024-2025 cut points**	**2025-2026 cut points**	**2024-2025 cut points**	**2025-2026 cut points**
Very low	Up to 1.5	Up to 2	Up to 1.6	Up to 2.7	Up to 4	Up to 2.5
Low	>1.5 and <=3	>2 and <=3.4	>1.6 and <=4.5	>2.7 and <=6.2	>4 and <=8	>2.5 and <=5.2

Moderate	>3 and <=4.5	>3.4 and <=5.3	>4.5 and <=12.2	>6.2 and <=11.2	>8 and <= 12	>5.2 and <=8
High	>4.5 and <=8	>5.3 and <=7.8	>12.2 and <=20.1	>11.2 and <=17.6	>12 and <=20	>8 and <=11
Very high	>8	>7.8	>20.1	>17.6	>20	>11

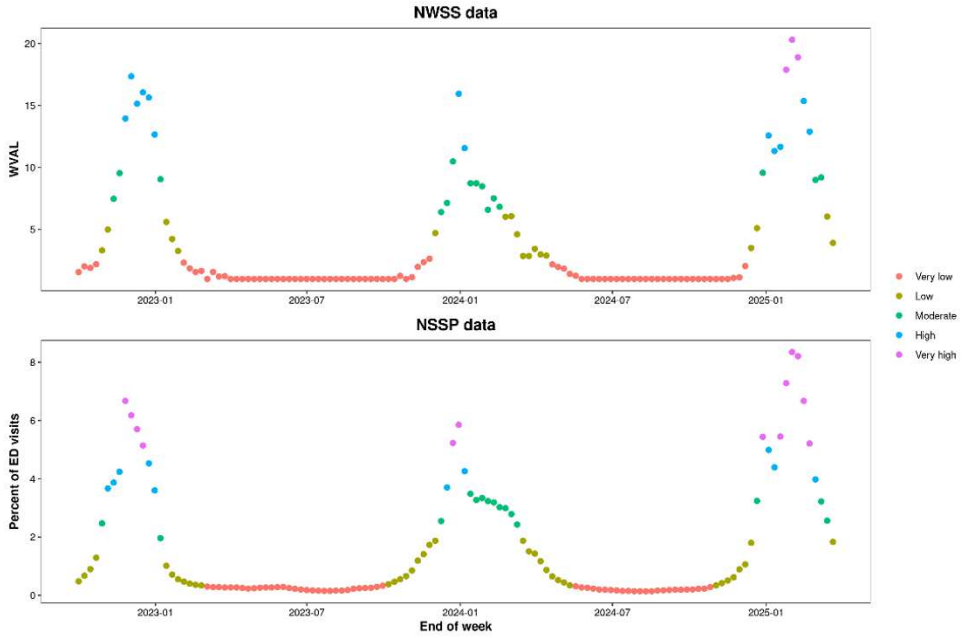
The table compares the 2024–2025 cut-points with the selected 2025–2026 cut-points for five WVAL categories: very low, low, moderate, high, and very high. The 2025–2026 cut-points were selected by comparing candidate category assignments with clinical surveillance data. WVAL, wastewater viral activity level.

The Jenks method at the regional level using all WVALs within each region led to the optimal alignment between influenza A WVAL activity levels and NSSP categories for influenza when determining activity level cut points. This resulted in the activity levels for influenza A WVALs to be assigned as follows: WVALs up to 2.7, very low; WVALs greater than 2.7 and up to 6.2, low; WVALs greater than 6.2 and up to 11.2, moderate; WVALs greater than 11.2 and up to 17.6, high; WVALs greater than 17.6, very high (Table 6).

The Jenks method at the regional level using all WVALs within each region led to the optimal alignment between RSV WVAL activity levels and NSSP categories for RSV when determining activity level cut points. This resulted in the activity levels for RSV WVALs to be assigned as follows: WVALs up to 2.5, very low; WVALs greater than 2.5 and up to 5.2, low; WVALs greater than 5.2 and up to 8, moderate; WVALs greater than 8 and up to 11, high; WVALs greater than 11, very high (Table 6).

The strength of Kendall's Tau between WVALs and NSSP data at the national level ranged from 0.74 ($p<0.0001$) for RSV to 0.81 ($p<0.0001$) for influenza A, with SARS-CoV-2 showing a correlation of 0.78 ($p<0.0001$) (Figures 4-6).

Figure 4. Temporal alignment of national wastewater viral activity levels and percentage of ED visits for influenza A



408

409 *Points show weekly influenza A wastewater viral activity level (WVAL) categories in the National*

410 *Wastewater Surveillance System (NWSS) data and weekly National Syndromic Surveillance Program*

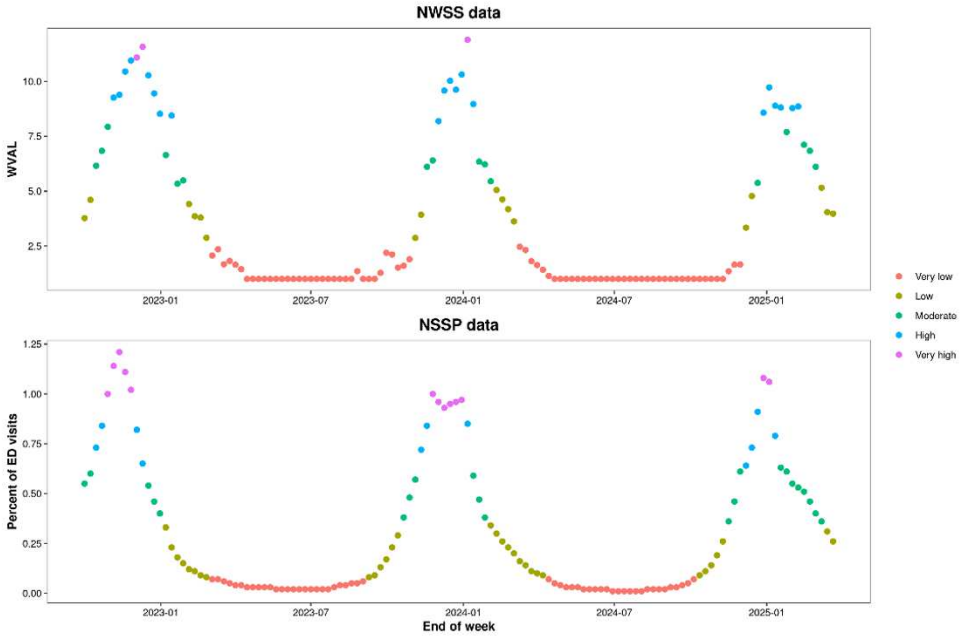
411 *(NSSP) percentage of influenza emergency visits categories. Both systems use five categories: very low,*

412 *low, moderate, high, and very high. Categories were identical in 63.8% of weeks and differed by no more*

413 *than one category in 97.7% of weeks.*

414 **Figure 5. Temporal alignment of national wastewater viral activity levels and percentage of ED visits**

415 **for RSV**



416

417 *Points show weekly RSV wastewater viral activity level (WVAL) categories in the National Wastewater*

418 *Surveillance System (NWSS) data and weekly National Syndromic Surveillance Program (NSSP)*

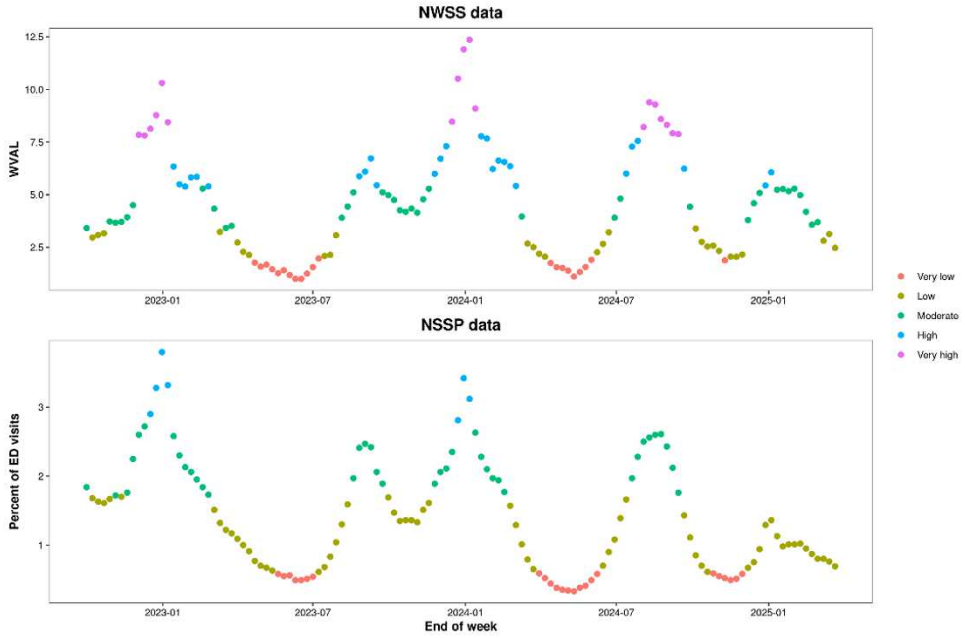
419 *percentage of RSV emergency visits categories. Both systems use five categories: very low, low,*

420 *moderate, high, and very high. Categories were identical in 53.8% of weeks and differed by no more than*

421 *one category (one category above or below) in 91.5% of weeks*

422 **Figure 6. Temporal alignment of national wastewater viral activity levels and percentage of ED visits**

423 **for SARS-CoV-2**



Points show weekly SARS-CoV-2 wastewater viral activity level (WVAL) categories in the National Wastewater Surveillance System (NWSS) data and weekly National Syndromic Surveillance Program (NSSP) percentage of COVID-19 emergency visits categories. Both systems use five categories: very low, low, moderate, high, and very high. Categories were identical in 33.1% of weeks and differed by no more than one category (one category above or below) in 86.9% of weeks

Discussion

In this evaluation of CDC NWSS WVALs, we found that adjustments to components of the WVAL led to moderate to high correlations between WVALs and clinical data, fewer extreme WVALs, and lower WVALs overall for the 2026-2027 respiratory virus season. Components of the WVAL that were adjusted for the U.S 2025-2026 respiratory virus season included using non-normalized data; the amount of time included and frequency of baseline and standard deviation updates; the length of time to stabilization; cut points for assigning activity levels to the WVAL; and methods for aggregating to state, regional and national geographic levels. The WVAL metric was designed to allow for the comparison of viral trends over time, including at higher geographic (state, regional, and national) levels, and to be used in

combination with clinical data to approximate viral levels in communities.(16) Annual re-validation of the WVAL allows for optimization of methods to meet these goals.

Though WVALs are intended to capture viral trends in wastewater, there are some sites where the WVAL was not accurately capturing these trends. One of the largest changes in the re-validation for the 2025–2026 respiratory season was moving from utilizing normalized data to non-normalized data for the WVAL. This was done because of variation in how data used for normalization, flowrate, and microbial data are reported to 1CDP and instances where the normalization method inflated or deflated the WVAL. Variation in data used for normalization came from differences in reporting sources and between sampling sites. The original WVAL calculation method took normalization into account, meaning that if the normalization value, such as flow-rate or microbial indicator level, for a wastewater treatment plant changed, the WVAL would be impacted.(17) Although this produced more variation in concentration data, which was beneficial for the WVAL because it is divided by the standard deviation, this also caused increases in the WVAL when there had been no change in the actual viral RNA concentration but the normalization value increased due to environmental factors such as hurricanes or flooding. Normalized concentration data are still useful and needed when working directly with those data; however for the WVAL metric, we determined that non-normalized data were more appropriate.

In addition to switching to non-normalized data to avoid spurious increases in viral activity, several other changes were made to the WVAL calculation following the re-validation for the 2025–2026 respiratory virus season.(17) These changes included aligning the length of time used for the baseline and the time until a site has a WVAL calculated between all three pathogens, shifting the timing of the baseline re-calculation to the clinical off-season, and updating the regional and national aggregation methods. Regional WVALs were not calculated for influenza A and RSV during the 2024–2025 respiratory season since the original WVAL aggregation method took the median WVAL for all sites in an area. This could skew the regional WVALs to follow that state's trend rather than the region as a whole

due to some states having many more sites. Though there were more sites testing for influenza A and RSV during the validation period, taking the median WVAL for all states within a region gave each state an equal weight in the regional WVAL and was selected as the preferred method. At the national level, the median of the WVALs for all states was selected to give each state an equal weight in the national WVAL. This resulted in WVALs that were lower than those calculated using linear weighting, and sensitive to increases in WVALs from states with fewer sites.(17)

In Canada's adaptation and validation of the WVAL, they found strong correlations between the WVAL and clinical testing results for SARS-CoV-2.(18) While one goal of the WVAL was to align designated wastewater level categorizations with clinical data sources and trends, there are certain instances where the WVAL did not align well. Differences in wastewater and clinical coverage may contribute to misalignment, along with different methods for aggregating to higher geographic scale such as state populations utilized when calculating national ILINet activity levels but not used for national WVAL aggregations. When comparing clinical RSV data and the RSV WVAL, misalignment may occur due to decreased wastewater contributions from diapered populations. RSV disease burden is highest in younger children or older individuals who may be in diapers, meaning they do not contribute as much to wastewater in their area.(19) Additionally, for influenza, the A(H5N1) outbreak among animals in 2024 demonstrated that animal-related inputs can be detected through wastewater surveillance.(20) Future work will try to assess the influence of environmental inputs and their impact on the WVAL, which is intended to estimate trends in human populations. Lastly, the lower category agreement between NSSP and NWSS for SARS-CoV-2 likely reflects changes in disease epidemiology over time. WVAL categories were derived using cut-points that included the pandemic period, when SARS-CoV-2 circulation and associated emergency department visits were higher than in recent years. As clinical presentation SARS-CoV-2 has become less severe, emergency department visits have declined despite continued community transmission(21). This shift may have altered the relationship between

wastewater concentrations and clinical indicators, contributing to the lower concordance between wastewater and emergency department based categories.

Limitations

WVALs have several limitations that should be considered when interpreting results. Data from CDC NWSS are submitted by STLT health departments, a CDC commercial contractor, and an academic partner program, each using distinct laboratory protocols and sampling methodologies, including different LOD methods. These differences may contribute to variability in measured wastewater concentrations across samples. Because LODs are used to impute wastewater concentrations for non-detects, the resulting concentrations may exhibit little or no variation, which can lead to higher-than-anticipated WVALs at sites with few or no detections. Additionally, since WVALs are calculated using relative statistics (e.g., percentiles, standard deviations, means, and medians), time series may be smoothed and may not provide as early a warning of pathogen increases or decreases as concentration-based metrics and WVALs cannot be compared across sites, jurisdictions, or regions with consistently higher or lower disease prevalence. Individuals who live in areas with non-sewered systems (septic) may not be represented in wastewater surveillance data and population mobility, including commuting through or traveling to different areas, may lead to wastewater contributions outside of a single sewershed. For RSV, wastewater surveillance data may not fully align with clinical surveillance data because of reduced wastewater contributions from diapered populations. For influenza, wastewater surveillance cannot distinguish between human and animal contributions within a sewershed. Additionally, a WVAL could not be calculated for influenza B because of the low number of influenza B detections in wastewater. Finally, pathogen prevalence within communities plays an important role in the interpretability of WVALs. Pathogens with low prevalence may yield WVALs that are difficult to interpret due to sporadic detections and unpredictable disease peaks.

Conclusions

The WVAL underwent re-validation prior to the U.S 2025–2026 respiratory virus season to account for changes in data availability and for shifts in clinical seasonality of the pathogens. Changes in how the WVAL was calculated included switching to using non-normalized data, aligning the length of time used for the baseline and time until a site has a WVAL calculated between pathogens, adjusting timing of the baseline re-calculations to be during the off-season, changing the aggregation method at the regional and national levels, and adjusting the category cut points. Future WVAL work will focus on validation of aggregation methods and the development of additional wastewater metrics. Specifically, determining the proportion of the population that should be covered when aggregating the WVAL, development of a metric for low prevalence pathogens since the WVAL is not suitable for pathogens with fewer detections in wastewater, and development of new metrics to effectively monitor wastewater during outbreaks of various emerging or re-emerging pathogens will be investigated.

Equations:

$$C_{norm} = \begin{cases} Ln(\frac{C_s * F}{P}), \text{flow} - \text{population normalization} \\ Ln(\frac{C_s}{C_h}), human\ fecal\ normalization \end{cases} \quad (1)$$

$$Z_o = \frac{C_{norm-\mu}}{\sigma} \quad (2)$$

$$WVAL = \frac{C_{norm-P_{0.10}}}{\sigma} \quad (3)$$

$$W_d = (\frac{1-0.8}{1-0.8^n}) * 0.8^{i-1} \quad (4)$$

$$W_l = \frac{\frac{i}{n(n+1)}}{2} \quad (5)$$

Where:

- C_{norm} is either flow (F)–population (P) normalized or human fecal (C_h) normalized natural log-transformed wastewater sample concentration (C_s).
- Z_o is the z-score for outlier detection.

- μ is the mean natural log-transformed wastewater concentration at the data submitter level.
- σ is the standard deviation of the natural log-transformed wastewater concentration at the data submitter level.
- $P_{0.10}$ is the 10th percentile of the natural log-transformed wastewater concentration at the data submitter level.
- W_d is the hierarchical weight decay.
- W_l is the linear weight.
- n is the number of total jurisdictions.
- i is the rank for that jurisdiction.

Declarations

Ethics approval and consent to participate

This study was reviewed by CDC for human subjects research and was given a "non-research" determination

Consent for publication

Not applicable

Availability of data and materials

State and territory and national WVAL data are publicly available on the CDC NWSS webpages (https://www.cdc.gov/wastewater/). NWSS site-level WVAL and wastewater concentration data, NSSP ED data based on facility location, IIINet data, NREVSS data, RSV-Net data, and COVID-Net data are available on CDC's Open Data Webpages (data.cdc.gov). WVAL R code is available on CDC NWSS GitHub (https://github.com/CDCgov/NWSS).

Funding statements

The authors received no financial support for the research, authorship, and/or publication of this article.

Competing interests

The authors declare no competing financial interest.

Author contributions

Jenna Webb and Megan Gerdes are co-first authors that contributed equally to the conception, methodology, analysis, and writing of the manuscript. Data analysis: Megan Bias, Heather Reese, Dan Cornforth, Igor Volkov, and Dennis Nichols. Wastewater surveillance subject-matter expertise: Megan Bias, Heather Reese, Dan Cornforth, Elisabeth Burnor, Kirsten Weisbeck, Rebecca Schneider, Nathan LaCross, Bradie Ahern, and Dustin Servello. Clinical influenza subject-matter expertise: Alexandra Busbee, Alicia Budd, Alexia Couture, and Matthew Biggerstaff. Clinical RSV and SARS-CoV-2 subject-matter expertise: Erin Anderson and Ben Silk. Lisa Oakley served as senior author.

Disclaimer

The findings and conclusions in this article are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Acknowledgements

We acknowledge the valuable contribution of all jurisdictions, territorial partners, laboratories, health systems, and utilities submitting data to the National Wastewater Surveillance System for making this work possible. Specifically for this study, we want to acknowledge Alabama Department of Public Health; Alaska Division of Public Health; Arizona Department of Health Services; Arkansas Department of Health; California Department of Public Health; Laboratory-Based Surveillance Program, Disease Control & Emergency Preparedness Bureau, Chicago Department of Public Health; Colorado Department of Public Health and Environment and participating wastewater utilities; Connecticut Department of Public Health; Delaware Division of Public Health; Department of Health of the District of Columbia (DC Health), the District of Columbia Water and Sewer Authority (DC Water), and the District of Columbia Department of Forensic Sciences - Public Health Laboratory; Florida Department of Health; Georgia

Department of Public Health; Guam Department of Public Health and Social Services, Program Name: Epidemiology and Laboratory Capacity Program, Wastewater Utility: Guam Waterworks Authority; Hawaii State Department of Health; Houston NWSS Center of Excellence, Houston Health Department, Houston Public Works, and Rice University; Idaho Department of Health and Welfare; Illinois Department of Public Health; Indiana Department of Health; Iowa Department of Public Health and the State Hygienic Laboratory at the University of Iowa. ; Kansas Division of Public Health and Environment; Kentucky Department of Public Health, Kentucky Wastewater Surveillance System (KYWSS); Louisiana Department of Health; Maine Department of Health and Human Services, Lewiston Auburn Clean Water Authority, Presque Isle Water District, Fort Kent Water & Wastewater Department, Houlton Water Company Wastewater Treatment Plant, Caribou WWTP, Brunswick Sewer District, Portland Water District (Westbrook), Portland Water District (East End), Town of Yarmouth Wastewater Department, Town of Falmouth Wastewater Department, Greater Augusta Utility District (GAUD), Rockland WWTF, Boothbay Harbor Sewer District, Town of Bethel Maine Wastewater Treatment Plant, City of Bangor Wastewater Treatment Plant, Guilford Sangerville Sanitary District, City of Bath WPCF, Belfast Wastewater Treatment Facility, York Sewer District, City of Biddeford Wastewater Department, Sanford Sewer District; Maricopa County Department of Public Health; Maryland Department of Health; Massachusetts Department of Public Health; Michigan Department of Health and Human Services; Minnesota Department of Public Health and the Minnesota Department of Health Wastewater Monitoring Program; Mississippi State Department of Health; Missouri Department of Health and Senior Services; Montana Department of Public Health and Human Services; Nebraska Department of Health and Human Services - Likhitha Duggirala, Derry Stover; Nevada Division of Public and Behavioral Health; New Hampshire Department of Health and Human Services; Department of Health for the State of New Jersey; New Mexico Department of Health, Scientific Laboratory and Environmental Health Epidemiology Bureau; New York State Department of Health, Daniel Lang, Haley Kappus Kron, Shazneen

Damani, Paul Dougall, Kristen St. George, Kim Musser, James Chithalen, Sylvia Byun, Samantha Long, Eli Rosenberg; New York City Department of Health and Mental Hygiene and the NYC Department of Environmental Protection; North Carolina Division of Public Health; North Dakota Department of Health and Human Services, Laboratory Services Section, and Disease Control and Forensic Pathology Section; Ohio Department of Health, Ohio Wastewater Monitoring Network (OWMN); Oklahoma State Department of Health; Oregon Health Authority, Public Health Division and Melissa Sutton, MD, MPH, Tyler Radniecki, PhD, Christine Kelly, PhD, Rebecca Falender, MPH, DVM; Pennsylvania Department of Public Health; Philadelphia Department of Public Health; Puerto Rico Public Health Institute; Rhode Island Department of Public Health, Richard Huard, Glen Gallagher, Brady Cunningham, Genevieve Caron, Stephanie Pollen, Gabrielle Kuba, Raul Guiterrez, Hayley Stauber, Stephanie Cromwell; South Carolina Department of Public Health (SCDPH) Wastewater Program; SCDPH CDES: Dr. Abdoulaye Diedhiou, Laureen Mitchell, Nusaiba Mohamed, Jack Roddey, and Katherine O'Shields-Free; and SCDPH PHL: Dr. Jennifer Meredith, Dr. Cory J. Weaver, Christy Greenwood, Samuel Evans, and Megan Woronko.; South Dakota Department of Health; Tennessee Department of Health; Texas Department of State Health Services, Heidi Bojes, Victoria A. Salinas, Layla R. Lustri and Audrey E. Robertson.; Utah Department of Health and Human Services, Program: Utah Wastewater Surveillance System; Vermont Department of Health, Laboratory Sciences and Infectious Diseases; Virginia Department of Health; Washington State Department of Health's Washington Wastewater-Based Epidemiology Program (WAWBE); WV Bureau of Public Health with Marshall U and West Virginia U as partners in Wastewater Testing for Community Health in WV (WaTCH-WV); Wisconsin Wastewater Monitoring Program, Wisconsin Department of Health Services, Wisconsin State Laboratory of Hygiene, University of Wisconsin-Milwaukee; Wyoming Department of Health. Some of these data were collected as part of the WastewaterSCAN / SCAN project, a partnership between Stanford University, Emory University, and Verily funded. philanthropically through a gift to Stanford University.

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