

Variability and Uncertainty in SARS-CoV-2 Wastewater-Based Surveillance Normalization: A  
Systematic Review

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A thesis  
Submitted in partial fulfillment of the  
requirements for the degrees of

Master of Public Health

University of Washington

2025

Committee:

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Program Authorized to Offer Degree:

Department of Environmental and Occupational Health Sciences

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**Abstract**

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Wastewater-based surveillance (WBS) for SARS-COV2 is a valuable tool for monitoring dynamics of community disease burden through detection and quantification of viral RNA shed in feces. Wastewater data can exhibit significant variability, making it difficult to compare results over time and across sewersheds. To address this, studies employ a range of normalization techniques, yet the effectiveness of these methods remain unclear. This study systematically reviewed the application of normalization techniques in SARS-CoV2 WBS to assess their impact on data variability and uncertainty. We found that recovery control threshold values were the most widely applied technique overall, though their use declined between 2022 and 2024, while biomarker adjustments, particularly using the Pepper Mild Mottle Virus (PMMoV), became the predominant method in recent years. Bovine Coronavirus (BCoV) was the most used recovery control method. Clinical case correlation analysis was conducted in the majority of studies. Our analysis found that the most frequently used normalization techniques exhibited variability that is orders of magnitude greater than the variability they aim to control for, thereby increasing uncertainty and not reliably accounting for their targeted variability. Standardizing normalization approaches could improve the reliability of WBS trends, but the lack of clear confidence thresholds limits their interpretability. This study discusses how uncertainty can be communicated to decision-makers and examines how standardization and resource availability impact the effectiveness of WBS in different policy contexts. These results provide insights for future WBS normalization standardization and program implementation by informing program design and highlighting the effectiveness and uncertainty of normalization techniques.

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## List of Terms

5-HIAA	5-Hydroxyindoleacetic Acid
BCoV	Bovine Coronavirus
BRSV	Bovine Respiratory Syncytial Virus
CAF	Caffeine
CRE	Creatinine
CDC	Centers for Disease Control and Prevention
CFU	Colony-Forming Unit
CrAss	Cross-Assembly Phage
<i>E. coli</i>	<i>Escherichia coli</i>
EPA	Environmental Protection Agency
F+	F+ Coliphage
HCoV	Human Coronavirus
HF183	Human-associated Bacteroides genetic marker
Lachno3	Lachnospiraceae bacterium 3
MS2	MS2 bacteriophage
MHV	Murine Hepatitis Virus
NWSS	National Wastewater Surveillance System
OC43	Human Coronavirus OC43
Phi6	Pseudomonas phage Phi6
PMMoV	Pepper Mild Mottle Virus
SARS-CoV2	Severe Acute Respiratory Syndrome Coronavirus 2
STV	Statistical Threshold Value
TKN	Total Kjeldahl Nitrogen
TSS	Total Suspended Solids
VSS	Volatile Suspended Solids
WBS	Wastewater-Based Surveillance

## **Acknowledgements**

Special thanks to Dr. Scott Meschke and Dr. Ann Bostrom for their invaluable guidance and support throughout this project, this work would not have been possible without them. I also sincerely thank the faculty and administration of the Department of Environmental and Occupational Health Sciences and Evans School for their support and for the knowledge and skills that informed this work. Additionally, I am grateful to the public health researchers whose work laid the foundation for this study. Finally, I would like to thank my family for their unwavering encouragement and support throughout this journey.

## 1. Introduction

### 1.1 Background

In the United States, managing the Covid-19 pandemic was complicated by limited access to comprehensive, rapid infection tracking systems.<sup>1</sup> Traditional clinical diagnostic tests, hampered by accessibility issues and the high rate of asymptomatic cases, were proven insufficient for real-time monitoring of transmission.<sup>2,3</sup> Compounding this issue, are the diverse epidemiological manifestations of COVID-19 across demographics like race, age, and pre-existing health conditions, that made it challenging to establish uniform guidelines for when individuals should seek testing.<sup>4</sup> As of January 2025, Covid-19 has infected over 110 million people and caused more than 1.2 million deaths in the United States.<sup>5</sup> Covid-19 causes a range of acute and potentially chronic effects including fever, cough, shortness of breath, fatigue, headache, chest pain, loss of smell, and confusion.<sup>6</sup> The chronic effects of Covid-19, “long covid”, are less understood but chronic fatigue, confusion, loss of taste or smell, Type I diabetes, and breathing difficulties have been reported in up to 14% of those infected.<sup>7,8</sup>

Wastewater based surveillance (WBS) emerged early in the pandemic as a potential tool through which to monitor changes in community levels through the detection of SARS-CoV2 RNA shed in fecal matter.<sup>9</sup> Long employed as an evaluative tool to monitor other health markers including chemical exposures, drug usage, and disease spread, wastewater data can supplement clinical surveillance.<sup>10,11</sup> Applied to the Covid-19 pandemic, WBS demonstrated its potential to offer a cost-effective and scalable approach for monitoring population-level trends of SARS-CoV2.<sup>9</sup> Moreover, as clinical testing rates and reporting of illness declined, WBS provided a surveillance approach that was not influenced by variations in clinical testing. Since 2022, more than 30 states across the US have stopped reporting clinical testing positivity or significantly scaled down their clinical testing reports.<sup>12</sup> There remain several challenges that need to be overcome to effectively utilize WBS, particularly in generating reliable and easily interpretable data. Variation in WBS data can arise from numerous sources, including differences in processing techniques, varying contributing populations, wastewater dilution, varying fecal shedding rates, and wastewater composition, all of which can complicate direct temporal and inter-site comparisons of samples.<sup>13,14</sup> Raw data alone may not provide consistent or comparable SARS-CoV2 concentrations, and researchers have sought normalization techniques to adjust for variability in the wastewater matrix. Common normalization techniques use markers of process

recovery, fecal content, population, and environmental conditions to standardize measurements of SARS-CoV2 concentrations.

Numerous laboratory workflows and testing methods have been employed to quantify SARS-CoV2 in wastewater. Briefly, these workflows generally include sampling, sample preparation, sample concentration, RNA extraction, and quantification. Sampling techniques typically include grab sampling or composite autosampling, with proper storage and homogenization being essential first steps to ensure sample integrity and accuracy. Concentration methods vary depending on the sample fraction selected for analysis, but may include ultrafiltration, electronegative filtration, polyethylene glycol (PEG) precipitation, ultracentrifugation, skim milk flocculation, or other techniques. RNA is extracted and purified to isolate SARS-CoV2, followed by quantification using RT-qPCR (reverse transcription-quantitative polymerase chain reaction) or RT-ddPCR (RT-droplet digital PCR), typically targeting the N1, N2, or E genes. In late 2021, the CDC issued general guidance on laboratory practices for wastewater surveillance, though a standardized method was not defined despite the well-established application of several methods.<sup>15</sup>

Wastewater is a variable sample matrix potentially containing several strains of SARS-CoV2 dispersed throughout the liquid and solid fractions. It is generally understood that the viral lipid envelope causes a greater portion of the virus to partition to the solid fraction, but studies have shown that viral RNA is present in the liquid fraction, even after concentration procedures. Studies suggest that adhesion-aggregation of SARS-CoV2 to the insoluble fraction can result in RNA concentrations within the solid fraction being orders of magnitude higher than in the liquid fraction.<sup>16-18</sup> Traditionally, RNA extraction has focused on the liquid fraction after removing large particles, though a significant portion of RNA may be in the removed fraction.<sup>16,19</sup> Recently, there has been a shift toward analyzing the solid fraction or sludge, as these may contain significantly higher concentrations of SARS-CoV2 RNA.<sup>20,21</sup> Although the partitioning dynamics of SARS-CoV2 RNA between these fractions are not fully understood and may vary by site-specific factors, it is clear that both fractions contain measurable RNA, necessitating tailored approaches to sample processing and quantification.

A common normalization technique is the use of a matrix recovery control to account for technical variability, specifically addressing concentration, extraction, and quantification processes that can create differences in viral recovery between samples. Commonly used matrix

recovery viruses are often biologically similar to SARS-CoV2, such as BCoV, OC43, Phi6, MHV, BRSV, HCoV, and inactivated SARS-CoV2. The CDC currently recommends including a recovery control when wastewater conditions or laboratory methods change, but does not provide a recommendation on normalization technique, methodology, or indicator choice.<sup>15</sup> While there is no standardized method, recovery controls (exogenous markers that are added to samples prior to concentration or extraction) are often seeded to qualitatively or quantitatively assess recovery. This contrasts with endogenous controls, which are naturally present in wastewater samples, such as fecal indicators or human genetic markers. For qualitative recovery assessment, researchers often apply the control in a binary fashion, requiring samples to meet an established recovery efficiency to be considered valid. Biologically similar viruses to SARS-CoV2 have been found to be reliable qualitative process controls.<sup>22</sup> For quantitative recovery assessment, per method or per sample recovery efficiency have been calculated and in some cases used to adjust the measured SARS-CoV2 concentrations. Endogenous fecal viruses such as PMMoV or F+ have also been used regularly as process controls despite having substantial morphological and biological differences. These viruses may result in inaccurate recovery estimates of SARS-CoV2, but may serve as qualitative markers of successful recovery, though they do not replace the effectiveness of coronavirus recovery controls as method validation.<sup>15,22</sup>

Using different extraction methods for the recovery control virus and SARS-CoV2 can introduce bias, resulting in efficiency discrepancies, and potentially recovery efficiencies exceeding 100%.<sup>23</sup> These discrepancies may also arise due to distributional issues and measurement uncertainties, such as with aggregates and imprecision in PCR methods. Substantial differences in recovery efficiencies have been observed in wastewaters, with acceptable recoveries reported as low as 0.1%, with reasons for such variability not well understood.<sup>24</sup> Additionally, though proxy viruses may be biologically similar to SARS-CoV2, they may not accurately reflect how SARS-CoV2 is affected by biological and physicochemical processes during concentration, extraction, and quantification, as different proxies can show varying recovery efficiencies in the same samples.<sup>25-27</sup> Consequently, the recovery efficiency measured with a proxy virus, varying by proxy, concentration method, and sample, and may differ from the actual recovery efficiency for SARS-CoV2.<sup>28</sup> Using inactivated SARS-CoV2 reduces these discrepancies, ensuring recovery data is more relevant to the target virus. Recovery controls have been used to verify the consistency of a method's performance across samples and

over time, monitoring changes in concentration and extraction efficiency.<sup>25,26,29–33</sup> However, uncertainty in SARS-CoV2 measurements can be introduced or amplified if the measured SARS-CoV2 concentration is adjusted by the recovery efficiency. Results can also be misleading if there are differences in recovery efficiencies between samples and methods, amplifying and potentially introducing errors in the final data.

Another common normalization technique is the use of human-associated biomarkers to account for sample variability. During wastewater sampling there are often changes in wastewater dilution, contributing population, and relative fecal content in wastewater that can result in variability between samples. Normalizing by the amount of human fecal content in a sample or population biomarkers that can indicate levels of human activity is thought to be a way to interpret SARS-CoV2 concentrations and compare concentrations across time and sites.

Human fecal normalization controls are organisms or biomarkers specific to human feces that can be measured in wastewater to quantify the fecal content.<sup>15</sup> Normalization by human fecal markers intends to account for population variability through the measurement of fecal content, as the concentration of fecal content can correlate with the number of individuals contributing to the wastestream in a sewershed. Common fecal markers used for normalization include PMMoV, CrAss, F+, and HF183. While there is no standardized method, it is common for researchers to divide unnormalized SARS-CoV2 concentrations by the measured concentration of the fecal marker.<sup>34,35</sup> <sup>36</sup> The CDC encourages NWSS partners to use a fecal marker if environmental normalization is not possible, though they do not specify a specific indicator.<sup>37</sup>

There are also several biomarkers that are excreted by humans and can correlate with the number of individuals contributing to sewersheds. Such biomarkers often include metabolites such as CAF, CRE, and 5-HIAA. Such metabolites can be excreted in both feces and urine, as well as introduced through various human activities and thus can correlate with human activity in a given area.<sup>36</sup> Normalizing SARS-CoV2 concentrations by metabolites adjusts for changes in local population. Studies have found chemical metabolites to be reliable and stable population indicators in wastewater, with improved normalization correlations with variable populations than fecal controls.<sup>38–40</sup> 5-HIAA and CAF are most often found in agreement with hydrochemical properties, while metabolites including CRE are found to be more unstable and variable.<sup>41</sup>

However, using human-associated biomarker normalization can introduce uncertainty as it relies upon the assumption that the chosen biomarker will correlate with fecal load and contributing population, and that the marker fate and measurement uncertainty will be correlated with attenuation of the fecal load. Variability in individual fecal shedding, heterogeneous marker distribution in wastewater, differences in viral decay rates, and differing responses to pretreatment and environmental conditions can result in inaccurate adjustments, potentially skewing data.<sup>19,42,43</sup> Additionally, seasonal trends along with sewershed characteristics can result in inconsistent fecal marker concentrations that can decrease comparability within and across sewersheds.<sup>44,45</sup> Similar temporal and site-specific trends have been found with metabolites.<sup>46</sup> Studies using markers including PMMoV and CrAss have shown inconsistent and site-specific results.<sup>47-49</sup> These issues, combined with cross-laboratory discrepancies in quantifying fecal markers, can limit the comparability of normalized results across studies and over time, requiring careful application to avoid introducing further data variability.

Environmental normalization is another common normalization technique that adjusts viral concentrations using environmental factors including wastewater flow, population estimates, conductivity, pH, and temperature to account for variability in sample conditions. By adjusting for these variables, environmental normalization seeks to account for population and dilution variability. Flow normalization, a common approach used in wastewater surveillance of drug use and other viruses including polio, norovirus, Hepatitis A, enterovirus, and Influenza A, can be effective if the sample accurately represents 24 hours of shedding.<sup>50-57</sup> However, if the sample approach does not accurately reflect 24 hours of shedding, due to fluctuating population or inconsistent flow, systematic error can be introduced. Other environmental parameters, such as population estimates, conductivity, or pH, are used to account for similar wastewater sample variability and have shown correlation with population and dilution factors, but they are less frequently applied.<sup>58</sup> Other types of environmental factors employed include TSS, VSS, and TKN which can help adjust for dilution and variation in wastewater quality. Variability in these factors, as well as inconsistencies in how they correlate with fecal load or wastewater strength, can contribute to heightened uncertainty in data interpretation. Furthermore, inaccuracies in the measurement of metadata can lead to misinterpretations and potentially introduce systematic errors.<sup>56</sup>

The CDC started offering informal guidance on SARS-CoV2 wastewater surveillance normalization in 2020 and released its first formal guidance in 2021, focusing on recovery controls, fecal normalization, and wastewater flow.<sup>15,37</sup> The CDC recommends normalizing samples by daily wastewater flow to compare samples over time, and normalizing by sewershed population estimates or fecal biomarkers, such as PMMoV, CrAss, HF183, and Lachno3, to compare samples from across sampling locations.<sup>15,37</sup> As of March, 2025, when both flow-population and fecal normalization values are available, the CDC prioritizes flow-population normalization for their own reporting.<sup>59</sup> Currently, there is no universally accepted or applied normalization technique.

Normalization validation for SARS-CoV2 wastewater surveillance is complicated as there is no “gold standard” against which data and techniques can be measured. In the absence of such a standard, researchers often rely on comparisons with clinical case data to assess the accuracy of normalization methods. However, as public testing and reporting have declined significantly, clinical data no longer provide a consistent or comprehensive reference. This reliance on incomplete clinical metrics introduces additional uncertainty, as wastewater concentrations may not align with reported case counts and be inaccurately normalized to better fit such trends. Without a reliable benchmark, the evaluation of normalization techniques remains largely speculative, underscoring the need for alternative validation frameworks that could better support comparability and accuracy across studies.

There is currently no standardized data reporting of WBS of SARS-CoV2. Despite no formal guidance, researchers and organizations have provided evaluations and recommendations on best practices to facilitate meaningful interpretation and comparison within and across datasets. Many researchers recommend reporting un-normalized data along with normalized data, if conducted.<sup>28,60</sup> Ambiguities in normalization techniques and methods reduce the utility of reporting only adjusted data. Additionally, by reporting un-normalized data, data can be more dynamic to changes in analysis strategies and thus facilitate interpretation and comparison.<sup>60</sup>

This review documents the current application of normalization techniques for WBS of SARS-CoV2. The primary objectives of this study are:

- (i) To conduct a systematic review of the different normalization techniques used in WBS,
- (ii) To evaluate the dynamics between normalization techniques, variability, and uncertainty,
- (iii) To give insight into the application of normalization techniques for WBS policy.

## 2. Methods

### 2.1 Systematic Review

An electronic search for eligible literature was conducted from 23 July 2024 to 1 August 2024 following PRISMA guidelines.<sup>61</sup> ER searched six health, medicine, environment, and science databases, names and date coverage are provided in Table 1. Backwards citation searching was conducted by searching the reference lists of studies included in full text screenings. Forward citation searching was conducted in October 2024 to identify studies citing eligible studies and access them for inclusion. Routine monitoring of new studies was also conducted through December 2024. The search strategies employed for this review were informed by previous reviews with additional terms identified through several strategies including literature review, database thesauri, and utilizing the PRISMA- search guidelines. Further details of the electronic search strategies are given in the supplementary appendices (Table S1).

Population: We included studies of wastewater surveillance systems that measured SARS-CoV2. We applied no restriction on the size of the sewershed included.

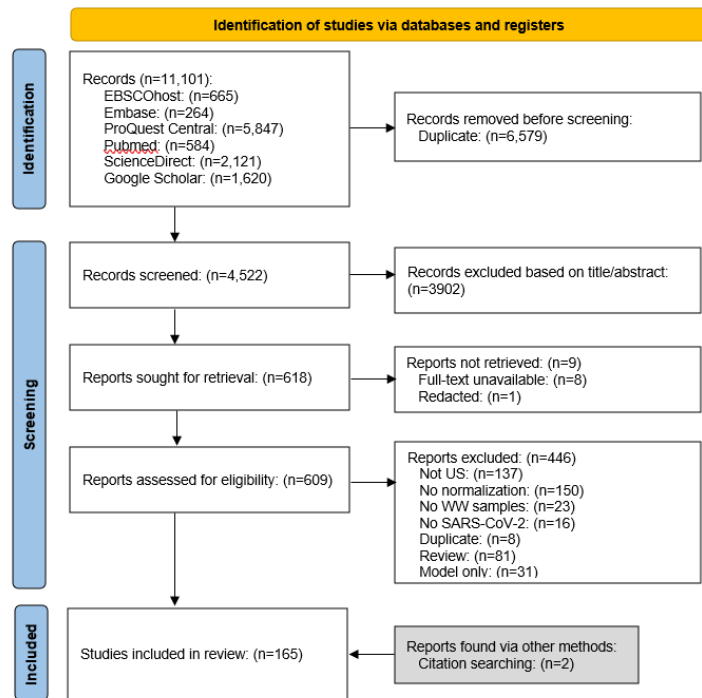
Database	Coverage
EBSCOhost	
Education Resources Information Center	1966 to present
Medline	1946 to present
Academic Search Complete	1887 to present
GeoRef	1785 to present
GeoRef In Process	1933 to present
Global Health	1910 to present
Primary Search	1990 to present
Embase	1947 to present
Google Scholar	1950 to present
ProQuest Central	
Coronavirus Research Database	2020 to present
Dissertations & Theses Global Database	1861 to present
Natural Science Collection	1946 to present
Publicly Available Content Database	2000 to present
TOXLINE	1965 to present
Medline	1946 to present
Pubmed	1966 to present
ScienceDirect	1823 to present

**Table 1.** Databases and the platform through which databases were searched and the dates of coverage.

Intervention: We considered all normalization methods used to adjust for variations in wastewater sampling, concentration methods, population, or environmental factors that could affect SARS-CoV2 viral load measurements. We excluded techniques that focused on sample processing and handling procedures rather than on data adjustments such as type of concentration method, sample type, or storage conditions. Studies that conducted no normalization were excluded.

Study type: To be included, studies had to contain primary data, including experimental designs and novel normalization techniques. Review articles, meta-analyses, and theoretical papers were excluded. Only studies conducted in the United States were included, though we applied no language restrictions. No restriction was placed on publication dates.

Studies were assessed for eligibility according to the inclusion criteria, with studies screened in several stages. All citations were imported to the reference manager Zotero, and duplicates (n=6,579) were identified and removed with the integrated deduplication tool. One reviewer independently reviewed titles and abstracts (n=4,522). Uncertainties about studies fulfilling eligibility criteria were discussed with an additional reviewer until consensus was reached. The same protocol was subsequently followed to determine full text eligibility (n=609). Studies excluded after full text assessment as well as reason for exclusion are given in the supplementary appendices. Additional records were identified from backwards and forward citation searching (n=2), a total of 165 records were included in this systematic literature review. Figure 1 summarizes the results of this systematic search as a PRISMA flow diagram.



**Figure 1.** PRISMA flow diagram of the search and selection process.

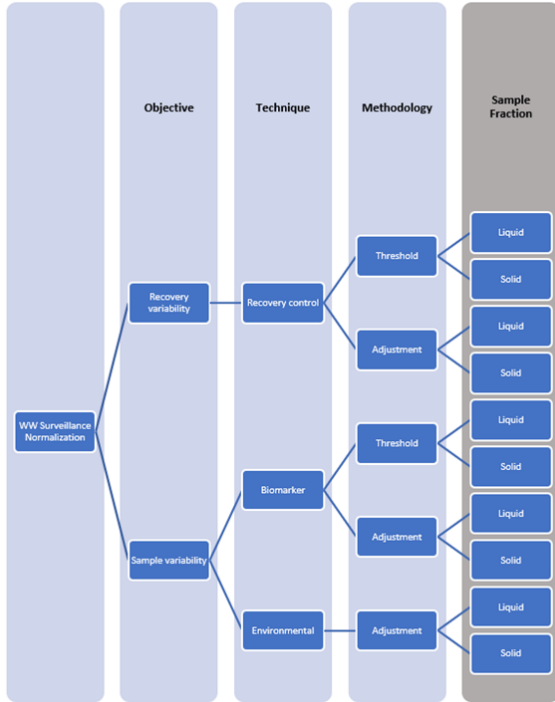
Data were extracted from the studies to inform understanding of the normalization techniques utilized and their application. One reviewer extracted study characteristics including population, location, date of study, methods used, normalization protocol and normalization objectives using a standard data extraction format. Any discrepancies were discussed with another reviewer and resolved with discussion. Normalization protocols were broadly categorized as follows: recovery control, human-associated biomarkers, and environmental normalization. Where multiple protocols were reported, all protocols were included for analyses and for reporting.

## 2.2 Normalization techniques and methods

Given the multitude of normalization techniques being investigated, we categorized the included studies along two dimensions: (1) what technique was used: recovery control, human biomarker, or environmental normalization and (2) how was the technique used to adjust data. Figure. 2 summarizes the dimensions along which records were categorized. We categorized included studies using the above dimensions into the following categories:

1. Recovery control threshold values,
2. Recovery control adjustment,

3. Human biomarker threshold value,
4. Human biomarker adjustment,
5. Environmental normalization adjustment.



**Figure 2.** Normalization dimensional categorization for SARS-CoV2 wastewater surveillance.

Viral or metabolite indicators that were measured by molecular assays and originate from biological processes were included as a human biomarker technique. Metadata measurements of wastewater or sewershed conditions such as flow, population estimations, wastewater compounds, or measure of wastewater solids were included as environmental normalization. Some of the studies employed multiple normalization techniques (e.g. marker and metadata normalization). These studies were categorized into each category for which they met the criteria. Threshold values were categorized separately as they are comprised of recovery controls utilized to validate PCR and extraction processes that are not typically considered normalization. As some studies have utilized such techniques to adjust reported values, all recovery controls were included in this study to understand the landscape of their usage.

We used authors' stated objectives in their methodology, when included, to classify techniques. Stated objectives included specifying the type of normalization used or the specific variability being addressed. When objectives were not explicitly included, methodology was used to classify techniques based on the similarity of the protocol to other studies e.g.

normalizing by PMMoV adjustment was assumed to be a fecal normalization unless otherwise stated. Studies that did not include methodology but included objectives of applied techniques such as Lancaster, et. al. 2022, Klevens, et.al. 2023, Fielding-Miller, et.al. 2023, and Anneser, et. al. 2022, were categorized and used in analysis of all eligible studies, but were not included in further analysis. The sample fraction used for analysis and quantification was collected for each included record but was not used in further categorization. Protocols were determined to use the liquid fraction of samples if they used the supernatant from centrifugation, the supernatant from settling, the filtrate from filtration, PEG precipitation, or if magnetic affinity separation particles such as nanotrap were added to the liquid sample. Protocols were determined to use the solid fraction of samples if they used the pellet from centrifugation, the solids suspended on the filter from filtration, the collected solids from settling, or direct extraction from solids or sludge.

### **2.3 Variability Analysis**

We assessed the included studies to understand how their chosen normalization techniques affect variation and uncertainty in the data. Techniques were separated by objectives; recovery control aims to account for technical variability while biomarker and environmental normalization aim to account for sample variability. This evaluation focused on two key aspects: (1) the effectiveness of the technique in addressing the variability it aims to control, and (2) the potential for the technique to introduce uncertainty into the data. To determine effectiveness at addressing target variability, the order of magnitude of variability of normalization techniques was compared to their targeted variability. For normalization techniques targeting technical variability, effectiveness was determined by evaluating applicability to replicate SARS-CoV2 physiochemical interactions. For sample variation, it was assessed that for most sewersheds, population variability is within one order of magnitude whereas dilution variability spans 1–3 orders of magnitude. For introduced uncertainty, it was evaluated the degree to which the normalization technique would introduce error into results. An overview of each technique is presented in Table 1 and Table 2. Each technique and method were rated on a scale from 1 to 5, with 1 indicating the lowest score and 5 representing the highest score for each evaluated dimension. Composite scores were calculated for each study by multiplying each dimension score by its respective weight, summing the results. For studies utilizing multiple techniques, we averaged the composite scores to determine an overall score. This approach allowed for a

systematic comparison of studies based on the robustness and reliability of their normalization methods.

Technique	Method	What is the likelihood that the technique will effectively account for the variability its targeting?	What is the likelihood that this technique will minimize introducing uncertainty into the data?
Recovery control threshold value	Coronaviruses: BCoV, OC43, BRSV, HCoV, MHV	High-Medium (4): Coronaviruses are biologically similar to SARS-CoV2, making them effective recovery controls for accounting for variability introduced during laboratory processes through the identification of outliers. However, differences in how proxy viruses and SARS-CoV2 interact with environmental and physicochemical processes can reduce accuracy. <sup>25,29-33,62</sup>	High (5): Final SARS-CoV2 concentrations are not adjusted and thus new error is unlikely to be introduced. <sup>28</sup>
	Fecal controls: e.g. PMMoV, CrAss, F+  Other viral controls: MS2, Phi6, TV, PURO	Medium-Low (2): Recovery controls aim to account for technical variability. Fecal markers have substantial biological differences from SARS-CoV2 limit their ability to accurately reflect viral losses specific to coronaviruses. They are better suited for general recovery assessment rather than SARS-CoV2-specific variability. <sup>15</sup>	High-medium (4): Fecal controls introduce additional variability due to their dissimilar recovery efficiencies and interactions with laboratory processes compared to SARS-CoV2, but using as a qualitative control can help identify outliers without adjusting data.
Recovery control adjustment	Coronaviruses: BCoV, PMMoV, OC43, BRSV, HCoV, MHV	High-Medium (4): Coronaviruses are biologically similar to SARS-CoV2, making them effective recovery controls for accounting for variability introduced during laboratory processes through the identification of outliers. However, differences in how proxy viruses and SARS-CoV2 interact with environmental and physicochemical processes can reduce accuracy. <sup>25,28,29-33,62</sup>	Low (1): Errors can be introduced and amplified by adjusting concentration due to proxy virus not reflecting SARS-CoV2 conditions. <sup>24,28,63,64</sup>

**Table 2.** Variability assessment of recovery control techniques. The color coding represents the effectiveness of a technique in addressing variability and uncertainty, with dark red indicating low effectiveness, light red for medium-low, yellow for medium, light green for medium-high, and dark green for high effectiveness.

Technique	Method	What is the likelihood that the technique will effectively account for the variability its targeting?	What is the likelihood that this technique will minimize the magnitude of variability introduced into the data?
Human-associated biomarker threshold value	Fecal markers: PMMoV, CrAss, F+	Medium-Low (2): Fecal markers aim to address variability in wastewater dilution and population size by correlating SARS-CoV2 concentrations with fecal content. However, inconsistencies such as uneven marker distribution, variable fecal shedding rates, and site-specific trends reduce their correlation with population. <sup>19,42-44,47,48,55</sup> Fecal markers exhibit variance within 1-3 orders of magnitude. <sup>65,65-67</sup>	High (5): Threshold values are used only for sample screening and do not adjust SARS-CoV2 concentrations, they are unlikely to introduce additional uncertainty into data.

Human-associated biomarker adjustment	Fecal markers: PMMoV, CrAss, F+, HF183	Medium-Low (2): These markers are used as proxies for fecal content. However, inconsistencies such as uneven marker distribution, variable fecal shedding rates, and site-specific trends reduce their correlation with population. <sup>19,42-44,47,48,55</sup> Fecal markers exhibit variance within 1-3 orders of magnitude. <sup>65,65-67</sup>	Low (1): Errors can be introduced and amplified by adjusting SARS-CoV2 concentrations to biomarker with different variability than population changes. <sup>19,42-45,47-49,55</sup>
	Metabolites: CRE, CAF, 5-HIAA, XAN, PARA	Low (1): These markers are used as proxies for fecal or urine content. However, excretion rates vary based on individual behavior, seasonal changes, and geographic region. Robust data on metabolite variability is limited, but studies suggest that CAF exhibits variance on the order of 1-4 while 5-HIAA, CRE, and PARA show variance on the order of 1-3. <sup>39, 46,68-71</sup> They are not reliable indicators of fecal content.	Low (1): Errors and uncertainty can be introduced and amplified by adjusting SARS-CoV2 concentration to metabolites that are unreliable indicators of fecal content.
Environmental adjustment	Flow, population estimate, flow-population estimate	High- Medium (4): Flow normalization is well-established for addressing dilution variability, as wastewater flow typically varies within 1 order of magnitude. <sup>44,72-75</sup> Population estimates, when derived from stable sewersheds, also generally vary within 1 order of magnitude. However, in small or highly transient catchments, population estimates can fluctuate by as much as 7 orders of magnitude, making them unreliable for normalization in those settings. <sup>55,57,71,76,77</sup>	Medium (3): Uncertainty can be introduced when SARS-CoV2 concentrations are adjusted when flow or population estimates do not reliably reflect contributing variability. For flow, single timepoint measurements or averages may misrepresent actual wastewater inputs. Population estimates are unreliable in certain use cases and fail to capture real-time fluctuations.
	TSS, VSS, BOD, TKN, NH3	Medium-Low (2): These parameters are used as proxies for fecal content. Studies suggest TSS, NH3, BOD, VSS and conductivity exhibit variance on the order of 1-2 while pH and temperature show variance within 1 order of magnitude. <sup>44,71,72,78-80</sup>	Medium-Low (2): Variability can be introduced due to measurement inaccuracies or external factors unrelated to viral concentrations, potentially leading to skewed adjustments. Additionally, inconsistencies in sampling or analytical techniques can amplify data variability.
	PO4, conductivity, pH, temperature	Low (1): These parameters are used as proxies for non-population variability. PO4 exhibits variance on the order of 1-2. <sup>78</sup> Conductivity, pH, and temperature exhibit variance within 1 order. <sup>78-80</sup> They are not reliable indicators of fecal content.	Low (1): Errors and uncertainty can be introduced and amplified by adjusting SARS-CoV2 concentrations to parameters that are not reliable indicators of sample variability sources and that are highly sensitive to external factors.

**Table 3.** Variability assessment of biomarker and environmental normalization techniques. The color coding represents the effectiveness of a technique in addressing variability and uncertainty, with dark red indicating low effectiveness, light red for medium-low, yellow for medium, light green for medium-high, and dark green for high effectiveness.

### 3. Results

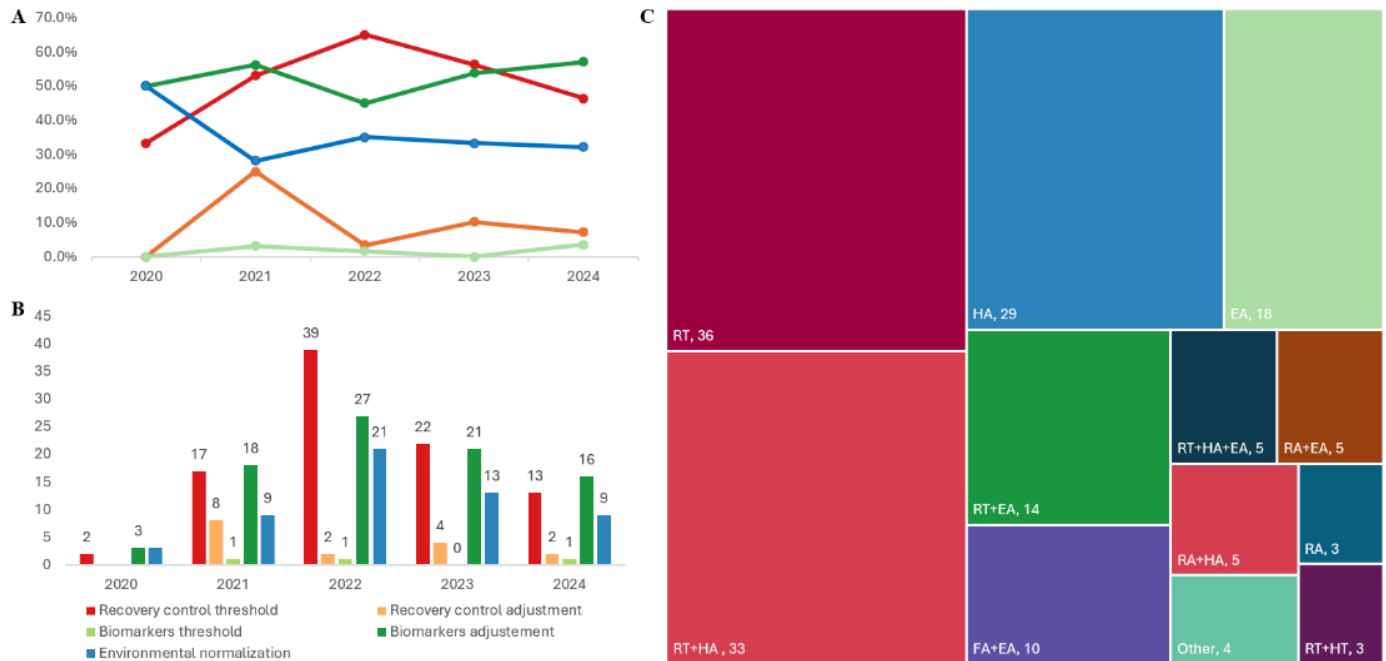
#### 3.1 Frequency Analysis

We found 11,101 records in database searching. After duplicate removal, we screened 4,522 records from which we reviewed 609 full-text documents and included 165 records (Table S2 and S3). Subsequently, we searched documents that cited any of the initially included studies

as well as the references of the initially included studies. Three additional studies were identified through this method.

We excluded 31 studies from our review that met all eligibility criteria but only utilized normalization in predictive modeling. The focus of this review is on normalization of wastewater samples and not intended to evaluate statistical modeling processes. Studies were not eligible for inclusion if they only conducted inhibition control techniques. Inhibition control typically is utilized to detect the presence of inhibitory substances in the laboratory process while normalization as it is used in this review is to adjust measurements to account for variability. There is considerable procedural overlap between inhibition control and recovery control in studies, particularly when using the bacteriophage Phi6. To determine eligibility, we relied on the authors' specified purpose for using the bacteriophage, whether as a recovery control or an inhibition control.

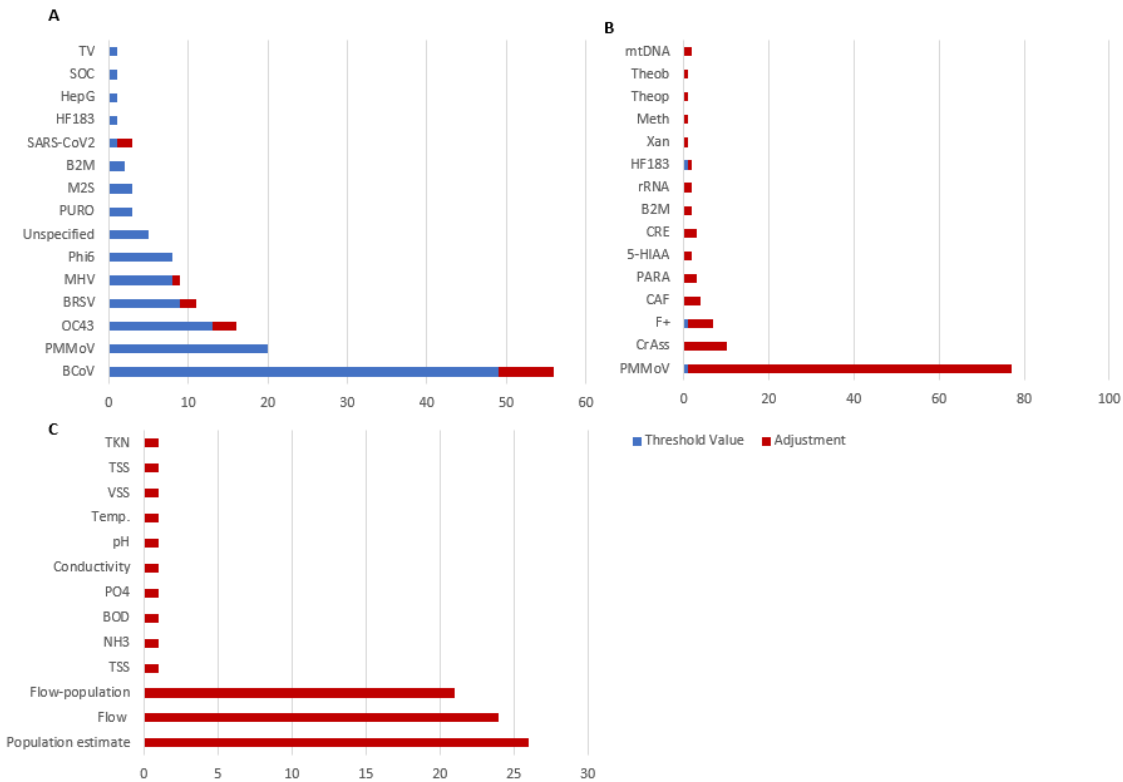
Recovery control was used in 66.06% (n=109/165) of all studies reviewed, with 56.36% (n=93/165) of all eligible studies applying a threshold technique, 9.70% (n=16/165) adjusting values, and 3.05% (n=5/165) not specifying methodology. Human-associated biomarker normalization was used in 53.33% (n=88/165) of all studies, predominantly through adjustment methods, 51.83% of total studies (n=85/165), with threshold methods appearing in only 1.83% (n=3/165). Environmental normalization was used in 33.33% (n=55/165) of studies and was solely utilized as an adjustment method, with no threshold approaches applied (Figure 3B). Biomarker adjustment and recovery control threshold were the predominant techniques employed each year. Recovery control threshold usage peaked in 2022 with 65% (n=39/86) of eligible studies from that year utilized the technique. Biomarker adjustment peaked in 2024 with 57.1% (n=16/41) of eligible studies from that year utilized the technique (Figure 3A).



**Figure 3.** A) Annual percentage of papers employing each normalization technique. B) Annual distribution of normalization techniques. C) Proportion of normalization schemes in included studies. RT, recovery threshold; RA, recovery adjustment; BT, human biomarker threshold; BA, human biomarker adjustment; EA, environmental normalization adjustment.

Most studies utilized multiple normalization techniques methods, with 38.27% (n=62/165) using only a single technique. 14 normalization schemes, combinations of techniques, were used; recovery threshold (n=36/165), recovery threshold plus fecal adjustment (n=33/165), and fecal adjustment (n=30/165) being the most common methodologies (Figure 3C). The highest number of methods used in a single study was 13, with a median of 2.11.

The most used normalization method was adjustment by PMMoV, applied in 43.3% (n=71/165) of studies. This was followed by threshold values using BCoV (29.9%, n=49/165) and adjustments using population estimates (16.5%, n=27/165) (Figure 4).



**Figure 4.** Frequency of normalization methods and methodologies for A) Recovery control techniques, B) Human-associated biomarkers, and C) Environmental normalization.

34.2%(n=56/165) of studies reported only their raw, un-normalized SARS-CoV2 data, 32.9% (n=54/165) of studies reported their un-normalized and normalized data, 27.4% (n=45/165) of studies reported their normalized data only, and 5.5% (n=9/165) reported their statistical data only. For studies that only reported their statistical data, the correlation coefficients of SARS-CoV2 concentrations with clinical cases was reported.

The liquid fraction of wastewater samples was the most utilized (44.24%, n=73/165), followed closely by the solid fraction (38.18%, n=63/165). A total of 12.1% (n=20/165) of studies utilized both the liquid and solid fractions, while 2.42% (n=4/165) alternated between fractions during sampling. The liquid sample was the most abundantly used sample fraction each year other than in 2023.

### 3.2 Use of Clinical Cases

A majority of studies, 75% (n=123/165), conducted correlation analysis of wastewater concentrations, un-normalized or normalized, with clinical Covid-19 cases. There was an increasing trend of reports using clinical case correlations, with a steady year over year increase in usage with over three quarters of studies using such correlation in 2024 (82.1%, n=23/41) compared to only 33.3% usage in 2020 (n=2/5) and 68.8% 2021 (n=22/54) (Figure S2).

### **3.3 Variability Analysis**

The variability analysis was conducted using the identified scoring system from 1 to 5, where a higher score represents lower variability and uncertainty concerns, and a lower score indicates greater variability and uncertainty concerns. The average combined score was 2.88 (n=162), with the most frequent scores being 3 (n=53/162) and 1.5 (n=26/162) indicating a wide range of variability and uncertainty concerns. Separating out variability analyzes of recovery and sample variability techniques, we find higher associated variability concerns with sample variability techniques (mean=2.13, n=121) than for recovery variability techniques (mean=3.85, n=100) (Table S4).

## **4. Discussion**

### **4.1 Trends in Normalization Practices**

This analysis outlines the evolving landscape of normalization practices in WBS, and the increasing reliance on biomarker adjustments and clinical case correlations within studies. There is a noted increase in biomarker adjustment usage following the publication of CDC guidance in late 2021 that included guidelines for using such biomarkers. Recovery control thresholds were the most applied normalization method overall, followed by biomarker adjustment. Recovery control thresholds usage peaked in 2022, then declined through 2024. Inversely, biomarker adjustment was at its lowest usage rate in 2022, with increased usage through 2024, surpassing recovery control thresholds as the most frequently used technique. The trends indicate a growing emphasis on addressing sample variability, which emerged as the most significant source of introduced variability in the data, with lower scores indicating heightened concerns (mean=2.03) compared to recovery variability techniques (mean=3.71). The increasing reliance on clinical case correlations, utilized in 75% of all eligible studies, also highlights a potential risk of amplifying introduced variability in data interpretation, particularly as these correlations often

hinge on error-prone normalization techniques. The steady rise in studies reporting clinical correlations, from 33% in 2020 to 82% in 2024, further reinforces the need for a more comprehensive understanding of the applicability of normalization methods.

The selection of sample fractions for analysis has remained relatively consistent over time. The liquid fraction has been predominantly utilized each year except for 2023, with minimal variation observed in the proportions of liquid, solid, and combined fraction usage.

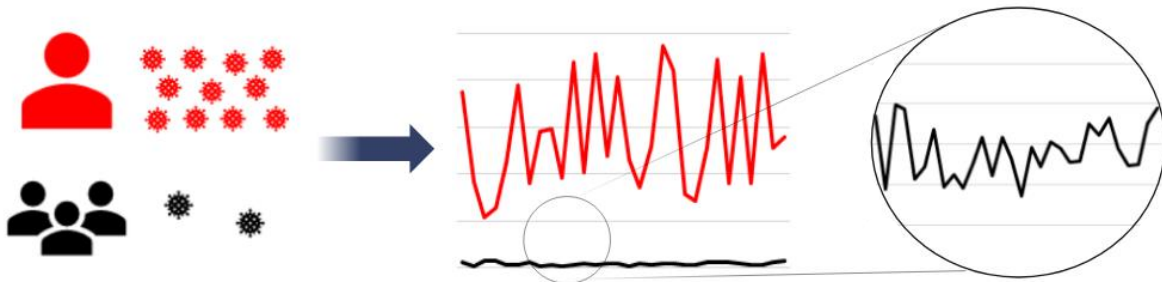
A more deliberate and systematic approach is required to ensure normalization methods deliver reliable and meaningful insights. Challenges to normalization implementation largely stem from two issues: (1) misalignment of normalization techniques with their target variability, and (2) misunderstandings of introduced uncertainty. By ensuring that a normalization technique adequately targets and accounts for the variability of interest and minimizes the risk of introduced variability, researchers can improve data quality and support more reliable applications of WBS. As noted above, recovery controls, aimed to address technical variability, in practice reflect the recovery efficiency of the indicator virus, not SARS-CoV2, misaligning with the intended goal of correcting variability in SARS-CoV2 measurements. Biomarker normalization, intended to mitigate sample variation, reflects the concentrations of the chosen marker introducing influence unrelated to SARS-CoV2. Similarly, environmental normalization aims to address sample variation resulting from differences in wastewater parameters. Some metadata normalization techniques align with specific contextual objectives, such as using census population estimates to represent a static population and flow measurements to estimate daily wastewater volume. However, these methods, along with others, have often been misapplied, for instance using flow, TSS, NH<sub>3</sub>, or BOD as proxies for dynamic population estimates

#### **4.2 Magnitude of Variability**

The two primary sources of variability that affect the comparability of wastewater samples at a single site are dilution and population changes, often assessed through proxies like flow and fecal markers, respectively. In WBS, there is expected variance in any given sewershed of flow and population that can produce uncertainty in data interpretations. Normalization techniques aim to account for such variability to reduce uncertainty in comparing samples. To avoid introducing errors, variability of normalization techniques must align with the magnitude of variability they aim to address. For most sewersheds, dilution variability typically spans 1–2

orders of magnitude, whereas population variability is generally within one order.<sup>74,81-83</sup> In large cities with relatively stable populations, population variability may be even smaller, often fluctuating by only a few percent. Effective normalization depends on the selection of markers whose variability matches the expected dynamics of the target sewershed.

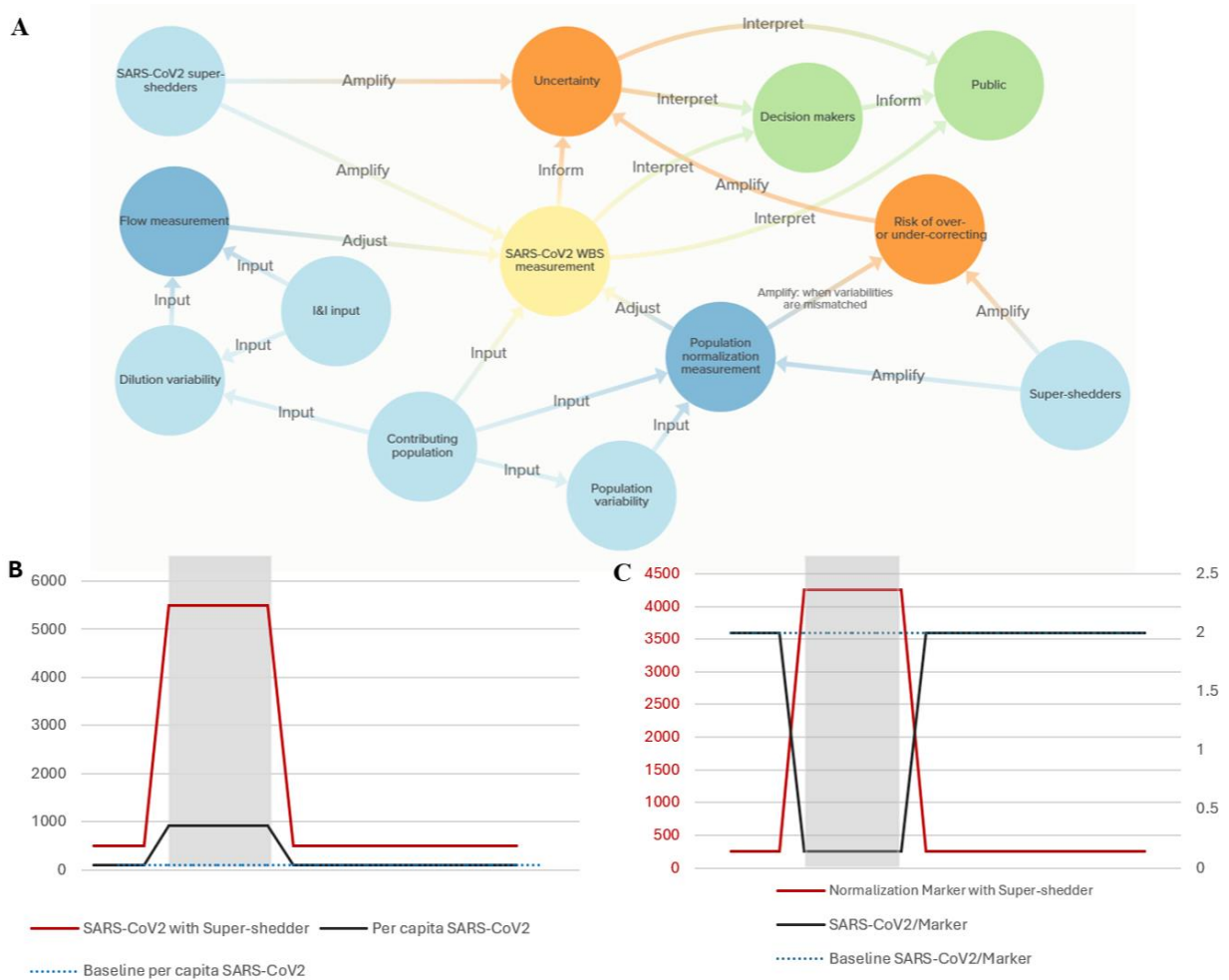
Flow measurements are the primary tool for addressing dilution variability. Within sites, flow typically varies within 1 order of magnitude with coefficients of variation (CVs) of up to 10%.<sup>44,72-75</sup> For techniques aimed at controlling for population variability there is a wider range of variability magnitudes. Fecal markers such as PMMoV and CrAssphage exhibit variance within 1-3 orders of magnitude.<sup>65,65-67</sup> In addition, commonly used fecal markers including PMMoV exhibit geographic variation within 1-2 orders of magnitude.<sup>65</sup> Data is less available for human metabolites but in-situ studies have exhibited variability within 1-3 orders for 5-HIAA, CRE, and PARA, with CAF having variability as high as 4 orders.<sup>39,68-71</sup> Variability of population estimates is dependent upon the size of the sewershed and methods employed but can be as high as 7 orders of magnitude in smaller sites, though is typically within 1-2 orders for most wastewater catchments.<sup>71,76,77</sup> Environmental markers, including TSS, NH<sub>3</sub>, and BOD, generally vary by 1-2 orders, while pH and temperature exhibit greater stability, remaining within one order of magnitude.<sup>84,85</sup>



**Figure 5.** Impact of super-shedders on trend identification.

When the variability of a normalization marker exceeds that of the viral signal, corrections risk amplifying noise and distorting trends. Overly variable markers may overcorrect, producing erroneous trends, while excessively stable markers may under correct, failing to account for legitimate fluctuations. Both scenarios impair interpretability, complicate cross-site comparisons, and undermine public health decision-making. For instance, overcorrection could underestimate viral prevalence during surges, while inconsistent normalization across sites could obscure regional trends. Many fecal markers commonly used by records included in this study, such as

PMMoV, CrAss, human metabolites, BOD, and TSS vary by orders greater than expected population changes likely overcorrecting SARS-CoV2 measurements. Highly variable markers including PMMoV and CrAss, also present a risk of under correction, as super-shedders of these viruses can overshadow the background signal from the broader population, leading to periods of low yet highly variable signals when super-shedders are not active (Figure 6).



**Figure 6.** A) Diagram of system relationships between variability, uncertainty, and stakeholders for WBS of SARS-CoV2. Blue nodes represent input elements; orange represents uncertainty risks; green represents stakeholders; and yellow represents the core element. B) Mock data illustrating the impact of a SARS-CoV2 super-shedder on per capita normalization with a constant population. The highlighted section represents the period during which the super-shedder is active resulting in over-correction in normalized values. C) Mock data illustrating the impact of a super-shedder on a normalization marker while holding SARS-CoV2 shedding rate constant. The highlighted section indicates the period of super-shedder input and over-correction.

One complicating factor is the relationship between wastewater flow variability and SARS-CoV2 concentrations. Ideally, holding SARS-CoV2 viral load constant, an inverse relationship between flow and concentration would be expected. However, real-world conditions, such as

population changes, irregular wastewater inputs, and variable shedding rates, introduce noise that decouples this relationship. SARS-CoV2 super-shedders further exacerbate these challenges by causing spikes in viral concentrations unrelated to flow variability, making normalization efforts more complex.

Mitigating these risks requires careful selection of selecting markers with variability profiles that complements SARS-CoV2 variability, developing site-specific correction factors, and employing composite approaches or temporal weighting to mitigate over- or under-normalization. Composite approaches and temporal weighting schemes may be viable approaches to address these variability patterns but would require further study and application. Success depends on understanding the variability of markers in detail and ensuring they align with SARS-CoV2 trends. If complete information is unavailable, normalization techniques should be approached cautiously, with an understanding of potential misalignment in variability magnitude that could skew data.

The lack of a gold standard further complicates normalization. Correlating normalized SARS-CoV2 concentrations with clinical case data is unreliable due to reduced testing rates and underreporting, which compromise diagnostic data as an accurate reflection of community infection rates. Efforts to identify the most effective or “best” normalization technique through correlations with clinical cases are similarly flawed, and unlikely to yield accurate best practices.

### **4.3 Ideal Normalization Technique**

An ideal normalization technique for WBS of SARS-CoV2 would be:

- Responsive to changes in wastewater volume
- Responsive to changes in fecal load
- Identical stability to SARS-CoV2 in wastewater over time
- Easy to measure
- Mathematically simple to use for adjusting viral concentrations
- Applicable across diverse sewersheds and populations

The most critical criteria are responsiveness to wastewater volume and fecal load variability. Responsiveness requires both consistent covariance with the factor it aims to correct for and a similar order of magnitude of variability. As noted above, for wastewater volume, the order of magnitude of variance is typically 1-2, while fecal load, used as a proxy for population, generally

varies within one order of magnitude for most sewersheds. A marker that exhibits consistent covariance but has a significantly different magnitude of variability may still be useful but would require additional scaling adjustments to avoid introducing distortions. Therefore, an ideal normalization marker should not only track changes in volume and fecal load reliably but also do so within a comparable range of variability to ensure accurate interpretation of WBS data.

A fundamental challenge in achieving an ideal normalization marker lies in balancing the need for dynamic responsiveness to population changes with the stability required to account for dilution effects. No single marker may perfectly achieve both objectives, making a combined approach an attractive solution. Pairing complementary normalization techniques, such as population estimates with flow data to account for dilution, can help address the inherent limitations of each method. Such composite approaches could leverage the strengths of multiple markers, providing a more robust and accurate normalization framework.

If a marker partially meets ideal criteria, there are challenges with interpretability though it can still provide valuable insights when paired with complementary data sources. For example, a marker that accurately reflects population size but fails to capture flow variability may yield inaccurate interpretations if used to adjust viral measurements in areas of high industrial input (I&I) or extreme weather events. Conversely, a marker sensitive only to flow variability, without accounting for population dynamics, could misrepresent trends in regions experiencing substantial population fluxes, such as during tourism peaks or large-scale events, though this limitation may be less critical in larger cities with relatively stable populations, they underscore the necessity of selecting markers that align with the specific characteristics and variability of the target sewershed. Such degraded techniques could flag outlier events or disruptions in wastewater dynamics, which might influence SARS-CoV2 concentrations. While the degraded marker may not offer precise normalization, it can help contextualize data, highlighting sites or samples where further investigation or omission may be needed.

Despite widespread use, current normalization techniques fall short of this ideal criteria. No single method fully meets all requirements. Combining flow and population estimates can approximate an ideal approach in large, stable sewersheds, as it captures both volume and fecal load variability. However, this method fails when applied to small or fluctuating populations, where population estimates exhibit greater variability, often exceeding the variability they aim to correct. Many widely used techniques, such as PMMoV and CrAss normalization, fail to meet

the most critical criteria, as they do not consistently respond to fecal load changes, with inconsistent covariance and greater orders of variability. Responsiveness to fecal load remains a major gap as no existing technique effectively captures these changes while maintaining broad applicability across different sewersheds.

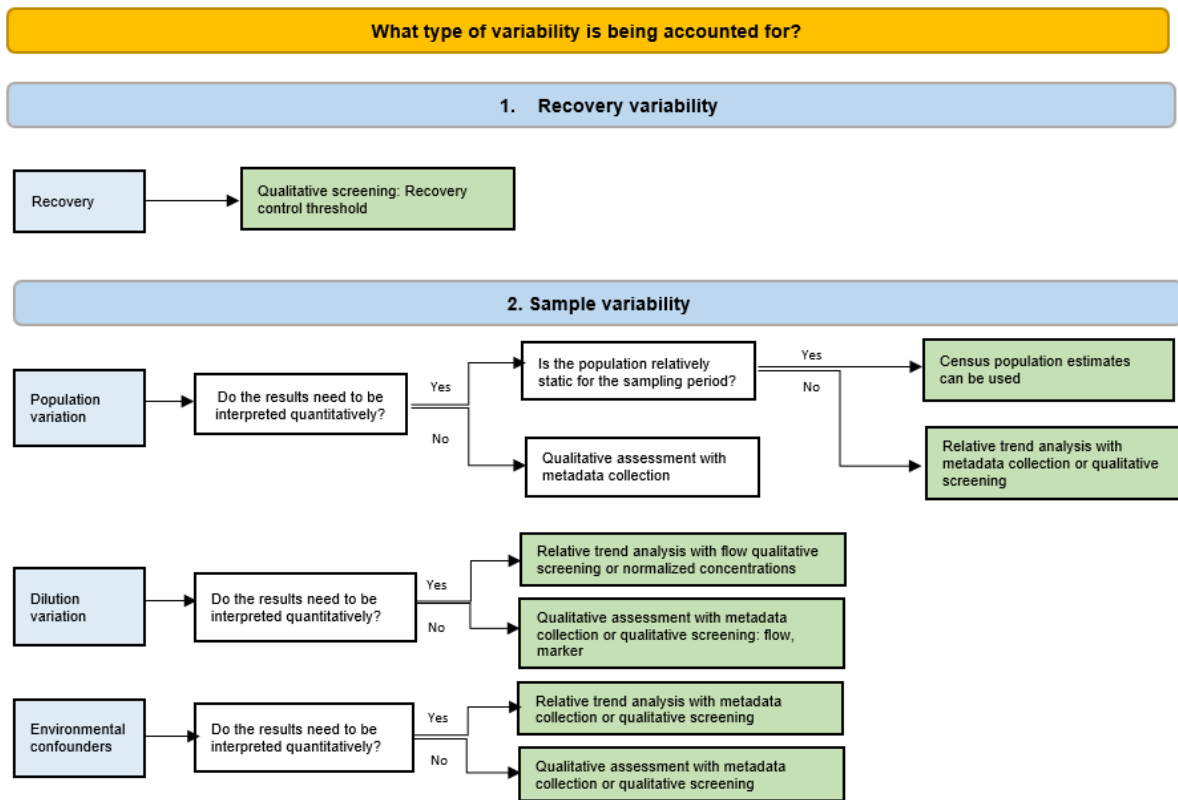
There are opportunities to augment the utility of degraded normalization techniques through pairings with additional methodologies or leveraging metadata. If limitations in techniques are well understood, complementary data sources, such as industrial discharge records or demographic data, can be integrated to address specific weaknesses. For example, pairing a population marker that is subject to seasonal trends with demographic data on tourism can help contextualize fluctuations in wastewater measurements. Similarly, pairing flow data with rainfall measurements to account for flow variation that might not be captured depending on flow measurement approaches; or population estimates paired with daily commuter estimates to capture true sewershed usage throughout a week. While such paired techniques should not be used to directly adjust SARS-CoV2 concentrations, they can enhance understanding of temporal or site-specific trends, providing insights into external factors that may influence measurements. These augmentations contribute to a more holistic normalization framework, maximizing the information extracted from imperfect techniques. If site-specific limitations are not fully understood, however, the implications of augmentations become more complex. In such cases, pairing a degraded marker with additional data sources could inadvertently introduce new uncertainties, as unrecognized biases or inconsistencies in the marker might compound rather than mitigate inaccuracies. This underscores the importance of rigorous characterization of marker behavior, supplementary data, and the sampling sewershed. Even so, metadata and complementary techniques could still provide value in this scenario, offering cross-validation opportunities or helping to flag anomalous results that warrant closer scrutiny. Ultimately, markers with compromised standards still hold potential for identifying anomalies particularly if site specific dynamics are well understood, but their application must be carefully considered to avoid misinterpreting data.

#### **4.4 A conceptual framework for implementation of normalization techniques**

It is critical to evaluate and validate the applicability of normalization techniques before employing them for wastewater surveillance. In Fig. 5, we present an approach to normalization

application to WBS studies to best describe and account for variability and minimize uncertainty in results. This workflow focuses on aligning site-specific variability and research aims with optimal techniques, methods, and data reporting to facilitate best practice.

Before applying the framework, researchers should clearly define and evaluate their research goals to determine whether qualitative or quantitative data is best suited to providing informative information. Matching the type of data collected to the specific goals and scope of the WBS program ensures efficient resource use and maximizes the impact of the surveillance effort.



**Figure 7.** Conceptual framework for integration of normalization techniques and methodologies to WBS programs.

Researchers should begin by determining the type of variability they aim to address in their workflow: recovery variability or sample variability. To address recovery variability, it is essential to gather information on the suitability of recovery control indicators, including their compatibility with the technical processes involved including sampling, concentration, extraction, and quantification. Using a proxy virus with physiochemical properties similar to SARS-CoV2 is recommended for recovery control such as other coronaviruses including BCoV, OC43, HCoV, or BRSV. While limited research has explored the effectiveness of using

inactivated SARS-CoV2 itself as the recovery control, doing so could address many limitations associated with proxy viruses, as the target virus would replicate the exact behaviors required for accurate control.

For researchers aiming to address sample variability in wastewater surveillance, it is essential to further differentiate this variability into population dynamics, dilution effects and those addressing broader environmental parameters. This distinction helps clarify the appropriate applications of wastewater data and ensures normalization techniques are aligned with the specific questions WBS is equipped to address, avoiding misapplications. The population dynamics within each sewershed must be carefully assessed to determine whether population normalization is necessary or feasible. In sewersheds where population demographics remain relatively stable, such as large catchment areas with minimal transient movement or in short-term studies, metadata sources like census data or other population estimates can often serve as effective proxies. These stable environments reduce the likelihood of significant population-driven variability in wastewater metrics, thereby simplifying normalization efforts. However, it is crucial to recognize the limitations of these approaches as not all sewersheds are static, and transient population dynamics, such as those resulting from tourism, commuting patterns, or university schedules may render static estimates inadequate. Researchers must evaluate the temporal and spatial dynamics of the population in their study area to determine whether normalization by population is appropriate or whether alternative methods may better account for variability.

For potential variability in dilution effects and environmental conditions, researchers must carefully assess sewershed-specific characteristics to identify potential sources of variability and determine whether normalization is appropriate or feasible for their study. For dilution variability, factors such as combined sewer systems, industrial contributions, precipitation patterns, and seasonal trends can all influence the applicability of dilution normalization methods. These characteristics must be evaluated to ensure that normalization is appropriate given the variability inherent to the sewershed. When addressing sewersheds that have high variability in dilution effects, flow normalization can be a valuable tool, provided it is used in conjunction with raw data reporting to maintain transparency. Flow normalization can aid in intra-site comparisons by accounting for dilution variability; however, its application for inter-site comparisons warrants significant caution. Flow measurements, while informative for

characterizing wastewater parameters, are highly site-specific and influenced by local dynamics. When comparing across different sewersheds, these differences can introduce additional variability, potentially confounding the interpretation of results and undermining the reliability of cross-site comparisons.

For researchers aiming to address broader environmental parameter variability in WBS, it is essential to first identify site-specific concerns before applying normalization techniques. Environmental factors such as temperature, chemical composition, and biological activity have been identified as factors that could influence viral survivability and the reliability of detected signals. While some researchers have attempted to normalize for these factors, such approaches must be applied with extreme caution. The degree to which these proxies truly reflect the conditions impacting viral decay or preservation remains poorly understood. Moreover, overreliance on unvalidated normalization methods can inadvertently introduce additional variability, potentially obscuring meaningful trends or misrepresenting the data. A more nuanced approach, informed by site-specific data and thorough evaluation of these proxies' applicability, is critical to avoid misinterpretation of results. Researchers can provide such metadata alongside quantified un-normalized SARS-CoV2 concentrations.

Previous research has largely focused on using sample normalization to adjust SARS-CoV2 concentrations in wastewater. Given the current understanding of sample variability normalization techniques, qualitative screening and the application of threshold values offer an improved alternative, helping to mitigate both inherent and introduced variability associated with traditional normalization methods. Unlike quantitative approaches, qualitative assessments focus on contextualizing data within specific local conditions and factors. This could include considering environmental influences, population dynamics, and seasonal variations that could affect wastewater composition. In situations where traditional biomarkers or environmental markers introduce variability due to their failure to align with the virus being studied, qualitative screening allows researchers to gain an understanding of the contextual factors influencing data and identify potential trends or outliers. For instance, evaluating the individual sewershed population dynamics, influent characteristics, or local environmental factors could complement and inform traditional SARS-CoV2 quantification methods but might introduce variability if used to adjust the actual value. By integrating qualitative insights, researchers can better

understand the external dynamics that can impact SARS-CoV2 quantifications and contextualized possible interpretations.

#### **4.5 Applications of data types**

Understanding the distinct roles and applications of qualitative, quantitative, and relative trend analysis in wastewater surveillance is essential for designing effective monitoring programs and interpreting data in ways that align with public health objectives. Previous research has heavily focused on absolute quantification in an attempt to allow for inter-site comparisons, often utilizing normalization techniques that have introduced variability. The appropriate application of data types is essential to ensure that WBS programs can provide interpretable data to safeguard public health without introducing sources of uncertainty.

Qualitative assessments are a valuable tool for WBS programs, particularly as an early warning system to detect viral presence in localized settings such as individual buildings, dormitories, or prisons. This approach is especially beneficial in identifying potential outbreaks at their onset, enabling timely interventions and resource allocation. During periods of low community transmission, qualitative assessments can be used to maintain baseline monitoring without the need for more resource-intensive quantitative analysis, ensuring cost-efficiency while preserving the ability to detect emerging infections. Qualitative data can also play a pivotal role in sentinel surveillance systems, where targeted locations serve as indicators of broader infection trends. By integrating qualitative assessments with metadata monitoring such as flow, occupancy rates, or environmental factors, researchers can contextualize detection data to refine outbreak prediction models. Additionally, qualitative methods are valuable for decision-making in settings with limited laboratory or financial resources, where actionable insights can still inform public health responses. These applications demonstrate the flexibility and utility of qualitative assessments in tailoring wastewater-based surveillance to specific monitoring needs and resource constraints.

Quantitative data can be useful to WBS programs, providing detailed insights into viral load dynamics particularly across larger or more complex sewersheds. Unlike qualitative assessments, which focus on the presence or absence of SARS-CoV2, quantitative data enables measurement of viral concentrations, allowing researchers to assess the scale and severity of infections within a community. This level of detail is especially valuable for tracking long-term

trends, monitoring the effectiveness of public health interventions, and identifying emerging hotspots. Additionally, quantitative analyses support efforts to estimate the severity of population-level infection rates by integrating viral load measurements with metadata such as flow rates and population estimates. While quantitative data often requires more advanced resources and technical expertise, its ability to deliver granular, actionable information makes it a valuable tool to address research questions, informing public health policy and response strategies.

Relative trend analysis, which examines changes in SARS-CoV2 concentrations over time within the same site, should play an increasingly important role in wastewater surveillance. Unlike absolute normalized concentration methods, which attempt to account for all system variations to allow for inter-site comparison, relative trend analysis focuses on tracking fluctuations in SARS-CoV2 concentrations within a given wastewater system. This approach helps mitigate issues related to sample variability and other uncertainties that can arise from inaccurate normalization. By focusing on localized trends, rather than absolute normalized concentrations, researchers can capture the direction and magnitude of viral presence over time, even in the face of confounding factors such as changes in sample composition, population, dilution, or reporting biases. This method has the added advantage of being less sensitive to the precise technical limitations of other normalization techniques and can provide more consistent and reliable insights for public health monitoring. As such, relative trend analysis offers a robust framework for detecting and understanding SARS-CoV2 transmission dynamics while reducing the risk of data misinterpretation.

Caution must be exercised when comparing SARS-CoV2 concentrations across different sites, as sewershed dynamics and other local factors can significantly influence wastewater composition. Variations in population size, wastewater infrastructure, and seasonal factors can all contribute to differences in SARS-CoV2 levels between sites. While normalization methods such as flow or metadata-based population estimates for static populations provide valuable site-specific insights, their applicability for comparisons across sewersheds with differing characteristics remains unsubstantiated. Currently, no fully validated normalization approaches exist to address these complexities and enable consistent, reliable comparisons across sewersheds without introducing significant variability. Until more robust and context-sensitive normalization

techniques are developed, such comparisons should be approached with caution, as they risk misrepresenting true trends and complicating data interpretation.

#### **4.6 Modeling approaches**

Various modeling approaches have been developed to estimate COVID-19 infection rates from wastewater concentrations, using single or multi-parameter frameworks to extrapolate data into incidence rates. However, such studies were not included in this analysis as they did not meet the eligibility criteria. Many of these studies incorporated normalization techniques for SARS-CoV2 wastewater concentrations within their models. For instance, Rainy et al., Cao et al., McMahan et al., Lewis-Borrell et al., and Rauch et al. utilized sewer flow data to estimate COVID-19 incidence rates.<sup>86-90</sup> Wong et al., Phan et al., Abelson et al., Huang et al., and Rauch et al. incorporated population estimates into their models.<sup>91-94</sup> Additionally, Acer et al. and Abelson et al. factored in recovery efficiencies using recovery controls.<sup>95,96</sup> Other normalization methods employed in modeling include the use of fecal indicators like PMMoV, as well as adjustments for socioeconomic factors, temperature, and environmental parameters.<sup>87,97,98</sup> The use of wastewater concentration normalization techniques in modeling highlights their widespread application and underscores the importance of employing these methods thoughtfully to align with research objectives and account for site-specific variability.

Models to estimate COVID-19 cases from wastewater data exhibit significant variability and limited applicability, raising concerns about their reliability for widespread use. The accuracy of such models is undermined by inherent variability in wastewater data, applied normalization techniques, and disease characteristics. Population dynamics, temporal fluctuations, and environmental influences introduce significant challenges, and normalization methods like biomarker adjustment or recovery control often fail to address these issues effectively. Additionally, the fecal shedding rate of SARS-CoV2, a key parameter for extrapolating incidence rates, remains poorly understood, further complicating the reliability of these models.<sup>99-101</sup> Reliance on clinical case correlations as a validation metric is also problematic due to inconsistencies in testing rates, underreporting, and the absence of a standardized gold standard. Without careful consideration of site-specific conditions and research objectives, these models are unlikely to provide accurate or consistent estimates of COVID-19

incidence. A more systematic and validated approach is necessary to ensure that WBS delivers meaningful and actionable insights.

#### **4.7 Signal Interpretation and Resource Allocation**

One effective approach for WBS interpretation is the use of major trends, such as increasing or decreasing signals, or the presence of concurrent signals across multiple sites. These signals are relatively straightforward to identify and require less precise normalization compared to absolute quantification. This simplicity makes them more resilient to variability and errors, reducing inconsistencies temporally and across sites, enhancing their utility for regional comparisons. Concurrent signals from multiple sites within a region, for example, increase confidence that observed trends reflect broader epidemiological changes rather than local anomalies. By focusing on overarching patterns instead of exact measurements, WBS data can provide more robust insights, even when normalization methods are imperfect or site-specific factors introduce variability. Specifically, neighboring municipalities could coordinate their efforts through data sharing, standardization of normalization techniques, or pooling of resources to monitor regional trends effectively. Such collaboration would enhance the reliability of interpretations, especially in the later stages of the COVID-19 pandemic when infection rates are relatively low but with periodic outbreaks that necessitate public health interventions. These signals are particularly useful for identifying significant epidemiological changes while minimizing the risks of misinterpretation due to technical limitations.

Interpreting WBS signals effectively can guide policy decisions, particularly for optimizing resource allocation. In the context of limited resources and diminished public engagement due to pandemic fatigue, prioritization becomes critical. Resources should be directed toward areas showing sharp increases in signals, enabling focused efforts such as expanded clinical testing, targeted public health messaging, or vaccination booster campaigns. Conversely, when signals show a consistent decrease, resources can be conserved and redirected toward maintaining baseline surveillance or addressing regions with inconsistent trends. Inconsistent signals across regions require careful interpretation, as they may stem from differences in normalization techniques or local population behaviors. Addressing these inconsistencies might involve deploying additional resources for data validation or cross-referencing with clinical testing data to clarify uncertainties. Ultimately, a nuanced

understanding of signal dynamics, resource availability, and targeted collaboration among stakeholders can optimize the effectiveness of WBS signal interpretation.

#### **4.8 Risk communication considerations**

When communicating WBS data, it is important to clearly convey that WBS is most effective for identifying trends but lacks the precision for determining precise disease prevalence. Effective risk communication about the confidence in WBS for viral monitoring should emphasize describing the inherent variability within WBS while addressing uncertainties to convey findings effectively to stakeholders. For public health decision-makers, WBS informed trends can provide insights for messaging campaigns and public guidance, including guidance for high-risk individuals, recommended prevention strategies, and guidance for infected persons. For the public, WBS trends can guide personal safety measures and community precautions, such as decisions about masking, interactions with vulnerable individuals, and travel plans.

A key challenge in risk communication of WBS data is obtaining and understanding sufficient confidence to provide actionable insights. Variability in normalization techniques, shedding rates, and sampling methods introduces uncertainty that complicates the interpretation of trends. Standardizing normalization methods offers a potential pathway to reduce this uncertainty, enabling more reliable trend detection and data interpretation. However, the lack of clear thresholds to distinguish meaningful trends from expected variance continues to hamper efforts to align WBS data with actionable decision-making. Effective communication of WBS trends must transparently address uncertainty while emphasizing the value of trends in guiding public health interventions. Current limitations in defining and understanding confidence levels and process controls hinder the ability to establish clear and actionable guidance, presenting a critical gap in the field. Drawing parallels to other surveillance systems, such as those used in environmental monitoring or economic forecasting, can provide a framework for understanding how uncertainty thresholds and process controls are set in similar scenarios. For example, in monitoring recreational water quality for fecal contamination, the EPA recommended bacterial limits for Enterococci and *E. coli* at a geometric mean of 30 cfu and 100 cfu per 100 mL, respectively; and STVs of 110 cfu and 320 cfu per 100 mL, respectively. These recommendations were based off estimates to achieve a desired estimated illness rate of 32/1000.<sup>102</sup> To trigger a standards violation, EPA recommends that the geometric mean should

not exceed the recommended geometric means in any 30-day interval and that no more than 10% of samples should exceed the recommended STVs.<sup>102</sup> . When these standards are exceeded met, beaches and swimming areas are closed, and signs are posted to warn the public and prohibit swimming. Similar standards are widely accepted and applied but critiqued on the thresholds and confidence levels applied.<sup>103</sup> Similar strategies could be developed for WBS SARS-CoV2 monitoring upon a more thorough understanding of variability, uncertainty, and trends.

The challenge of effectively communicating uncertainty in information so that it is understood and appropriately used by audiences has long been recognized. While some critiques suggest that acknowledging uncertainty could undermine trust or confidence in results, research increasingly supports that transparent communication of uncertainty, when done effectively, does not adversely impact trust and can assist audience understanding and decision-making.<sup>104,105</sup> Specifically, presenting uncertainty to decision-makers can assist in aligning data with actionable insights. For instance, continuing the Enterococci and *E. coli* example, researchers could explain to public health officials that there is a 70% chance that a single sample with a measure concentration above the recommended STV was accurate and reflective, informing the decision to require at least 10% of samples to trigger an exceedance and warning issuance. The decision to communicate uncertainty and the method to do so with the public should be considered by WBS program managers. There is broad consensus in technical literature that communicating numerical uncertainty about data does not elicit psychological reactance and can increase perceived trustworthiness among the public.<sup>104,106</sup> WBS program managers should carefully consider whether and how to communicate uncertainty to the public. Studies indicate that the public can effectively interpret uncertainty when it is presented both verbally and numerically, with only a small decrease in trust observed, primarily for verbal uncertainty communication.<sup>104,107</sup> However, relying solely on verbal qualifiers such as “estimated” or “about” can further reduce trust and perceived reliability in both the data and its source.<sup>104,108</sup> For communicating with technical audiences, publishing raw data alongside numerical uncertainty enhances transparency, reproducibility, and trustworthiness.<sup>109</sup> Publishing raw data alongside clear explanations of any normalization equations is likely to achieve such benefits. Additionally, making raw data accessible promotes the robustness of results, enabling reinterpretation and reanalysis as normalization techniques evolve.

An important consideration for WBS data is its connection to actionable insights, particularly in the context of public health decision-making and risk communication. Several dimensions must be addressed to make WBS data actionable. First, it is essential to clarify the types of decisions the data could support and what confidence is needed or desired to determine action; such decisions could include issuing warnings, guidance for infected individuals, vaccination campaigns, or targeted interventions for vulnerable populations.<sup>110</sup> Among these applications, the development and refinement of warning systems stand out as a critical use case for WBS data. Effective warning systems must translate data into clear, actionable messages.<sup>110</sup> Communicating absolute risks with contextual data is considered best practice to help the public understand potential dangers and modify behaviors accordingly.<sup>111,112</sup> Research emphasizes the critical need for warning messages to be specific, consistent, and credible, while offering clear, actionable guidance.<sup>110</sup> The findings stress that effective warnings must not only communicate the nature of the risk but also provide explicit instructions for mitigating it, as this greatly enhances public compliance and leads to better outcomes.<sup>113</sup> While the development of robust warning systems lies beyond the scope of this analysis, frameworks such as that of Mileti and Sorenson offer valuable insights into effective alert and warning practices.<sup>113</sup> Program managers leveraging WBS data can consider such research and guidance to ensure their systems and messages effectively translate surveillance insights into public health action.

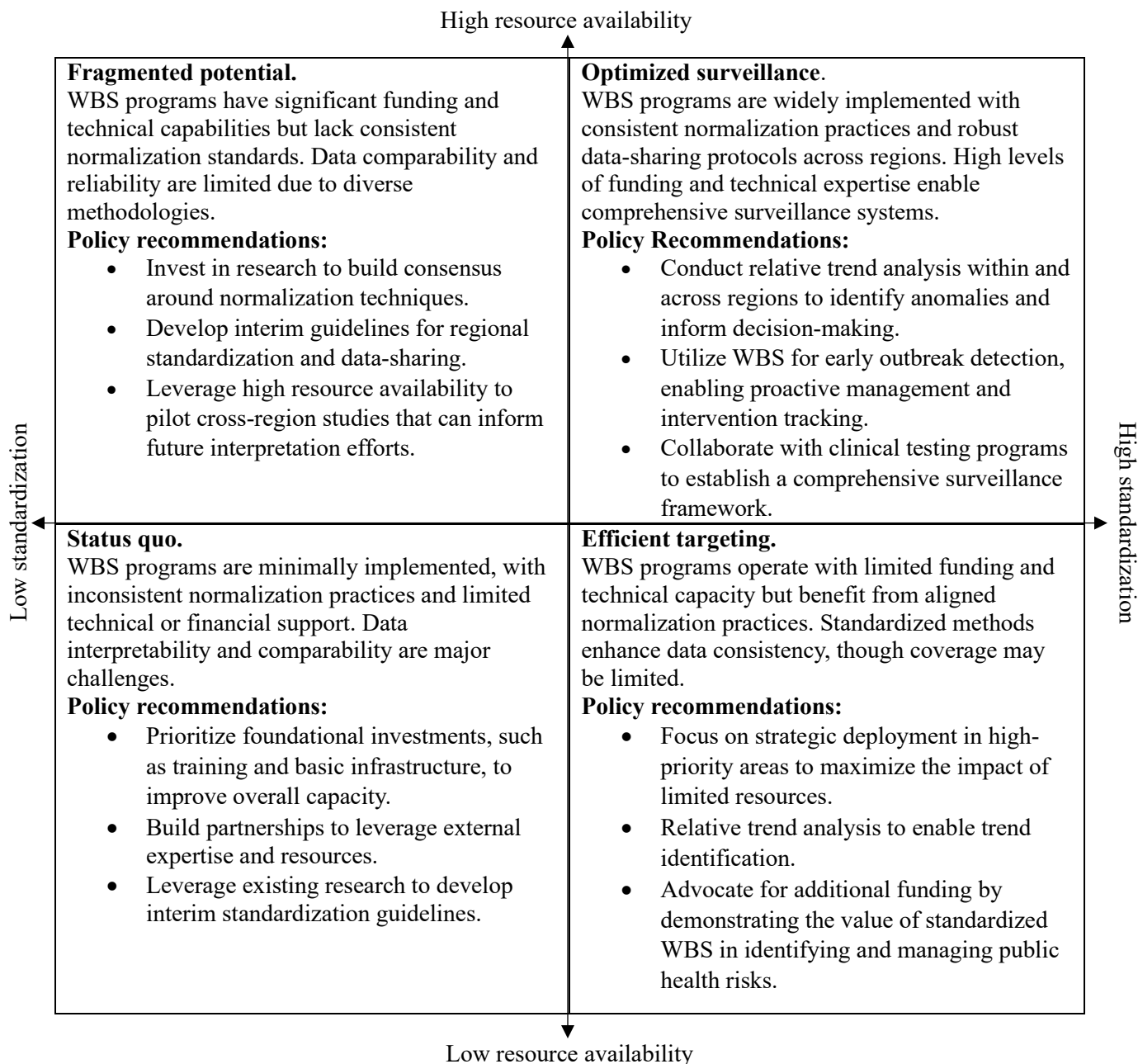
Further analysis is essential to advance the development of confidence intervals, process controls, warning systems, and the integration of WBS results with public health actions. Given that most WBS programs engage with diverse audiences, from technical experts to decision-makers and the public, this research underscores the importance of tailoring communication to facilitate interpretation and trustworthiness. To achieve this, the following specific recommendations are proposed:

- (i) Position WBS as a tool primarily suited for population-level trends, recognizing its limitations in estimating disease prevalence,
- (ii) Publish raw data alongside explanations of any normalization equations, normalized results, and contextual metadata,
- (iii) Provide numerical uncertainty wherever possible.

#### **4.9 Policy implications**

The core policy challenge for WBS of SARS-CoV-2 lies in its limited effectiveness due to inconsistent normalization techniques and uncertainty in data interpretation. Without standardized and universally accepted normalization methods, the reliability and comparability of data across sites are compromised, reducing WBS's overall utility for public health decision-making. While WBS demonstrates the greatest potential with a high degree of standardization and high resource availability, it can still offer value under varying resource constraints and degrees of standardization, though in different ways. This dynamic will likely depend on the level of data use, local, regional, or federal. Less normalization is likely required for local decision-making compared to monitoring across multiple sites or when using aggregate data at larger scales.

To explore these dynamics, this analysis examines policy scenarios through two critical dimensions: the degree of standardization in normalization methods and the level of resource availability. For this framework, resources encompass financial investments, technical capacity, and access to clinical testing. These factors are treated as unidimensional for simplicity, though they are inherently complex and influenced by multiple, interrelated variables in practice. For example, while clinical testing is assumed in this analysis to be universally representative, it often reflects disparities in access and coverage across different communities. Similarly, the need for less standardization for single-site, local data use can be integrated into the higher standardization dimension when analyzing the policy implications.



**Figure 8.** A 2x2 scenario matrix of WBS adaptation scenario for SARS-CoV2. Each axis represents a critical driver; the x-axis is resource availability; the y-axis is normalization technique standardization. The combination of these two variables leads to four plausible scenarios.

This framework underscores the critical interplay between normalization refinement and resource availability in shaping the effectiveness of WBS as a policy tool. By aligning resources with the inherent variability of normalization techniques, policymakers can strategically position WBS to deliver maximum public health utility, from anomaly detection in resource limited settings to comprehensive surveillance in high-capacity systems.

The implementation considerations for SARS-CoV2 WBS are influenced by several factors that impact the role of WBS data and available normalization techniques. Understanding how WBS can be utilized under varying conditions including resource and clinical testing availability, provides critical insights into its policy implications. By examining potential scenarios, this analysis explores how decision-making and public health strategies can adapt to these contexts to maximize the utility of WBS for diverse public health landscapes.

### **Scenario 1: Optimized surveillance**

In an optimized surveillance scenario, the landscape of WBS is marked by widespread implementation underpinned by consistent normalization practices and robust data-sharing protocols. Across regions, governments and public health agencies invest heavily in the infrastructure, funding, and technical expertise necessary to operate comprehensive surveillance systems. Standardized normalization techniques ensure results are temporally comparable within and across sites. In this scenario, policy recommendations emphasize leveraging the strengths of an optimized system. First, conducting relative trend analysis within and across regions allows for the identification of trends including localized outbreaks or shifts in viral transmission. This comparative approach aids in pinpointing areas that may necessitate a public health response or focus as well as timely updates to decision-makers and the public. Second, WBS should be utilized for early outbreak detection, enabling public health authorities to implement proactive management strategies. Furthermore, WBS can be used to track the effectiveness of interventions. Finally, collaboration with clinical testing programs can enable a comprehensive surveillance framework, aligning environmental signals with individual-level case data to enhance the accuracy and timeliness of public health decision-making. These policies collectively ensure that optimized surveillance systems function as a cornerstone of resilient public health infrastructure.

### **Scenario 2: Efficient targeting**

In a high-standardization but low-resources scenario, WBS operates within constrained funding and technical capacity. Despite these limitations, the alignment of normalization practices across regions ensures consistent and comparable data, which strengthens the reliability of surveillance efforts. Coverage is necessarily limited, focusing on select locations rather than widespread monitoring. Standardized methods enable the extraction of high-quality insights even with fewer resources, providing valuable information for public

health decision-making. The policy recommendations for this scenario emphasize efficiency and impact. First, WBS deployment should target high-priority areas, such as densely populated regions, vulnerable communities, or locations with a history of outbreaks. Concentrating resources in these areas maximizes the impact of surveillance, ensuring that limited funding delivers the greatest possible public health benefits. Second, conducting relative trend analysis wherever possible to interpret temporal trends within and across regions allows for identification of outbreaks or epidemic progression data to inform decision-makers and the public. Lastly, programs can advocate for increased funding by demonstrating the value of standardized WBS. Highlighting the system's ability to identify and manage public health risks through clear, consistent data can build the case for additional investment, ultimately expanding the reach and efficacy of surveillance programs. These policies strive to maximize the effectiveness of WBS within resource-constrained contexts.

### **Scenario 3: Status quo.**

In a low-resource, low-standardization scenario, WBS programs are minimally implemented and struggle with inconsistent normalization techniques. These constraints significantly hinder the interpretability and comparability of data across regions, reducing the reliability of WBS as a public health tool. With limited infrastructure, expertise, and coordination, efforts are fragmented, and actionable insights are limited. Policy recommendations in this scenario focus on establishing the groundwork for future improvement. First, foundational investments, such as training public health personnel and developing basic sampling and testing infrastructure, are critical for building capacity. These steps create a base from which WBS efforts can expand over time. Second, building partnerships with academic institutions or private sector companies can help leverage external expertise and resources in developing capacity. Such collaborations can fill immediate gaps in knowledge and funding while fostering longer-term sustainability. Lastly, interim standardization guidelines can be developed using existing research and best practices. These guidelines, even if temporary, can improve data interpretability and comparability in the short term, offering a clearer picture of WBS's potential and demonstrating its value. Despite challenges, WBS retains potential as an emerging tool for monitoring public health risks, but progress requires deliberate investments and collaborations to move beyond this status quo state.

### **Scenario 4: Fragmented potential.**

In a fragmented potential scenario, WBS programs operate with substantial funding and technical capabilities, but the lack of consistent normalization standards undermines their effectiveness. The diversity of normalization techniques used within and across regions results in data that are difficult to compare and limits the reliability of insights derived from surveillance efforts. Despite the high resource availability, the absence of standardized approaches creates challenges in identifying trends across time and regions. This scenario highlights the tension between capability and cohesion, where the full potential of WBS remains unrealized due to methodological fragmentation. Policy recommendations for this scenario emphasize leveraging existing resources to address gaps in standardization and coordination. First, investing in research to build consensus around normalization techniques is essential for aligning practices within and across regions. By bringing together stakeholders to evaluate and validate methodologies, a foundation for consistent practices can be established. Second, developing interim regional guidelines for standardization and data-sharing can provide a temporary framework to enhance data comparability while long-term solutions are explored. These guidelines can include best practices for normalization and reporting, ensuring that data are at least partially harmonized. Lastly, high resource availability presents an opportunity to pilot cross-region studies. These studies can serve as a testing ground to provide valuable insights into how to interpret data across different levels of normalization standardization, optimizing the quality and usefulness of the resulting insights. Together, these actions can help bridge the gap between capability and consistency, unlocking the full potential of WBS programs in managing public health risks.

These scenarios underscore the flexibility and potential of WBS as a public health tool. While high-resource settings maximize the utility of WBS through sophisticated surveillance systems and standardization, low-resource scenarios demonstrate capacity to provide critical signals in the absence of other data. Policymakers must navigate the trade-offs inherent in each scenario, tailoring their strategies to the specific constraints and opportunities of their context. By understanding the capabilities and limitations of WBS across these scenarios, public health officials can make informed decisions that enhance preparedness and response efforts.

#### **4.10 Limitations**

One notable limitation of this systematic review was that a single reviewer independently screened identified records due to time and resource constraints. However, any conflicts or uncertainties were discussed with a second reviewer (SM) to mitigate potential biases. While this approach may increase the risk of overlooking relevant studies, the consistency and robustness of the findings lend confidence to their validity. Another challenge was categorizing variability across studies, stemming from both the lack of detailed information on some normalization techniques and the inherent differences between study locations. The heterogeneity in sampling methods, population sizes, and environmental factors across study sites complicates direct comparisons and synthesis of findings. Additionally, the absence of standardized reporting for normalization approaches further limits the ability to accurately evaluate and categorize sources of variability.

## **5. Conclusion**

This study identified key trends in the application of normalization techniques for WBS of SARS-CoV2. Recovery control threshold values were the most commonly applied technique overall, though their use declined between 2022 and 2024, while biomarker adjustments became the dominant method in recent years. However, this shift presents concerns, as biomarker normalization techniques introduce significant uncertainty into WBS data. Similarly, the increasing reliance on clinical case correlation analysis, underscores the challenges in validating normalization techniques as such analyzes risk amplifying uncertainty in data. There is a great opportunity to improve WBS reliability and potentially interpretability through the adoption of standardized normalization techniques that best account for variability and minimize uncertainty. Methods prone to high uncertainty, such as adjustments using PMMoV, CrAss, F+ coliphage, TSS, and COD, should be used cautiously and primarily as performance indicators to flag samples for comparison rather than for direct adjustments. Similarly, recovery controls like BCoV and OC43 should be applied to identify outlier samples rather than to directly adjust SARS-CoV2 concentrations.

For effective implementation of WBS programs, developing a structured framework that aligns site-specific variability with appropriate normalization methods would support best practices. Recognizing the limitations of different normalization approaches can help researchers design more robust surveillance programs while maintaining awareness of data uncertainty. Even

standardizing normalization techniques at a subregional or regional level could improve relative trend analysis, enhance confidence in data interpretation, and facilitate the dissemination of best practices. Without such standardization, reliable temporal and cross-site comparisons remain challenging.

Looking ahead, further research is needed in several areas. First, a deeper understanding of how normalization methods contribute to variability is necessary to refine techniques and reduce uncertainty. Second, research should focus on determining the confidence levels required to support decision-making based on WBS data. Finally, defining process controls for WBS will help establish acceptable variability thresholds, improving the reliability of trend analyses. Finally, while normalization methods are essential for interpreting WBS data, they also introduce variability and uncertainty. Addressing these challenges through further research and standardization efforts is crucial for maximizing the utility of WBS in public health decision-making.

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## Appendix A: Supplementary Data

Table S1. Search terms used to identify records for eligibility screening for this analysis

1. Normalization and SARS-CoV-2 and wastewater
2. Normalization and covid-19 and wastewater
3. Normalization and SARS-CoV-2 and wastewater surveillance
4. Normalization and Covid-19 and wastewater surveillance
5. 3 and biomarkers
6. 4 and biomarkers
7. Wastewater trends and SARS-CoV-2
8. Wastewater dashboards and SARS-CoV-2
9. Wastewater trends and Covid-19
10. Wastewater dashboards and Covid-19

Table S2. Each included study in this analysis and characteristics of interest including citation, study location, study participants, normalization techniques, normalization methods, sample fraction, and data reported.

Study ID	Population			Normalization technique and method			Sample type	Reported data		
	Author	Year	Location	Source of participants	Recovery control	Human-associated biomarkers	Environmental	Sample fraction	Wastewater concentrations	Correlation with clinical cases
Abelson, et. al.	2023	Key Biscayne, FL	Community	OC43 <sup>b</sup>			Population estimate <sup>a</sup>	Liquid	Raw	✓
Acer, et.al	2022	US	University				Flow <sup>a</sup>	Liquid	Adjusted	✓
Ai, et. al.	2021	OH	Community			PMMoV <sup>a</sup> , CrAss <sup>a</sup>		Solid then liquid	Raw, Adjusted	✓
Al-Duroobi, et. al.	2023	San Antonio, TX	Community	BCoV <sup>a</sup>				Solid	Raw, Adjusted	✓
Al-Duroobi, et. al.	2021	Bexar County, TX	Community	BCoV <sup>b</sup>				Solid	Raw	✓
Al-Faliti, et. al.	2022	US	Community	BCoV <sup>b</sup> , PMMoV <sup>b</sup>			Flow <sup>a</sup>	Both	Raw, Adjusted	✓
Amirali, et. al.	2024	Coral Gables, FL	University	B2M <sup>b</sup> , OC43 <sup>f</sup> , PMMoV <sup>b</sup>				Solid	Statistical analysis only	✓
Anderson-Coughlin, et. al.	2022	DE	University				Flow-population estimate <sup>a</sup>	Liquid	Raw	-
Anderson-Coughlin, et. al.	2021	New Castle County, DE	Community, University	TV <sup>b</sup>		PMMoV <sup>a</sup>		Liquid	Raw	✓
Anneser, et. al.	2022	CT, MA, ME	Community	BRSV <sup>†*</sup>		PMMoV <sup>a*</sup>		Both	Raw	✓

Ash, et. al.	2023	Knoxville, TN	University	PMMoV <sup>b</sup>		Liquid	Raw	✓
Baldwin, et. al.	2023	Shreveport, LA	Community	BRSV <sup>b</sup>	PMMoV <sup>a</sup>	Solid	Raw, Adjusted	✓
Barich, et. al.	2021	Mount Vernon, OH	University	OC43 <sup>a*</sup> , SARS-CoV2 <sup>a</sup>	Flow <sup>a</sup>	Both	Raw, Adjusted	✓
Barua, et. al.	2022	Charlotte, NC	Community	BCoV <sup>b</sup>		Solid	Raw	✓
Bayati, et. al.	2022	MO	Community	PURO <sup>b</sup>	Flow <sup>a</sup>	Liquid	Adjusted	✓
Beattie, et. al.	2022	NC	Community	BCoV <sup>b</sup>		Solid	Raw	-
Bernard, et. al.	2022	OH	Community	HF183 <sup>b</sup>		Liquid	Raw	-
Betancourt, et. al.	2021	Tucson, AZ	University	HCoV <sup>b</sup>		Liquid	Raw	-
Bhattarai, et. al.	2021	UT	Community	SARS-CoV2 <sup>a</sup>	Flow-population estimate <sup>a</sup>	Both	Raw, Adjusted	-
Bivins, et. al.	2021	IN	Community	BRSV <sup>a</sup>	PMMoV <sup>a</sup>	Solid	Raw, Adjusted	-
Bivins, et. al.	2021	South Bend, ID	University	BRSV <sup>a</sup>	PMMoV <sup>a</sup>	Solid	Raw, Adjusted	✓
Boehm, et. al.	2023	CA	Community	BCoV <sup>b</sup>	PMMoV <sup>a</sup>	Solid	Raw, Adjusted	✓
Boehm, et. al.	2022	CA	Community	BCoV <sup>b</sup> , PMMoV <sup>b</sup>		Solid	Raw	✓
Bowes, et. al.	2021	Tempe, AZ	Community		Flow-population estimate <sup>a</sup>	-	Raw	✓
Bowes, et. al.	2023	Tempe, AZ	Community	MHV <sup>b</sup>	Flow <sup>a</sup>	Liquid	Adjusted	✓
Boza, et. al.	2024	Coral Gables, FL	University	OC43 <sup>b</sup> , MS2 <sup>b</sup>		Liquid	Raw	✓
Brighton, et. al.	2024	New Orleans, LA	Community	PMMoV <sup>b</sup>		Liquid	Raw	✓
Brooks, et. al.	2023	Yarmouth, ME	Community	BRSV <sup>a</sup>	Flow <sup>a</sup>	Liquid	Raw, Adjusted	✓
Cavany, et. al.	2022	South bend, IN	University	BRSV <sup>a</sup>		Solid	Adjusted	✓
Cha, et. al.	2023	US	University	BCoV <sup>b</sup>	PMMoV <sup>a</sup>	Solid	Adjusted	✓
Chan, et. al.	2024	CA	Community		PMMoV <sup>a</sup>	Solid	Raw, Adjusted	✓
Chan, et. al.	2023	San José, CA	Community	BCoV <sup>b</sup>	PMMoV <sup>a</sup>	Solid	Adjusted	✓
Ciesielski, et. al.	2021	VA	Community	BCoV <sup>b</sup>		Liquid	Raw	-
Cohen, et. al.	2022	Blaskcбург, VA	University	BCoV <sup>b</sup>	Population estimate <sup>a</sup>	Liquid	Raw, Adjusted	✓
Conway, et. al.	2023	Mount Pleasant, MI	University, Community		PMMoV <sup>a</sup>	Solid	Raw, Adjusted	✓

Curtis, et. al.	2020	Norfolk, VA	Community			Flow <sup>a</sup>	Solid	Raw, Adjusted	-
Daza-Torres, et. al.	2024	CA	Community	BCoV <sup>b</sup>	PMMoV <sup>a</sup>		Solid	Adjusted	✓
Daza-Torres, et. al.	2023	CA	Community	BCoV <sup>b</sup>	PMMoV <sup>a</sup>		Solid	Adjusted	✓
Duvallet, et. al.	2022	US	Community		PMMoV <sup>a</sup>		Liquid	Adjusted	✓
Fahrenfeld, et. al.	2022	US	University		PMMoV <sup>a</sup>		Liquid	Raw, Adjusted	✓
Fahrenfeld, et. al.	2022	US	Community		PMMoV <sup>a</sup>	Flow-population estimate <sup>a</sup>	Liquid	Adjusted	✓
Feng, et. al.	2021	WI	Community	BCoV <sup>b</sup>	PMMoV <sup>a</sup> , HF183 <sup>a</sup>	Population estimate <sup>a</sup>	Solid	Raw	✓
Fielding-Miller, et. al.	2023	San Diego County, CA	Building	PMMoV <sup>†</sup>			Liquid	Statistical analysis only	-
Flood, et. al.	2023	MI	Community			Flow-population estimate <sup>a</sup>	Solid	Adjusted	✓
Gerrity, et. al.	2021	NV	Community	BCoV <sup>a</sup>	PMMoV		Solid	Adjusted	✓
Ghanbari, et. al.	2024	CO	Community	BCoV <sup>a</sup> , BCoV <sup>b</sup>	F <sub>+</sub> <sup>a</sup>	Flow <sup>a</sup>	Liquid	Raw	✓
Gibas, et. al.	2021	Charlotte, NC	University	BCoV <sup>b</sup>			Solid	Raw	✓
Gonzalez, et. al.	2020	VA	Community	BCoV <sup>b</sup> , BRSV <sup>b</sup>		Flow-population estimate <sup>a</sup>	Liquid then solid	Raw, Adjusted	-
Goodwin, et. al.	2023	El Paso County, CO	University		PMMoV <sup>†</sup>		Liquid	Raw	✓
Graham, et. al.	2020	Santa Clara County, CA	Community	BCoV <sup>b</sup> , MHV <sup>b</sup> , PMMoV <sup>b</sup>	PMMoV <sup>a</sup>		Both	Raw, Adjusted	✓
Greenwald, et. al.	2021	San Francisco, CA		BCoV <sup>b</sup> , SOC <sup>b</sup>	PMMoV <sup>a</sup> , CrAss <sup>a</sup> , Bacteroides rRNA <sup>a</sup>	Flow-population estimate <sup>a</sup>	Liquid	Raw, Adjusted	✓
Grube, et. al.	2023	NC	Community	BCoV <sup>b</sup> , HepG <sup>b</sup>		Flow-population estimate <sup>a</sup>	Solid	Raw, Adjusted	✓
Haak, et. al.	2022	Washoe County, NV	Community	OC43 <sup>b</sup> , MS2 <sup>b</sup>			Liquid	Raw	-
Han, et. al.	2024	KY	Building		PMMoV <sup>a</sup>		-	Adjusted	-
Han, et. al.	2023	KY	Building		PMMoV <sup>a</sup>		-	Adjusted	-
Hoar, et. al.	2022	New York, NY	Community	BCoV <sup>b</sup>		Flow-population estimate <sup>a</sup>	Liquid	Adjusted	✓

Hoffman, et. al.	2023	NC	Community			Flow-population estimate <sup>a</sup>	Solid	Adjusted	✓
Holm, et. al.	2024	Jefferson County, KY	Community		PMMoV <sup>a</sup>		Both	Adjusted	✓
Holm, et. al.	2022	Louisville and Jefferson County, KY	Community	BCoV <sup>b</sup>	PMMoV <sup>a</sup> , CrAss <sup>a</sup>		Liquid	Raw, Adjusted	✓
Hopkins, et. al.	2023	Houston, TX	Community			Flow <sup>a</sup>	Liquid	Adjusted	✓
Hsu, et. al.	2022	MO	Community		PMMoV <sup>a</sup> , CAF <sup>a</sup> , PARA <sup>a</sup> , 5-HIAA <sup>a</sup>	Flow <sup>a</sup>	Liquid	Raw, Adjusted	✓
Huisman, et. al.	2022	San Jose, CA	Community	BCoV <sup>b</sup> , PMMoV <sup>b</sup>		Flow <sup>a</sup>	Solid	Adjusted	-
Hutchison, et. al.	2022	Lawrence, KS	Community	MHV <sup>b</sup> , PURO <sup>b</sup>	CRE <sup>a</sup> , Mit <sup>a</sup>	Flow <sup>a</sup> , NH <sub>3</sub> <sup>a</sup> , BOD <sup>a</sup>	Liquid	Adjusted	✓
Janssen, et. al.	2023	WI	Community		PMMoV <sup>a</sup>		Solid	Raw, Adjusted	-
Jarvie, et.al	2022	MI	Building, Community, University	Phi6 <sup>b</sup>			Solid	Raw	✓
Jeon, et. al.	2023	Honolulu, HI	Community		PMMoV <sup>a</sup> , F <sup>+</sup> <sup>a</sup>		Both	Statistical analysis only	✓
Jiang, et. al.	2022	UT	Community			Flow-population estimate <sup>a</sup>	Liquid	Statistical analysis only	-
Jimenez, et. al.	2023	Volusia County, Florida	Community		PMMoV <sup>a</sup>		Liquid	Raw, Adjusted	✓
Juel, et. al.	2021	Charlotte, NC	University	BCoV <sup>b</sup>			Solid	Raw	-
Kadonsky, et. al.	2023	Merced, Modesto, and Davis Counties, CA	Community	BCoV <sup>b</sup>	PMMoV <sup>a</sup>		Solid	Adjusted	✓
Kanchan, et. al.	2024	Boise, ID	Community			Flow <sup>a</sup>	Solid	Adjusted	✓
Kappus-Kron, et. al.	2024	Jefferson County, NY	Building		CrAss <sup>a</sup>		Solid	Adjusted	✓
Karthikeyan, et. al.	2022	San Diego, CA	University		PMMoV <sup>a</sup>		Solid	Raw, Adjusted	-
Karthikeyan, et. al.	2021	San Diego, CA			PMMoV <sup>a</sup>		Liquid	Adjusted	✓
Kaya, et. al.	2022	MD	Community	BRSV <sup>b</sup>			Both	Raw	-

Keshaviah, et. al.	2023	NC	Community	BCoV <sup>a</sup>	PMMoV <sup>a</sup>	Flow-population estimate <sup>a</sup>	Solid	Statistical analysis only	✓
Khan, et. al.	2023	Reno-Sparks, NV	Community	OC43 <sup>b</sup> , PMMoV <sup>b</sup>			Liquid	Raw	-
Kim, et. al.	2022	CA	Community	BCoV <sup>b</sup> , PMMoV <sup>b</sup>			Solid	Raw	✓
Kim, et. al.	2023	CA	Building	BCoV <sup>b</sup> , PMMoV <sup>b</sup>	PMMoV <sup>a</sup>		Both	Raw, Adjusted	-
Klaassen, et. al.	2024	Louisville and Jefferson County, KY	Community		PMMoV <sup>a</sup>	Flow <sup>a</sup>	Liquid	Raw, Adjusted	✓
Klevens, et. al.	2023	MA	Building	†, PMMoV <sup>b</sup>	PMMoV <sup>†</sup>		Liquid	Raw	✓
Kuhn, et. al.	2022	Oklahoma City, OK	Community	BCoV <sup>b</sup> , PMMoV <sup>b</sup>			Liquid	Raw	✓
Lamm, et. al.	2024	Miami-Dade County, FL	Community, University	OC43 <sup>b</sup>			Solid	Raw	✓
Lancaster, et. al.	2022	OH	Community	†			Liquid	Raw	✓
Langan, et. al.	2022	TX	University	BCoV <sup>b</sup>	PMMoV <sup>a</sup>		Liquid	Raw, Adjusted	✓
Larsen, et. al.	2022	NY	Community		CrAss <sup>a</sup>		Solid	Statistical analysis only	✓
Lee, et. al.	2024	Suffolk County, NY	Community	BCoV <sup>b</sup>	CAF <sup>a</sup>		Liquid	Adjusted	✓
Lee, et. al.	2022	Lincoln Parish, Louisiana	Community		PMMoV <sup>a</sup>		Liquid	Raw, Adjusted	✓
Li, et. al.	2021	Honolulu, HI	Community	BCoV <sup>b</sup>	PMMoV <sup>a</sup> , F <sup>+</sup> <sup>a</sup>		Both	Raw, Adjusted	-
Li, et. al.	2022	MI	Community		CRE <sup>a</sup> , 5-HIAA <sup>a</sup> , CAF <sup>a</sup> , Xan <sup>a</sup> , Meth <sup>a</sup> , PARA <sup>a</sup> , Theop <sup>a</sup> , Theob <sup>a</sup>	Flow <sup>a</sup> , BOD <sup>a</sup> , TKN <sup>a</sup> , TSS <sup>a</sup> , VSS <sup>a</sup>	Solid	Raw, Adjusted	✓
Li, et. al.	2024	Knoxville, TN	University		PMMoV <sup>a</sup>		Liquid	Raw, Adjusted	✓
Li, et. al.	2022 <sup>b</sup>	Reno-Sparks, NV	Community	OC43 <sup>b</sup> , PMMoV <sup>b</sup>			Liquid	Raw	✓
Li, et. al.	2024 <sup>b</sup>	NV	Community	OC43 <sup>b</sup> , PMMoV <sup>b</sup>			Liquid	Raw	✓

Li, et. al.	2022	MO	Community		CAF <sup>a</sup> , PARA <sup>a</sup> , PMMoV <sup>a</sup>	Population estimate <sup>a</sup>	Liquid	Raw, Adjusted	✓
Lininger, et. al.	2024	OR	Community			Flow- population estimate <sup>a</sup>	-	Adjusted	✓
Lott, et. al.	2023	Athens- Clarke County, Georgia	Community	BCoV <sup>a</sup>		Flow <sup>a</sup>	Liquid	Raw, Adjusted	✓
Lou, et. al.	2022	Houston, TX	Community	BCoV <sup>b</sup>			Liquid	Raw	✓
Lu, et. al.	2022	Columbu s, OH	University		PMMoV <sup>a</sup> , CrAss <sup>a</sup>		Liquid	Raw, Adjusted	✓
Lu, et. al.	2022	Portland, OR	Community			Population estimate <sup>a</sup>	Solid	Adjusted	-
Ma, et. al.	2022	Dayton, OH	Community	BCoV <sup>b</sup> , PMMo V <sup>b</sup>	PMMoV <sup>a</sup>	Flow <sup>a</sup>	Solid	Raw, Adjusted	✓
Majumdar, et. al.	2024	Rochester , MN	Community		PMMoV <sup>a</sup>	Flow <sup>a</sup>	Liquid	Adjusted	✓
McGowan, et. al.	2022	Toldeo, OH	University	MHV <sup>b</sup> , PURO <sup>b</sup>	CrAss <sup>a</sup>		Liquid	Raw, Adjusted	✓
Meadows, et. al.	2024	ID	Community	BCoV <sup>b</sup>			Solid	Raw	✓
Melvin, et. al.	2021	MN	Community		PMMoV <sup>a</sup>		Liquid	Adjusted	✓
Mondal, et. al.	2021	Dane County, WI	Community	MS2 <sup>b</sup>	PMMoV <sup>a</sup>		Liquid	Raw, Adjusted	✓
Montesinos -López, et. al.	2023	Davis, CA	Community		PMMoV <sup>a</sup>		Solid	Adjusted	✓
Nagarkar, et. al.	2022	Hamilton County, OH	Community	OC43 <sup>a</sup>	PMMoV <sup>a</sup> , CrAss <sup>a</sup>	Flow <sup>a</sup>	Both	Raw, Adjusted	✓
Nelson, et. al.	2022	Austin, TX	Community			Flow <sup>a</sup>	Solid	Adjusted	✓
Nguyen, et. al.	2022	Seattle, WA	Community			NH3 <sup>a</sup> , PO4 <sup>a</sup>	Liquid	Raw, Adjusted	-
O'Brien, et. al.	2021	Waco, TX	University			Population estimate <sup>a</sup>	Both	Raw, Adjusted	-
Oh, et. al.	2022	Champai gn County, IL		BCoV <sup>b</sup>	PMMoV <sup>a</sup>		Solid	Adjusted	-
Osborn, et. al.	2024	MN	Community		PMMoV <sup>a</sup>	Flow <sup>a</sup>	Liquid	Raw	✓
Pasha, et. al.	2024	Raleigh, NC	Community	BCoV <sup>b</sup>		Flow- population estimate <sup>a</sup>	Solid	Adjusted	✓
Peccia, et. al.	2020	New Haven, CT	Community		RNA <sup>a</sup>		Solid	Raw	-
Pecson, et. al.	2021	Los Angeles	Community	OC43 <sup>a</sup>			Both	Raw, Adjusted	-

		County, CA						
Phan, et. al.	2023	MA	Community			Flow <sup>a</sup>	Liquid	Raw -
Rabe, et. al.	2023	CA	Community	BCoV <sup>b</sup>	PMMoV <sup>a*</sup>		Both	Raw ✓
Rainey, et. al.	2023	US	Community		PMMoV <sup>a</sup> , CrAss <sup>a*</sup> , F <sub>+</sub> <sup>a*</sup>	Flow <sup>a</sup>	Both	Statistical analysis only ✓
Rainey, et. al.	2022	Gainesville, FL	University	OC43 <sup>b</sup>			Solid	Raw ✓
Rainey, et. al.	2022	Levy County, FL	Community	OC43 <sup>b</sup>			Solid	Raw -
Rao, et. al.	2024	Atlanta, GA	Community	BCoV <sup>b</sup> , MS2 <sup>b</sup>	PMMoV <sup>a</sup> , mtDNA <sup>a</sup>		Both	Raw -
Reeves, et. al.	2021	Boulder, CO	University	BCoV <sup>b</sup>	F <sub>+</sub> <sup>b</sup>		Liquid	Raw ✓
Rezaeitavebe, et. al.	2024	Athens, OH	Community	BCoV <sup>a</sup>	PMMoV <sup>a</sup>		Liquid	Raw ✓
Robbins, et. al.	2024	Concord, NH	Community	PMMoV <sup>b</sup>			Liquid	Raw -
Rouchka, et. al.	2021	Louisville, KY	Community		PMMoV <sup>a</sup>		Liquid	Adjusted ✓
Safford, et. al.	2022	Davis, CA	Community	Phi6 <sup>b</sup>	PMMoV <sup>a</sup>		Solid then liquid	Adjusted ✓
Safford, et. al.	2022	Davis, CA	Community	Phi6 <sup>b</sup>	PMMoV <sup>a</sup>		Solid then liquid	Adjusted ✓
Saingam, et. al.	2023	Seattle, WA	Community	BCoV <sup>b</sup>			Liquid	Raw ✓
Saldana, et. al.	2024	Los Angeles, CA	University		PMMoV <sup>a</sup>		Solid	Raw, Adjusted ✓
Schafer, et. al.	2024	WI	Community	BCoV <sup>b</sup>	PMMoV <sup>b</sup>		Liquid	Raw -
Schill, et. al.	2023	CA	Community	BCoV <sup>b</sup>	PMMoV <sup>a</sup>	Flow <sup>a</sup> , Conductivity <sup>a</sup> , TSS <sup>a</sup>	Liquid	Raw, Adjusted ✓
Schoen, et. al.	2022	CA	Community	BCoV <sup>b</sup>	PMMoV <sup>a</sup>		Solid	Adjusted ✓
Schussman, et. al.	2022	Milwaukee County, WI	Community	BCoV <sup>b</sup>	PMMoV		Solid	Statistical analysis only -
Scott, et. al.	2021	New Orleans, LA	University	Phi6 <sup>b</sup>	PMMoV <sup>a</sup>		Solid	Raw, Adjusted ✓
Sellers, et. al.	2022	Columbia, SC	University		PMMoV <sup>a</sup>		Liquid	Raw ✓
Sharkey, et. al.	2021	Miami-Dade County, FL	University	OC43 <sup>b</sup>			Both	Raw ✓
Silva, et. al.	2022	AR	Community	BCoV <sup>b</sup>	PMMoV <sup>a</sup>		Liquid	Adjusted -

Simpson, et. al.	2021	CA	Community	BCoV <sup>b</sup>	PMMoV <sup>a</sup>		Solid	Raw, Adjusted	-
Solo-Gabriele, et. al.	2023	Coral Gables, FL	University	OC43 <sup>b</sup>			Solid	Raw	✓
Stadler, et. al.	2020	Houston, TX	Community			Flow-population estimate <sup>a</sup>	Liquid	Adjusted	✓
Straathof, et. al.	2024	OH	Community		PMMoV <sup>a</sup>	Flow <sup>a</sup> , Population estimate <sup>a</sup> , pH <sup>a</sup> , Temp <sup>a</sup> , TSS <sup>a</sup>	Solid	Adjusted	✓
Swift, et. al.	2023	SC	Community	BRSV <sup>b</sup>	PMMoV <sup>a</sup>		Liquid	Raw	✓
Swift, et. al.	2022	Columbia, SC	Community	BRSV <sup>b</sup>			Liquid	Raw	-
Terry, et.al.	2022	King County, WA	Community			Flow-population estimate <sup>a</sup>	Liquid	Adjusted	-
Thafkali, et. al.	2024	New Orleans, LA	Building		PMMoV <sup>a</sup>		Both	Adjusted	-
Vadde, et. al.	2022	Bexar County, TX	Community	BCoV <sup>b</sup>	HF183 <sup>b</sup>		Solid	Raw	✓
Wartell, et. al.	2022	US	Community	BRSV <sup>b</sup>	PMMoV <sup>a</sup>		Liquid	Raw, Adjusted	✓
Wartell, et. al.	2022	MD	University	BRSV <sup>b</sup>			Liquid	Raw	-
Weidhaas, et. al.	2021	UT	Community	MHV <sup>a</sup>		Flow-population estimate <sup>a</sup>	Liquid	Adjusted	✓
Welling, et. al.	2022	Durham, NC	University	BCoV <sup>b</sup>			Liquid	Statistical analysis only	✓
West, et. al.	2023	Detroit, MI	Community	Phi6 <sup>b</sup>			Liquid	Raw	-
Wilder, et. al.	2021	NY	Community	BCoV <sup>b</sup> , SARS-CoV2 <sup>b</sup>	CrAss <sup>b</sup>		Solid	Raw	✓
Wolfe, et. al.	2021	CA, IL, NY	Community	BCoV <sup>a</sup>	PMMoV <sup>a</sup>		Solid	Raw, Adjusted	✓
Wolfe, et. al.	2021	CA	Community		PMMoV <sup>a</sup>	TSS <sup>a</sup>	Solid	Raw, Adjusted	✓
Wright, et. al.	2022	US	University	MHV <sup>b</sup>		Flow-population estimate <sup>a</sup>	Liquid	Adjusted	✓
Wu, et. al.	2021	US	Community	MHV <sup>b</sup> , PMMoV <sup>b</sup>		Flow <sup>a</sup>	Liquid	Raw, Adjusted	✓
Wu, et. al.	2021	MA	Community	MHV <sup>b</sup>	PMMoV <sup>a</sup>		Liquid	Adjusted	✓
Wu et. al.	2020	MA	Community		PMMoV <sup>a</sup>		Both	Raw, Adjusted	-
Yang, et. al.	2024	New York, NY	Community			Flow-population estimate <sup>a</sup>	-	Adjusted	✓

Zambrana, et. al.	2022	Stanford, CA	University	B2M <sup>b</sup> , PMMoV <sup>a</sup>		Solid	Raw	✓
Zhan, et. al.	2022	FL	Community, University		PMMoV <sup>a</sup> , B2M <sup>a</sup> , F <sup>+</sup> <sup>a</sup>	Both	Raw, Adjusted	✓
Zhan, et. al.	2023	FL	Community, University	B2M <sup>b</sup> , OC43 <sup>b</sup>	PMMoV <sup>a</sup> , B2M <sup>a</sup> , F <sup>+</sup> <sup>a</sup>	Solid	Raw, Adjusted	✓
Zhao, et. al.	2023	Detroit, MI, b	Community	Phi6 <sup>b</sup>	Flow-population estimate <sup>a</sup> , TSS <sup>a</sup>	Solid	Raw, Adjusted	✓
Zhao, et. al.	2022	Detroit, MI	Community	Phi6 <sup>b</sup>	Flow <sup>a</sup>	Solid	Raw, Adjusted	✓
Zhao, et. al.	2023	Detroit, MI	Community	Phi6 <sup>b</sup>		Solid	Raw	✓

Note: \*Indicates multiple protocols utilized and technique was used for only a portion of the data. <sup>a</sup> Indicates the normalization technique was used to adjust SARS-CoV2 concentrations. <sup>b</sup> Indicates threshold value only, includes those that exclude samples due to insufficient detection and method validation. <sup>†</sup> Authors do not specify methodology of technique.

Table S3. Records assessed for full text eligibility that were excluded from this analysis and the reason for exclusion.

Study ID	Year	Title	Reason for Exclusion
Abelson, et. al.	2023	COVID-19 cases and mortality were modelled with respect to SARS-CoV-2 ...	No WW
Aberi, et. al.	2021	Quest for Optimal Regression Models in SARS-CoV-2 Wastewater ...	No SARS
Acosta, et. al.	2021	A multicenter study investigating SARS-CoV-2 in tertiary-care ...	No WW
Adams, et. al.	2024	The National Wastewater Surveillance System (NWSS): From inception ...	Review
Aghababaei, et. al.	2023	The fate of SARS-CoV-2 viral RNA in coastal New England wastewater ...	Review
Ahmed, et. al.	2022	Intraday variability of indicator and pathogenic viruses in ...	No normalization
Ahmed, et. al.	2020	Comparative analysis of Adsorption-Extraction (AE) and Nanotrap ...	No normalization
Ahmed, et. al.	2021	Influence of membrane pore-size on the recovery of endogenous ...	No normalization
Ahmed, et. al.	2020	SARS-CoV-2 RNA monitoring in wastewater as a potential early ...	No SARS
Ahmed, et. al.	2023	Surveillance of SARS-CoV-2 RNA in wastewater: Methods optimization ...	No normalization
Ahmed, et. al.	2021	Evaluation of process limit of detection and quantification ...	Review
Ahmed, et. al.	2021	Comparative analysis of rapid concentration methods for the ...	No SARS
Ahmed, et. al.	2020	Comparison of virus concentration methods for the RT-qPCR-based ...	No WW
Ahmed, et. al.	2023	Decay of SARS-CoV-2 and surrogate murine hepatitis virus RNA ...	No SARS
Ahmed, et. al.	2022	RT-qPCR and ATOplex sequencing for the sensitive detection of ...	Review
Ai, et. al.	2021	Wastewater-based epidemiology for tracking COVID-19 trend and ...	No normalization

	202		
Ai, et.al.	2	Application of machine learning for multi-community COVID-19 ...	No normalization
Alamin, et. al.	202 4	Reduction of SARS-CoV-2 by biological nutrient removal and disinfection ...	No normalization
Alamin, et. al.	202 3	Improved passive sampling methods for wastewater to enable more ...	No normalization
Al-Duroobi, et. al.	202 2	Wastewater-Based Surveillance of Sars-Cov-2 for Monitoring Covid-19 ...	No normalization
Alhama, et. al.	202 2	Monitoring COVID-19 through SARS-CoV-2 quantification ...	No normalization
Ali, et. al.	202 1	Occurrence of various viruses and recent evidence of SARS-CoV-2 ...	No normalization
Alygizakis, et. al.	202 1	Analytical methodologies for the detection of SARS-CoV-2 in ...	No normalization
Amman, et. al.	202 2	Wastewater is a robust proxy for monitoring circulating SARS-CoV-2 ...	No normalization
Amman, et. al.	202 2	National-scale surveillance of emerging SARS-CoV-2 variants ...	Model only
Amoah, et. al.	202 2	Effect of selected wastewater characteristics on estimation ...	Not US
Anand, et. al.	202 4	NYC metropolitan wastewater reveals links between SARS-CoV-2 ...	Review
Anand, et.al.	202 1	A review of the presence of SARS-CoV-2 RNA in wastewater and ...	No normalization
Ando, et. al.	202 2	The Efficient and Practical virus Identification System with ...	No normalization
Ando, et. al.	202 3	Wastewater-based prediction of COVID-19 cases using a highly ...	Review
Antkiewicz, et. al.	202 4	Wastewater-based protocols for SARS-CoV-2: insights into virus ...	No normalization
Arabzadeh, et. al.	202 1	Data filtering methods for SARS-CoV-2 wastewater surveillance	Review
Armenise, et. al.	202 4	Adjusting for dilution in wastewater using biomarkers: A practical ...	Model only
Armstrong, et. al.	202 2	U.S. Sewer Data Warns of a New Bump in Covid Cases After Lull.	Review
Armstrong, et. al.	202 2	The U.S. Is Expanding Its Hunt for Early Warnings of Covid in ...	No normalization
Arts, et. al.	202 3	Longitudinal and quantitative fecal shedding dynamics of SARS-CoV-2, ...	Review
Asian, et. al.	202 0	Sewage Monitoring in Rural Communities: A Powerful Strategy ...	Review
Augusto, et. al.	202 2	Sampling strategies for wastewater surveillance: Evaluating ...	No normalization
Baaijens, et. al.	202 2	Lineage abundance estimation for SARS-CoV-2 in wastewater using ...	No SARS
Babler, et. al.	202 3	Comparison of electronegative filtration to magnetic bead-based ...	Review
Babler, et. al.	202 2	Degradation rates influence the ability of composite samples ...	Review
Bayati, et. al.	202 4	The different adsorption-degradation behaviors of SARS-CoV-2 ...	No normalization
Baz Lomba, et. al.	202 4	Effectiveness of environmental surveillance of SARS-CoV-2 as ...	No SARS
Bertanza, et. al.	202 2	Improving the quality of wastewater treatment plant monitoring ...	No SARS
Bhattacharya, et. al.	202 1	Prevalence of SARS-CoV-2 in Communities Through Wastewater Surveillance-a ...	Review
Bibby, et. al.	202 1	Making waves: Plausible lead time for wastewater based epidemiology ...	Review

Bischel, et. al.	0	Model training periods impact estimation of COVID-19 incidence	Model only
	202		
Bivins, et. al.	2	Passive sampling to scale wastewater surveillance of infectious ...	Review
	202		
Bivins, et. al.	1	Variability in RT-qPCR assay parameters indicates unreliable ...	Model only
	202		
Bivins, et. al.	2	Building-level wastewater surveillance using tampon swabs and ...	Review
	202		
Boehm, et. al.	2	Regional replacement of SARS-CoV-2 variant BA. 1 with BA. 2 ...	No SARS
	202		
Bognich, et. al.	4	Fate-and-transport modeling of SARS-CoV-2 for rural wastewater-based ...	No SARS
	202		
Bohrerova, et. al.	3	Ohio Coronavirus Wastewater Monitoring Network: implementation ...	No normalization
	202		
Boogaerts, et. al.	4	Current state and future perspectives on de facto population ...	No WW
	202		
Bowes, et.al.	2	A framework for wastewater sample collection from a sewage cleanout ...	No WW
	202		
Breadner, et. al.	3	A comparative analysis of the partitioning behaviour of SARS-CoV-2 ...	No normalization
	202		
Breadner, et. al.	3	The Apparent Partitioning Behaviour of SARS-CoV-2 RNA in Municipal ...	No SARS
	202		
Burnet, et. al.	3	Persistence of endogenous RNA biomarkers of SARS-CoV-2 and PMMoV ...	Model only
	202		
Calderón-Franco , et. al.	2	Monitoring SARS-CoV-2 in sewage: Toward sentinels with analytical ...	Review
	202		
Câmara, et. al.	3	Detecting SARS-CoV-2 in sludge samples: A systematic review	No WW
	202		
Cao, et. al.	1	On forecasting the community-level COVID-19 cases from the concentration ...	Model only
	202		
Castro-Gutierrez, et. al.	2	Monitoring occurrence of SARS-CoV-2 in school populations: A ...	No normalization
	202		
Ceolotto, et. al.	4	Assessment of restriction measures implemented during COVID ...	No normalization
	202		
Cervantes-Avilés, et. al.	1	Approaches applied to detect SARS-CoV-2 in wastewater and perspectives ...	No WW
	202		
Champredon, et. al.	4	ern: An R package to estimate the effective reproduction number ...	No normalization
	202		
Chassalevris, et. al.	2	SARS-CoV-2 wastewater monitoring using a novel PCR-based method ...	No WW
	202		
Chen, et. al.	4	Wastewater-based epidemiology for covid-19 surveillance: A survey	Review
	202		
Chik, et. al.	2	Design and Validation of Sample Splitting Protocol for Comparison ...	Review
	202		
Child, et. al.	3	Optimised protocol for monitoring SARS-CoV-2 in wastewater using ...	Review
	202		
Choi, et. al.	3	Estimating the prevalence of COVID-19 cases through the analysis ...	No SARS
	202		
Choi, et. al.	3	Performance Evaluation of PMMV as an internal control for the ...	Other
	202		
Choi, et. al.	3	Performance Evaluation of PMMV as an internal control for the ...	No WW
	202		
Chowdhury, et. al.	4	Effectiveness of Wastewater-Based Epidemiology as an Early Warning ...	Review
	202		
Ciannella, et. al.	3	Recent progress on wastewater-based epidemiology for COVID-19 ...	No SARS
	202		
Clark, et. al.	3	Wastewater pandemic preparedness: Toward an end-to-end pathogen ...	Not US
	202		
Cluzel, et. al.	2	A nationwide indicator to smooth and normalize heterogeneous ...	Review

	202		
Cluzel, et. al.	1	Mathematical modeling and adequate environmental sampling plans ...	Review
	202		
Coil, et. al.	2	SARS-CoV-2 RNA Is Readily Detectable at Least 8 Months after ...	Review
	202		
Collivignarelli, et. al.	0	SARS-CoV-2 in sewer systems and connected facilities	No normalization
	202		
Conway, et. al.	4	Chronic shedding of a SARS-CoV-2 Alpha variant in wastewater	No normalization
	202		
Cricelio, et. al.	3	Bayesian sequential approach to monitor COVID-19 variants through ...	No normalization
	202		
Cutrupi, et. al.	3	Evaluation of concentration procedures, sample pre-treatment, ...	No normalization
	202		
Dafalla, et. al.	1	Improving SARS-CoV-2 analyses from wastewater	Duplicate
	202		
Dai, et. al.	4	Statistical framework to support the epidemiological interpretation ...	Review
	202		
Dai, et. al.	4	A Bayesian framework for modeling COVID-19 case numbers through ...	No normalization
	202		
Dai, et. al.	2	A Bayesian framework for modeling COVID-19 case numbers through ...	Not US
	202		
Daigle, et. al.	2	A sensitive and rapid wastewater test for SARS-COV-2 and its ...	No normalization
	202		
D'Aoust, et. al.	1	Catching a resurgence: Increase in SARS-CoV-2 viral RNA identified ...	No normalization
	202		
D'Aoust, et. al.	1	Catching a resurgence: Increase in SARS-CoV-2 viral RNA identified ...	Review
	202		
D'Aoust, et. al.	0	Quantitative analysis of SARS-CoV-2 RNA from wastewater solids ...	Review
	202		
de Araújo, et. al.	2	Long-term monitoring of SARS-CoV-2 RNA in sewage samples from ...	Review
	202		
Delatolla, et. al.	1	COVID-19 monitoring in rural communities: First comparison of ...	Duplicate
	202		
Deng, et. al.	2	Sars-CoV-2 Wastewater Surveillance at North Carolina Agricultural ...	Review
	202		
Di, et. al.	3	Comparing solid-based concentration methods for rapid and efficient ...	No normalization
	202		
Ding, et. al.	4	Comparison of RT-ddPCR and RT-qPCR platforms for SARS-CoV-2 ...	Review
	202		
Donner, et. al.	1	Wastewater monitoring for SARS-CoV-2	Duplicate
	202		
Drake, et. al.	4	Phylogenomic early warning signals for SARS-CoV-2 epidemic waves	No normalization
	202		
Dumke, et. al.	1	Evaluation of Two Methods to Concentrate SARS-CoV-2 from Untreated ...	No normalization
	202		
Dutta, et. al.	2	Wastewater-based epidemiology: a new frontier for tracking environmental ...	No SARS
	202		
Endo, et. al.	4	Explaining the impact of mutations on quantification of SARS-CoV-2 ...	Duplicate
	202		
Endo, et. al.	4	Enabling Quantitative Comparison of Wastewater Surveillance ...	Model only
	202		
Ensor, et. al.	4	Online trend estimation and detection of trend deviations in ...	No normalization
	202		
Fahrenfeld, et. al.	2	Year-long wastewater monitoring for SARS-CoV-2 signals in combined ...	No normalization
	202		
Faraway, et. al.	2	Challenges in realizing the potential of wastewater-based epidemiology ...	Review
	202		
Farkas, et. al.	0	Wastewater and public health: the potential of wastewater surveillance ...	Review
	202		
Farkas, et. al.	1	Concentration and Quantification of SARS-CoV-2 RNA in Wastewater ...	No WW

	202		
Farkas, et. al.	4	Near-source passive sampling for monitoring viral outbreaks ...	Review
Fatima, et. al.	202 3	Guidelines in Wastewater-based Epidemiology of SARS-CoV-2 with ...	No normalization
Fazil, et. al.	202 3	Normalization Practices for SARS-CoV-2 Data in Wastewater-based ...	No normalization
Feng, et. al.	202 3	Intensity of sample processing methods impacts wastewater SARS-CoV-2 ...	Review
Ferreira, et. al.	202 2	Evaluation of methods and processes for robust monitoring of ...	Review
Fielder, et. al.	202 1	Wastewater tracks community infection.	No WW
Fitzgerald, et. al.	202 1	Site specific relationships between COVID-19 cases and SARS-CoV-2 ...	Review
Flood, et. al.	202 3	Understanding the efficacy of wastewater surveillance for SARS-CoV-2 ...	No normalization
Focosi, et. al.	202 4	Online dashboards for SARS-CoV-2 wastewater-based epidemiology.	Review
Foladori, et. al.	202 4	Normalization of Viral Loads in Wastewater-Based Epidemiology ...	Review
Folkes, et. al.	202 4	Source to sink wastewater surveillance of severe acute respiratory ...	Not US
Folkes, et. al.	202 2	Campus source to sink wastewater surveillance of severe acute ...	Model only
Fontenele, et. al.	202 3	Wastewater surveillance uncovers regional diversity and dynamics ...	No SARS
Fuschi, et. al.	202 1	Wastewater-Based Epidemiology for Managing the COVID-19 Pandemic.	Review
Gagliano, et. al.	202 3	Wastewater-based epidemiology approach: The learning lessons ...	No normalization
Galani, et. al.	202 2	SARS-CoV-2 wastewater surveillance data can predict hospitalizations ...	Not US
Georgas, et. al.	202 3	Advancements in SARS-CoV-2 Testing: Enhancing Accessibility ...	No normalization
Gibas, et. al.	202 1	Implementing building-level SARS-CoV-2 wastewater surveillance ...	Review
Gonzalez, et. al.	202 1	Redesigning SARS-CoV-2 clinical RT-qPCR assays for wastewater ...	Review
Gosnell, et. al.	202 2	Wastewater Surveillance of SARS-CoV-2 on American University ...	Review
Green, et. al.	202 4	Community-scale surveillance of SARS-CoV-2: Optimizing sampling ...	Model only
Greenwald, et. al.	202 1	One-Step RT-qPCR for SARS-CoV-2 Wastewater Surveillance: NI, ...	Review
Gregory, et. al.	202 2	Genetic diversity and evolutionary convergence of cryptic SARS- ...	No normalization
Guérin-Rechdaoui, et. al.	202 2	Fate of SARS-CoV-2 coronavirus in wastewater treatment sludge ...	No normalization
Guerrero-Esteban, et. al.	202 2	Sensitive SARS-CoV-2 detection in wastewaters using a carbon ...	No normalization
Gupta, et. al.	202 3	Wastewater genomic surveillance captures early detection of ...	Duplicate
Gupta, et. al.	202 2	Wastewater Genomic Surveillance Captures Early Detection of ...	Not US
Gwenzi, W.	202 2	Wastewater, waste, and water-based epidemiology (WWW-BE): A ...	Review
Hamouda, et. al.	202 1	Wastewater surveillance for SARS-CoV-2: Lessons learnt from ...	No normalization
Haramoto, et. al.	202 2	Editorial: SARS-CoV-2 in water	No normalization

Harris-Lovett, et. al.	202 1	Wastewater Surveillance for SARS-CoV-2 on College Campuses: ...	No normalization
Hart, et. al.	202 0	Computational analysis of SARS-CoV-2/COVID-19 surveillance by ...	Not US
Hart, et. al.	202 3	Frequency and degradation of SARS-CoV-2 markers N1, N2, and ...	No normalization
Haskell, et. al.	202 3	Assessing the utility of passive sampling for building-scale ...	Review
Haskell, et. al.	202 4	Implementing an adaptive, two-tiered SARS-CoV-2 wastewater surveillance ...	No normalization
Hayase, et. al.	202 3	Near full-automation of COPMAN using a LabDroid enables high-throughput ...	Not US
Hayes, et. al.	202 2	Adsorption of SARS-CoV-2 onto granular activated carbon (GAC) ...	Not US
Hayes, et. al.	202 3	Enhanced detection of viruses for improved water safety	No WW
Hayes, et. al.	202 3	Simultaneous detection of SARS-CoV-2, influenza A, respiratory ...	No normalization
Hegazy, et. al.	202 4	Understanding the dynamic relation between wastewater SARS-CoV-2 ...	Duplicate
Hegazy, et. al.	202 2	Impact of coagulation on SARS-CoV-2 and PMMoV viral signal in ...	No normalization
Hegazy, et. al.	202 2	Minimizing Variability of SARS-CoV-2 Wastewater Measurements ...	Review
Heijnen, et. al.	202 1	Droplet digital RT-PCR to detect SARS-CoV-2 signature mutations ...	Model only
Helm, et. al.	202 2	Regional and temporal differences in the relation between SARS-CoV-2 ...	No WW
Helm, et. al.	202 3	Regional and temporal differences in the relation between SARS-CoV-2 ...	No SARS
Helm, et. al.	202 3	Regional and temporal differences in the relation between SARS-CoV-2 ...	Not US
Henriques, et. al.	202 3	Contribution of wastewater-based epidemiology to SARS-CoV-2 ...	Review
Hill, et. al.	202 3	Operationalizing an open-source dashboard for communicating ...	No WW
Hill, et. al.	202 3	Wastewater surveillance provides 10-days forecasting of COVID-19 ...	Review
Hinkle, et. al.	202 2	Comparison of RT-qPCR and digital PCR methods for wastewater-based ...	Not US
Hinz, et. al.	202 2	SARS-CoV-2 detection from the built environment and wastewater ...	No normalization
Hoar, et. al.	202 2	Assessment of Commonly Measured Wastewater Parameters to Estimate ...	No normalization
Hoar, et. al.	202 2	Monitoring SARS-CoV-2 in wastewater during New York City's ...	No normalization
Hoffman, et. al.	202 3	Using detrending to assess SARS-CoV-2 wastewater loads as a ...	No normalization
Holcomb, et. al.	202 4	Estimating Rates of Change to Interpret Quantitative Wastewater ...	Review
Holm, et. al.	202 3	SARS-CoV-2 wastewater concentration and linked longitudinal ...	No normalization
Holm, et. al.	202 2	Surveillance of RNase P, PMMoV, and CrAssphage in wastewater ...	Review
Holst, et. al.	202 2	Rapid implementation of high-frequency wastewater ...	No normalization
Hong, et. al.	202 1	Estimating the minimum number of SARS-CoV-2 infected cases needed ...	No normalization
Huang, et. al.	202 3	Optimization of correction factors for WBS in SARS-COV-2 uncertainty ...	Review

Hui, et. al.	2020	Paper-based devices for rapid diagnostics and testing sewage ...	Review
Hunawill, et. al.	2023	Assessment of Frequency, Degradation, Normalization, Inhibition, ...	No normalization
Hutchison, et. al.	2021	SARS-CoV-2 Wastewater Measurements Normalized Using Biomarkers	No normalization
Isaksson, et. al.	2022	Thw use of alternative normalization approaches to understand ...	Review
Isanovic, et. al.	2023	Integrated Wastewater Surveillance for SARS-CoV-2 and Antibiotic ...	Not US
Islam, et. al.	2022	The role of catchment population size, data normalization, and ...	Review
Islam, et. al.	2024	Pasteurization, storage conditions and viral concentration methods ...	No normalization
Jagadeesan, et. al.	2024	Wastewater-based proteomics: A proof-of-concept for advancing ...	No normalization
Jain, et. al.	2022	Long-term passive wastewater surveillance of SARS-CoV-2 for ...	Not US
Janssen, et. al.	2023	Lessons Learned from 2-Years of Sars-Cov-2 Wastewater Surveillance: ...	Review
Jarvie, et. al.	2023	Monitoring of COVID-19 in wastewater across the Eastern Upper ...	Not US
Jeng, et. al.	2023	Application of wastewater-based surveillance and copula time-series ...	No normalization
Jimenez, et.al.	2024	Protocol to longitudinally quantify SARS-CoV-2 RNA in wastewater ...	No normalization
Jin, et. al.	2024	Combining wastewater surveillance and case data in estimating ...	Model only
Jones, et. al.	2022	Estimating Wastewater Dilution Using Chemical Markers and Incomplete ...	Review
Jones, et. al.	2022	Critical Evaluation of Different Passive Sampler Materials and ...	No normalization
Jones, et. al.	2024	Critical Evaluation of Different Passive Sampler Materials and ...	Not US
Juel, et. al.	2023	Development and Optimization of Virus Concentration and Detection ...	No WW
KabdaÅŸIÄ±, et. al.	2021	Concentration techniques tailored for the detection of SARS-CoV-2 ...	Review
Kanamori, et. al.	2024	SARS-CoV-2 detection in pediatric dental clinic wastewater reflects ...	No normalization
Kanneganti, et. al.	2022	Estimating sewage flow rate in Jefferson County, Kentucky, using ...	No normalization
Kantor, et. al.	2022	Operationalizing a routine wastewater monitoring laboratory ...	No normalization
Kantor, et. al.	2021	Challenges in measuring the recovery of SARS-CoV-2 from wastewater	Review
Kaplan, et. al.	2021	Aligning SARS-CoV-2 indicators via an epidemic model: application ...	No normalization
Kapoor, et. al.	2022	Wastewater surveillance for SARS-CoV-2 to support return to ...	No normalization
Karami, et. al.	2022	SARS-CoV-2 in municipal wastewater treatment plant, collection ...	No normalization
Kasprzyk-Hordern, et. al.	2023	Wastewater-based epidemiology for comprehensive community health ...	Model only
Katayama, et. al.	2023	COPMAN: A novel high-throughput and highly sensitive method ...	Model only
Kazenelson, et. al.	2023	Detection of SARS-CoV-2 RNA in wastewater from an enclosed college ...	Review
Keck, et. al.	2023	Wastewater Surveillance for SARS-CoV-2 at Long-Term Care Facilities: ...	Review

Keck, et. al.	2023	Wastewater Surveillance-"Messy" Science With Public Health Potential	Model only
Keshaviah, et.al.	2021	Developing a Flexible National Wastewater Surveillance System ...	No WW
Kevill, et. al.	2022	A comparison of precipitation and filtration-based SARS-CoV-2 ...	No SARS
Kevill, et. al.	2022	Assessment of two types of passive sampler for the efficient ...	Model only
Kim, et. al.	2022	Optimizing Application of Wastewater-Based Epidemiology for ...	Model only
Kirby, et. al.	2021	Using Wastewater Surveillance Data to Support the COVID-19 Response ...	No normalization
Kiyar, et. al.	2023	Developing Wastewater-based Early Warning System for the Detection ...	Not US
Kopperi, et. al.	2021	Defining the methodological approach for wastewater-based epidemiological ...	Review
Kotay, et. al.	2022	Building-Level Wastewater Surveillance for SARS-CoV-2 in Occupied ...	No normalization
Kotlarz, et. al.	2023	Timing and Trends for Municipal Wastewater, Lab-Confirmed Case, ...	Not US
Krivoňáková, et. al.	2021	Mathematical modeling based on RT-qPCR analysis of SARS-CoV-2 ...	No normalization
Kumar, et. al.	2021	First comparison of conventional activated sludge versus root-zone ...	Review
Kumar, et. al.	2021	Lead time of early warning by wastewater surveillance for COVID-19: ...	Model only
Kumar, et. al.	2022	Unravelling the early warning capability of wastewater surveillance ...	Not US
Kumblathan, et. al.	2021	An efficient method to enhance recovery and detection of SARS-CoV-2 ...	No normalization
Kumblathan, et. al.	2023	Wastewater-Based Epidemiology for Community Monitoring of SARS-CoV-2: ...	Model only
Kuroita, et. al.	2024	Quantitative analysis of SARS-CoV-2 RNA in wastewater and evaluation ...	Review
Lai, et. al.	2023	A time series based machine learning strategy for wastewater-based ...	Review
Laicans, et. al.	2024	Precision and Accuracy Limits of Wastewater-Based Epidemiology: Lessons ...	Not US
Lamba, et. al.	2022	SARS-CoV-2 infection dynamics and genomic surveillance reveals ...	No normalization
Lambert-Slosarska, et. al.	2023	Use of passive samplers for the capture of SARS-CoV-2 and other ...	Review
Langan, et. al.	2022	Quantitative reverse transcription pcr surveillance of SARS-CoV-2 ...	Review
Langeveld, et. al.	2023	Normalisation of SARS-CoV-2 concentrations in wastewater: The ...	Other
Langeveld, et. al.	2021	Normalisation of SARS-CoV-2 concentrations in wastewater: The ...	Model only
Langone, et. al.	2021	SARS-CoV-2 in water services: Presence and impacts	No WW
Lara-Jacobo, et. al.	2021	First detection of SARS-CoV-2 proteins in wastewater samples ...	No normalization
Larsen, et. al.	2020	Tracking COVID-19 with wastewater	Review
Lastra, et. al.	2022	SARS-CoV-2 detection in wastewater as an early warning indicator ...	No normalization
Lee, et. al.	2023	Space/time estimation of precipitation and evaluation of daily ...	Not US
Lee, et. al.	2021	Early warning and rapid public health response to prevent COVID-19 ...	Not US

Lesne, et. al.	2021	Wastewater monitoring in support of health surveillance of the ...	Not US
Lewis-Borrell, et. al.	2023	Robust smoothing of left-censored time series data with a dynamic ...	No normalization
Li, et. al.	2023	Wastewater-based epidemiology predicts COVID-19-induced weekly ...	Not US
Li, et. al.	2024	Correlation between SARS-CoV-2 RNA concentration in wastewater ...	No normalization
Li, et. al.	2024	Detecting SARS-CoV-2 variants in wastewater and their correlation ...	Model only
Li, et. al.	2022	Uncertainties in estimating SARS-CoV-2 prevalence by wastewater-based ...	Model only
Li, et. al.	2023	A spatio-temporal framework for modelling wastewater concentration ...	No WW
Li, et. al.	2021	Integrating wastewater and randomised prevalence survey data ...	Model only
Li, et. al.	2022	Monitoring of SARS-CoV-2 in sewersheds with low COVID-19 cases ...	No WW
Li, et. al.	2023	Effect of wastewater collection and concentration methods on ...	Not US
Lin, et. al.	2023	Optimizing campus-wide COVID-19 test notifications with interpretable ...	Model only
Lisboa, et. al.	2024	Beyond Campus Borders: Wastewater Surveillance Sheds Light on ...	No normalization
Liu, et. al.	2023	Design and Simulation of Quarantine Policy and Wastewater Early ...	No WW
Liu, et. al.	2023	A sensitive, simple, and low-cost method for COVID-19 wastewater ...	No normalization
Liu, et. al.	2024	Quantitatively assessing early detection strategies for mitigating ...	Duplicate
Liu, et. al.	2022	An infodemiological framework for tracking the spread of SARS-CoV-2 ...	No normalization
Liu, et. al.	2022	Longitudinal Fecal Shedding of SARS-CoV-2, Pepper Mild Mottle ...	Not US
López-Peñalverr, et. al.	2023	Predictive potential of SARS-CoV-2 RNA concentration in wastewater ...	No normalization
Lott, et. al.	2024	Toilet Talk: Evaluating Tools of the Trade for Wastewater-Based ...	No normalization
Lott, et. al.	2022	Comparison of three tiled amplicon sequencing approaches for ...	No normalization
Lou, et. al.	2022	Direct comparison of RT-ddPCR and targeted amplicon sequencing ...	Review
Lowe, et. al.	2022	Using wastewater-based epidemiology as a potential instrument ...	No normalization
Lu, et. al.	2022	University SARS-CoV-2 wastewater surveillance and vaccination ...	Review
Lucansky, et. al.	2023	Comparison of the methods for isolation and detection of SARS-CoV-2 ...	No normalization
Lucansky, et. al.	2023	Comparison of the methods for isolation and detection of SARS-CoV-RNA ...	No normalization
Ma, et. al.	2024	Data-driven systematic analysis of waterborne viruses and health ...	No normalization
Machelor, et. al.	2022	Pima County to use wastewater data as COVID-19 'early warning ...	No normalization
Mackul'ak, et. al.	2021	Wastewater-Based Epidemiology as an Early Warning System for ...	No normalization
Maere, et. al.	2022	Normalization practices for SARS-CoV-2 data in wastewater-based ...	No normalization
Maida, et. al.	2024	Wastewater-Based Epidemiology for Early Warning and Surveillance ...	No normalization

Malla, et. al.	202 4	Beyond COVID-19: Wastewater-based epidemiology for multipathogen ...	No normalization
Mangwana, et. al.	202 4	Delineating the transmission and evolution of SARS-CoV-2 through ...	No normalization
Mantilla-Calderon, et. al.	202 2	Emerging investigator series: meta-analyses on SARS-CoV-2 viral ...	No normalization
Manuel, et. al.	202 1	Wastewater-Based Surveillance of Sars-Cov-2: Short-Term Projection, ...	No normalization
Manuel, et. al.	202 4	The role of wastewater testing for SARS-CoV-2 surveillance	No normalization
Marchini, et. al.	202 3	New RT-PCR Assay for the Detection of Current and Future SARS-CoV-2 ...	No normalization
Marin-Ramirez, et. al.	202 4	Predicting wastewater treatment plant influent in mixed, separate, ...	No normalization
Martin, et. al.	202 3	Optimization of sewage sampling for wastewater-based epidemiology ...	No normalization
Mattei, et. al.	202 3	Analysis of SARS-CoV-2 in wastewater for prevalence estimation ...	No normalization
Mazumder, et.al.	202 2	Sewage surveillance for SARS-CoV-2: Molecular detection, quantification, ...	No normalization
Mbogo, et. al.	202 3	Monitoring of Escherichia coli and SARS-CoV-2 in Municipal Wastewater ...	No normalization
McCalder , et. al.	202 3	Comparing methods for recovering genomic RNA from SARS-CoV-2 ...	No normalization
McClary-Gutierrez, et. al.	202 1	SARS-CoV-2 Wastewater Surveillance for Public Health Action.	No normalization
McClary-Gutierrez, et. al.	202 1	Standardizing data reporting in the research community to enhance ...	No normalization
McLerran, et. al.	202 2	Long-Term Surveillance Testing of SARS-CoV-2: Implementation ...	No normalization
McMahan, et. al.	202 2	Predicting COVID-19 infected individuals in a defined population ...	No normalization
McSparron, et. al.	202 3	Wastewater Flow Estimation, Using Localised Rainfall Data, for ...	No normalization
Medema, et. al.	202 0	Implementation of environmental surveillance for SARS-CoV-2 ...	No normalization
Mendonça-Gomes, et. al.	202 1	Shedding light on toxicity of SARS-CoV-2 peptides in aquatic ...	No normalization
Mercier, et. al.	202 4	Effective Method to Mitigate Impact of Rain or Snowmelt Sewer ...	No normalization
Michael-Kordatou, et. al.	202 0	Sewage analysis as a tool for the COVID-19 pandemic response ...	No normalization
Mogensen, et. al.	202 3	LISTENING TO OUR GUTS.	No normalization
Mohapatra, et. al.	202 3	The novel SARS-CoV-2 pandemic: Possible environmental transmission, ...	No normalization
Mohapatra, et. al.	202 1	Wastewater surveillance of SARS-CoV-2 and chemical markers in ...	No normalization
Mohapatra, et. al.	202 3	Wastewater surveillance of SARS-CoV-2 and chemical markers in ...	No normalization
Mohring, et. al.	202 4	Estimating the COVID-19 prevalence from wastewater.	No normalization
Monteiro, et. al.	202 2	Recovery of SARS-CoV-2 from large volumes of raw wastewater ...	No normalization
Moon, et. al.	202 4	A gold standard dataset and evaluation of methods for lineage ...	No normalization
Morecchiato, et. al.	202 4	Evaluation of different molecular systems for detection and ...	No normalization
Mullins, et. al.	202 4	Polyethylenimine mediated recovery of SARS-CoV-2 and total viral ...	No normalization

Munteanu, et. al.	202 3	SARS-CoV-2 Wastewater Genomic Surveillance: Approaches, Challenges, ...	No normalization
Muralidharan, et. al.	202 4	Evaluating Health Equity in Sub-City Wastewater Monitoring of ...	No normalization
Natarajan, et. al.	202 1	Standardized preservation, extraction and quantification techniques ...	No normalization
Naughton, et. al.	202 3	Show us the data: global COVID-19 wastewater monitoring efforts, ...	No normalization
Nauta, et. al.	202 3	Early detection of local SARS-CoV-2 outbreaks by wastewater ...	No normalization
Navarro, et. al.	202 1	SARS-CoV-2 detection in wastewater using multiplex quantitative ...	No WW
Neault, et. al.	202 0	SARS-CoV-2 protein in wastewater mirrors COVID-19 prevalence	No normalization
Nelson, et. al.	202 2	What poo tells us: wastewater surveillance comes of age amid ...	Duplicate
Neyra, et. al.	202 3	Establishing a statewide wastewater surveillance system in response ...	No normalization
Nguyen, et. al.	202 1	Label-free liquid crystal biosensor based on specific DNA aptamer ...	No normalization
Ni, et. al.	202 1	Novel multiplexed amplicon-based sequencing to quantify SARS-CoV-2 ...	Model only
Nørgaard, et. al.	202 3	Detection of SARS-CoV-2 in wastewater: normalization for dilution ...	No normalization
Novoa, et. al.	202 2	Wastewater and marine bioindicators surveillance to anticipate ...	Model only
Oh, et. al.	202 1	Regional and temporal variations affect the accuracy of variant-specific ...	No normalization
Okada, et. al.	202 4	Estimating the effective reproductive number $R_e$ for Covid-19: ...	No normalization
Olesen, et. al.	202 1	Making waves: defining the lead time of wastewater-based epidemiology ...	No normalization
Olesen, et. al.	202 1	Applications of wastewater-based epidemiology as a leading indicator ...	No normalization
Olivares-Pacheco, et. al.	202 2	Detection and quantification of sars-cov-2 wastewater treatment ...	Not US
Oloye, et. al.	202 3	Understanding common population markers for SARS-CoV-2 RNA normalization ...	Review
Oloye, et. al.	202 3	Understanding common population markers for SARS-CoV-2 RNA normalization ...	Not US
Organization, et. al.	202 2	Wastewater surveillance of SARS-CoV-2: questions and answers ...	Review
Osman, et. al.	202 3	Assessment of biomarkers for normalization of SARS-CoV-2 concentrations ...	Not US
Pant, et. al.	202 4	Mathematical assessment of wastewater-based epidemiology to ...	Review
Pardo-Figueroa, et. al.	202 2	Spatiotemporal Surveillance of SARS-CoV-2 in the Sewage of Three ...	Model only
Parkins, et. al.	202 4	Wastewater-based surveillance as a tool for public health action: ...	Not US
Peinado, et. al.	202 2	Improved methods for the detection and quantification of SARS-CoV-2 ...	Not US
Pellegrinelli, et. al.	202 3	Wastewater-based epidemiology revealed in advance the increase ...	Not US
Pellett, et. al.	202 4	Multi-factor normalisation of viral counts from wastewater improves ...	Model only
Pérez-Cataluña, et. al.	202 2	Spatial and temporal distribution of SARS-CoV-2 diversity circulating ...	Not US
Perez-Zabaleta, et. al.	202 3	Long-term SARS-CoV-2 surveillance in the wastewater of Stockholm: ...	Not US

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Petala, et. al.	2	Relating SARS-CoV-2 shedding rate in wastewater to daily positive ...	Not US
	202		
Petros, et. al.	2	Multimodal surveillance of SARS-CoV-2 at a university enables ...	Not US
	202		
Phan, et. al.	3	Prolonged viral shedding from noninfectious individuals confounds ...	Not US
	202		
Phan, et. al.	3	Making waves: Integrating wastewater surveillance with dynamic ...	Not US
	202		
Phan, et. al.	3	A simple SEIR-V model to estimate COVID-19 prevalence and predict ...	No normalization
	202		
Phillips, et. al.	2	SARS-CoV-2 cycle threshold (Ct) values predict future COVID-19 ...	Not US
	202		
Philo, et.al.	4	Wastewater surveillance for bacterial targets: current challenges ...	Not US
	202		
Pileggi, et. al.	2	Quantitative trend analysis of SARS-CoV-2 RNA in municipal wastewater ...	Not US
	202		
Pillay, et. al.	2	Enhanced Molecular Methods for the Detection and Quantification ...	Not US
	202		
Pillay, et. al.	2	Potential and challenges encountered in the application of wastewater-based ...	Not US
	202		
Pino, et. al.	1	Detection of SARS-CoV-2 in wastewater is influenced by sampling ...	Not US
	202		
Pino, et. al.	0	High Throughput SARS-COV-2, PMMOV, and BCoV quantification in ...	Not US
	202		
Polo, et. al.	0	Making waves: Wastewater-based epidemiology for COVID-19 approaches ...	Not US
	202		
Polo, et. al.	4	Making waves: wastewater-based epidemiology for COVID-19 approaches ...	Not US
	202		
Porter, et. al.	2	SARS-CoV-2 wastewater surveillance at two university campuses: ...	Not US
	202		
Proverbio, et. al.	2	Model-based assessment of COVID-19 epidemic dynamics by wastewater ...	Not US
	202		
Puggioni, et. al.	3	Effect of hydraulic retention time on the electro-bioremediation ...	Not US
	202		
Qasmieh, et. al.	2	The prevalence of SARS-CoV-2 infection and other public health ...	Not US
	202		
Qiu, et. al.	2	Validating and optimizing the method for molecular detection ...	Not US
	202		
Rachmadi, et. al.	2	W-SPHERE (Wastewater-SARS Public Health Environmental Response) ...	Not US
	202		
Rainey, et. al.	4	Assessment of a mass balance equation for estimating community-level ...	Not US
	202		
Rashid, et. al.	4	A scoping review of global SARS-CoV-2 wastewater-based epidemiology ...	Not US
	202		
Rauch, et. al.	2	Estimating actual SARS-CoV-2 infections from secondary data	Not US
	202		
Rauch, et. al.	2	Data modelling recipes for SARS-CoV-2 wastewater-based epidemiology.	Not US
	202		
Reynolds, et. al.	4	Coprostanol as a Population Biomarker for SARS-CoV-2 Wastewater ...	Not US
	202		
Ribeiro, et.al.	3	Assessment of crAssphage as a biological variable for SARS-CoV-2 ...	Not US
	202		
Rioux, et. al.	1	Wastewater-based epidemiology: the crucial role of viral shedding ...	Not US
	202		
Rusiñol, et. al.	3	Monitoring waves of the COVID-19 pandemic: Inferences from WWTPs ...	Not US
	202		
Rusková, et. al.	4	Comparison of ordinary reverse transcription real-time polymerase ...	Not US
	202		
Ryon, et. al.	2	Influences of 23 different equations used to calculate gene ...	Not US

Safford, et. al.	2021	Wastewater analysis can be a powerful public health toolâ€”if ...	Not US
Saguti, et. al.	2021	Surveillance of wastewater revealed peaks of SARS-CoV-2 preceding ...	Not US
Saingam, et. al.	2021	Integrating socio-economic vulnerability factors improves neighborhood-scale ...	Not US
Saini, et. al.	2022	Wastewater-based epidemiology for novel Coronavirus detection ...	Not US
Sakarovitch, et. al.	2023	Monitoring of SARS-CoV-2 in wastewater: what normalisation for ...	Not US
Sala, et. al.	2023	Systematic Review on the Correlation Between SARS-CoV-2 Real-Time ...	Not US
Salmoiraghi, et. al.	2022	Viral surveillance of wastewater as a promising tool to assess ...	Not US
Salvo, et. al.	2021	One-Year Surveillance of SARS-CoV-2 and Rotavirus in Water Matrices ...	Not US
Sangkham, et. al.	2023	A review on detection of SARS-CoV-2 RNA in wastewater in light ...	Not US
Sanjak, et. al.	2024	Wastewater surveillance pilot at US military installations: ...	No WW
Schang, et. al.	2021	Passive sampling of SARS-CoV-2 for wastewater surveillance	No normalization
Schenk, et. al.	2023	Prediction of hospitalisations based on wastewater-based SARS-CoV-2 ...	Not US
Schenk, et. al.	2023	Integrating Wastewater-Based Epidemiology and Mobility Data ...	Not US
Schenk, et. al.	2020	Impact of drainage quantity on SARS-CoV-2 viral concentration	Review
Schmidt, et. al.	2023	Watcher in the wastewater	Not US
Schmitz, et. al.	2022	Hospital-onset COVID-19: Associations with population- and hospital-level ...	Not US
Schumann, et. al.	2022	SARS-CoV-2 infection dynamics revealed by wastewater sequencing ...	Not US
Schussman, et. al.	2022	Wastewater surveillance using ddPCR reveals highly accurate ...	Not US
Schussman, et. al.	2023	Wastewater surveillance using ddPCR accurately tracked Omicron ...	Not US
Scott, et. al.	2024	The inhibition and variability of two different RT-qPCR assays ...	Not US
Senaratna, et. al.	2022	Estimating COVID-19 cases on a university campus based on Wastewater ...	Not US
Shah, et. al.	2023	Wastewater surveillance to infer COVID-19 transmission: A systematic ...	Not US
Sharaby, et. al.	2021	Whole campus wastewater surveillance of SARS-CoV-2 for COVID-19 ...	Not US
Sharara, et. al.	2024	Wastewater network infrastructure in public health: applications ...	Not US
Sharma, et. al.	2021	Real-Time On-Site Monitoring of Viruses in Wastewater Using ...	Not US
Sharma, et. al.	2023	COVID-19 epidemiologic surveillance using wastewater	Not US
Sheets, et. al.	2023	Modeling Household Epidemics and Analysis of Links From Testing ...	Not US
Slegers, et. al.	2022	Forecasting SARS-CoV-2 Virus Load in Sewage Using Autoregression ...	Not US
Smith, et. al.	2022	Quantifying the relationship between sub-population wastewater ...	Not US
Smith, et. al.	2023	Baseline sequencing surveillance of public clinical testing, hospitals ...	No normalization

	202		
Soni,et.al.	2	Surveillance of omicron variants through wastewater epidemiology: ...	Not US
	202		
Sookaromdee, et. al.	2	Malacological Epidemiology of Opisthorchis spp. and Sewage Viral ...	Not US
Sosa-Hernández, et. al.	202		
	4	Extensive Wastewater-Based Epidemiology as a Resourceful Tool ...	Not US
soufi, et. al.	202		
	1	Highly efficient and sensitive membrane-based concentration ...	No normalization
Spurbeck, et. al.	202		
	4	Feasibility of neighborhood and building scale wastewater-based ...	No SARS
Starke, et. al.	202	Measuring SARS-CoV-2 RNA concentrations in neighborhood	
	1	wastewater	Not US
Steele, et. al.	202		
	3	Sources of variability in methods for processing, storing, and ...	Not US
Stockdale, et. al.	202	RNA-Seq of untreated wastewater to assess COVID-19 and emerging	
	4	...	Not US
Subroyen, et. al.	202		
	3	Evaluating storage conditions and enhancement strategies on ...	Review
Swain, et. al.	202	The Implementation and Utilization of Wastewater-Based	
	3	Epidemiology: ...	Review
Sweetapple, et. al.	202	Dynamic population normalisation in wastewater-based epidemiology	
	1	...	No WW
Sweetapple, et. al.	202		
	2	Population normalisation in wastewater-based epidemiology for ...	Review
Tamáš, et. al.	202	Wastewater Sequencing-An Innovative Method for Variant Monitoring	
	4	...	No normalization
Tandukar, et. al.	202		
	3	Application of wastewater-based epidemiology for monitoring ...	Not US
Tang, et. al.	202		
	4	Exploration on wastewater-based epidemiology of SARS-CoV-2: ...	Not US
Thakali, et. al.	202		
	3	Real-time evaluation of signal accuracy in wastewater surveillance ...	Not US
Thapar, et. al.	202		
	2	Influence of storage conditions and multiple freeze-thaw cycles ...	Not US
Tharak, et. al.	202	Longitudinal and Long-Term Wastewater Surveillance for COVID-19:	
	0	...	Not US
Thompson, et. al.	202	Making waves: Wastewater surveillance of SARS-CoV-2 for	
	3	population-based ...	Not US
Thongpradit, et. al.	202	SARS-CoV-2 Surveillance in Hospital Wastewater: CLEIA vs. RT-	
	1	qPCR	Not US
Torabi, et. al.	202		
	3	Wastewater-based surveillance models for COVID-19: A focused ...	Not US
Torabi, et. al.	202		
	3	Stabilization of SARS-CoV-2 RNA in wastewater via rapid RNA ...	Not US
Tran, et. al.	202	Identifying spatiotemporal trends of SARS-CoV-2 RNA in	
	4	wastewater: ...	Not US
Triska, et. al.	202	WAVES (Web-based tool for Analysis and Visualization of	
	3	Environmental ...	Review
Trujillo, et. al.	202		
	1	Protocol for safe, affordable, and reproducible isolation and ...	Not US
Van Poelvoorde, et. al.	202		
	1	Strategy and performance evaluation of low-frequency variant ...	Not US
Varkila, et. al.	202		
	3	Use of wastewater metrics to track COVID-19 in the US	Not US
Varkila, et. al.	202		
	3	Use of wastewater metrics to track COVID-19 in the US: a national ...	Not US
Vaughan, et. al.	202		
	3	An exploration of challenges associated with machine learning ...	Review
Wang, et. al.	202		
	0	Removal performance of SARS-CoV-2 in wastewater treatment by ...	No WW

	202		
Wang, et. al.	2	Removal performance of SARS-CoV-2 in wastewater treatment by ...	No normalization
Wang, et. al.	202	The amount of SARS-CoV-2 RNA in wastewater relates to the	
	3	development ...	Not US
Wang, et. al.	202	SARS-CoV-2 wastewater-based epidemiology in an enclosed	
	2	compound: ...	Not US
Wang, et. al.	202		
	2	High-throughput wastewater analysis for substance use assessment ...	Not US
Wang, et. al.	202		
	2	Early warning of a COVID-19 surge on a university campus based ...	Not US
Wani, et. al.	202	Wastewater-based epidemiology of SARS-CoV-2: Assessing	
	3	prevalence ...	Not US
Wannigama, et. al.	202	COVID-19 monitoring with sparse sampling of sewerred and non-	
	3	sewerred ...	Not US
Wen, et. al.	202		
	3	Stability and WBE biomarkers possibility of 17 antiviral drugs ...	Not US
West, et. al.	202		
	2	Sensitive detection of SARS-CoV-2 molecular markers in urban ...	No normalization
Westcott, et. al.	202	The Detection of Periodic Reemergence Events of SARS-CoV-2 Delta	
	2	...	Not US
White, et. al.	202		
	4	Wastewater based epidemiology as a surveillance tool during ...	Not US
Wilde, et. al.	202		
	2	Accounting for Dilution of SARS-CoV-2 in Wastewater Samples ...	Not US
Wilhelm, et. al.	202		
	3	Interlaboratory comparison using inactivated SARS-CoV-2 variants ...	Not US
Williams, et. al.	202	Detection of SARS-CoV-2 RNA in wastewater from dormitory	
	3	buildings ...	Not US
Williams, et. al.	202		
	4	Simultaneous detection and characterization of common respiratory ...	No WW
Wolfe, et. al.	202		
	2	Detection of SARS-CoV-2 variants mu, beta, gamma, lambda, delta, ...	Not US
Wolken, et. al.	202		
	3	Wastewater surveillance of SARS-CoV-2 and influenza in preK-12 ...	Review
Wong, et. al.	202		
	1	Evaluating the sensitivity of SARS-CoV-2 infection rates on ...	No normalization
World Health Organization	202		
	2	Environmental surveillance for SARS-CoV-2 to complement other ...	Not US
World Health Organization	202		
	3	Environmental surveillance for SARS-COV-2 to complement public ...	Not US
Wu, et. al.	202	Technical Framework for Wastewater-Based Epidemiology of SARS-	
	2	CoV-2: ...	Not US
Wu, et. al.	202	SARS-CoV-2 RNA concentrations in wastewater foreshadow	
	0	dynamics ...	Not US
Wu, et. al.	202		
	2	Conditional Independence Test with Neural Network	Not US
Wu, et. al.	202	Technical framework for wastewater-based epidemiology of SARS-	
	2	CoV-2 ...	Not US
Wu, et.al.	202	Technical framework for wastewater-based epidemiology of SARS-	
	1	CoV-2.	No normalization
Wurtzer, et. al.	202		
	2	SARS-CoV-2 genome quantification in wastewaters at regional ...	Not US
Wurtzer, et. al.	202		
	2	From Alpha to Omicron BA.2: New digital RT-PCR approach and ...	Review
Xiao, et. al.	202		
	2	Metrics to relate COVID-19 wastewater data to clinical testing ...	Not US
Xiao, et. al.	202		
	3	Surveillance of SARS-CoV-2: From Sewage to the Clinic	No normalization
Xu, et. al.	202		
	3	Evaluation of RT-qPCR Primer-Probe Sets to Inform Public Health ...	No normalization

Xu, et. al.	2022	Refining detection methods for emerging SARS-CoV-2 mutants in ...	Not US
Yaglom, et. al.	2022	One health genomic surveillance and response to a university-based ...	Not US
Yanaç, et. al.	2022	Detection of SARS-CoV-2 RNA throughout wastewater treatment ...	Not US
Yang, et. al.	2024	Preconcentration and detection of SARS-CoV-2 in wastewater: ...	Not US
Zahmatkesh, et. al.	2022	Review of method and a new tool for decline and inactive SARS-CoV-2 ...	Not US
Zamarreño, et. al.	2024	Wastewater-based epidemiology for COVID-19 using dynamic artificial ...	Review
Zhang, et. al.	2023	Analytical performance comparison of four SARS-CoV-2 RT-qPCR ...	Not US
Zhang, et. al.	2022	Ultra-fast and onsite interrogation of Severe Acute Respiratory ...	Not US
Zhang, et. al.	2022	Unlocking the Potential of Public Datasets: Wastewater-Based ...	Not US
Zhang, et. al.	2021	SARS-CoV-2 in wastewater: From detection to evaluation	No normalization
Zheng, et. al.	2023	Wastewater Surveillance Provides Spatiotemporal SARS-CoV-2 Infection ...	Model only
Zheng, et. al.	2024	A rapid, high-throughput, and sensitive PEG-precipitation method ...	Model only
Zhu, et. al.	2021	Early warning of COVID-19 via wastewater-based epidemiology: ...	Model only

Figure S1. Annual percentage of eligible studies that correlated SARS-CoV2 wastewater concentrations to clinical Covid-19 cases.

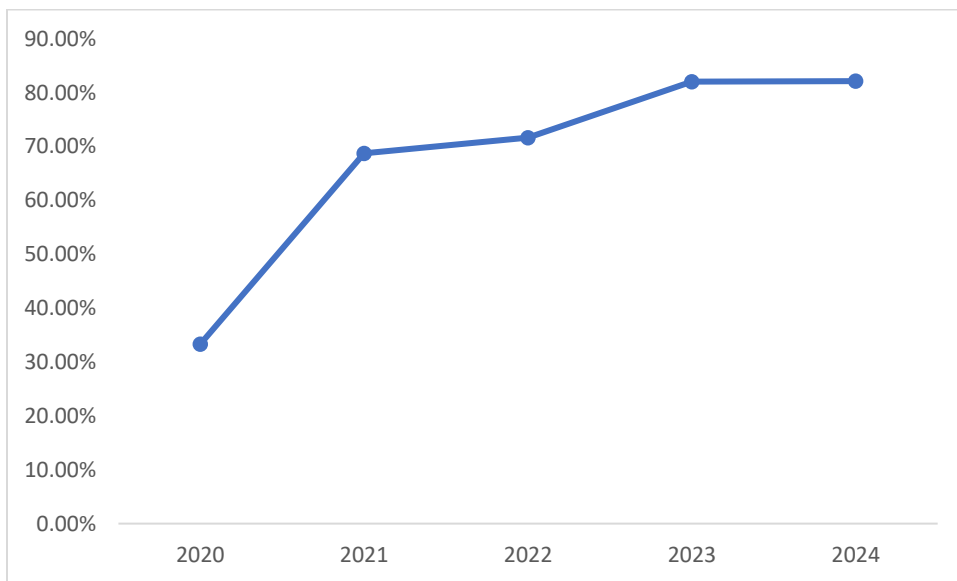


Table S4. Variability concern scores for included studies separated by objectives, recovery variability and sample variability. N/A indicates study did not employ technique.

Study ID	Variability Score
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Author	Year	Recovery variability techniques	Sample variability techniques
Abelson, et. al.	2023	4.5	3
Acer, et.al	2022	N/A	3
Ai, et. al.	2021	N/A	2.5
Al-Duroobi, et. al.	2023	2.5	1.5
Al-Duroobi, et. al.	2021	4.5	N/A
Al-Faliti, et. al.	2022	N/A	3
Amirali, et. al.	2024	N/A	N/A
Anderson-Coughlin, et. al.	2022	N/A	3
Anderson-Coughlin, et. al.	2021	3	1.5
Anneser, et. al.	2022	N/A	1.5
Ash, et. al.	2023	3	N/A
Baldwin, et. al.	2023	4.5	1.5
Barich, et. al.	2021	2.5	3
Barua, et. al.	2022	4.5	N/A
Bayati, et. al.	2022	3	3
Beattie, et. al.	2022	4.5	N/A
Bernard, et. al.	2022	4.5	N/A
Betancourt, et. al.	2021	4.5	N/A
Bhattarai, et. al.	2021	N/A	3
Bivins, et. al.	2021	2.5	1.5
Bivins, et. al.	2021	2.5	1.5
Boehm, et. al.	2023	4.5	1.5
Boehm, et. al.	2022	3.75	N/A
Bowes, et. al.	2021	N/A	3
Bowes, et. al.	2023	4.5	3
Boza, et. al.	2024	3.75	N/A
Brighton, et. al.	2024	3	N/A
Brooks, et. al.	2023	2.5	3
Cavany, et. al.	2022	2.5	N/A
Cha, et. al.	2023	4.5	1.5
Chan, et. al.	2024	N/A	1.5
Chan, et. al.	2023	4.5	1.5
Ciesielski, et. al.	2021	4.5	N/A
Cohen, et. al.	2022	4.5	3
Conway, et. al.	2023	N/A	1.5
Curtis, et. al.	2020	N/A	3
Daza-Torres, et. al.	2024	4.5	1.5
Daza-Torres, et. al.	2023	4.5	1.5
Duvallet, et. al.	2022	N/A	1.5
Fahrenfeld, et. al.	2022	N/A	1.5
Fahrenfeld, et. al.	2022	N/A	2.25
Feng, et. al.	2021	4.5	2.25
Fielding-Miller, et. al.	2023	N/A	N/A

Flood, et. al.	2023	N/A	3
Gerrity, et. al.	2021	2.5	N/A
Ghanbari, et. al.	2024	3.5	2.25
Gibas, et. al.	2021	4.5	N/A
Gonzalez, et. al.	2020	4.5	3
Goodwin, et. al.	2023	N/A	N/A
Graham, et. al.	2020	3.75	1.5
Greenwald, et. al.	2021	3.75	2.25
Grube, et. al.	2023	3.75	3
Haak, et. al.	2022	3.75	N/A
Han, et. al.	2024	N/A	1.5
Han, et. al.	2023	N/A	1.5
Hoar, et. al.	2022	4.5	3
Hoffman, et. al.	2023	N/A	3
Holm, et. al.	2024	N/A	1.5
Holm, et. al.	2022	4.5	1.5
Hopkins, et. al.	2023	N/A	3
Hsu, et. al.	2022	N/A	2
Huisman, et. al.	2022	3.75	3
Hutchison, et. al.	2022	N/A	2.17
Janssen, et. al.	2023	N/A	1.5
Jarvie, et.al	2022	3	N/A
Jeon, et. al.	2023	N/A	1.5
Jiang, et. al.	2022	N/A	3
Jimenez, et. al.	2023	N/A	1.5
Juel, et. al.	2021	4.5	N/A
Kadonsky, et. al.	2023	4.5	1.5
Kanchan, et. al.	2024	N/A	3
Kappus-Kron, et. al.	2024	N/A	1.5
Karthikeyan, et. al.	2022	N/A	1.5
Karthikeyan, et. al.	2021	N/A	1.5
Kaya, et. al.	2022	4.5	N/A
Keshaviah, et. al.	2023	2.5	2.25
Khan, et. al.	2023	3.75	N/A
Kim, et. al.	2022	3.75	N/A
Kim, et. al.	2023	3.75	1.5
Klaassen, et. al.	2024	N/A	2.25
Klevens, et. al.	2023	3	N/A
Kuhn, et. al.	2022	3.75	N/A
Lamm, et. al.	2024	4.5	N/A
Lancaster, et. al.	2022	N/A	N/A
Langan, et. al.	2022	4.5	1.5
Larsen, et. al.	2022	N/A	1.5
Lee, et. al.	2024	4.5	1.5
Lee, et. al.	2022	N/A	1.5

Li, et. al.	2021	4.5	1.5
Li, et. al.	2022	N/A	2.17
Li, et. al.	2024	N/A	1.5
Li, et. al.	2022	3.75	N/A
Li, et. al.	2024	3.75	N/A
Li, et. al.	2022	N/A	2
Lininger, et. al.	2024	N/A	3
Lott, et. al.	2023	2.5	3
Lou, et. al.	2022	4.5	N/A
Lu, et. al.	2022	N/A	1.5
Lu, et. al.	2022	N/A	3
Ma, et. al.	2022	3.75	2.25
Majumdar, et. al.	2024	N/A	2.25
McGowan, et. al.	2022	3	1.5
Meadows, et. al.	2024	4.5	N/A
Melvin, et. al.	2021	N/A	1.5
Mondal, et. al.	2021	3	1.5
Montesinos-López, et. al.	2023	N/A	1.5
Nagarkar, et. al.	2022	2.5	2.25
Nelson, et. al.	2022	N/A	3
Nguyen, et. al.	2022	N/A	2
O'Brien, et. al.	2021	N/A	3
Oh, et. al.	2022	4.5	1.5
Osborn, et. al.	2024	N/A	2.25
Pasha, et. al.	2024	4.5	N/A
Peccia, et. al.	2020	N/A	1.5
Pecson, et. al.	2021	2.5	N/A
Phan, et. al.	2023	N/A	3
Rabe, et. al.	2023	4.5	1.5
Rainey, et. al.	2023	N/A	2.25
Rainey, et. al.	2022	4.5	N/A
Rainey, et. al.	2022	4.5	N/A
Rao, et. al.	2024	3.75	1.5
Reeves, et. al.	2021	4.5	3.5
Rezaeitavabe, et. al.	2024	2.5	1.5
Robbins, et. al.	2024	3	N/A
Rouchka, et. al.	2021	N/A	1.5
Safford, et. al.	2022	3	1.5
Safford, et. al.	2022	3	1.5
Saingam, et. al.	2023	4.5	N/A
Saldana, et. al.	2024	N/A	1.5
Schafer, et. al.	2024	4.5	3.5
Schill, et. al.	2023	4.5	1.83
Schoen, et. al.	2022	4.5	1.5
Schussman, et. al.	2022	4.5	N/A

Scott, et. al.	2021	3	1.5
Sellers, et. al.	2022	N/A	1.5
Sharkey, et. al.	2021	4.5	N/A
Silva, et. al.	2022	4.5	1.5
Simpson, et. al.	2021	4.5	1.5
Solo-Gabriele, et. al.	2023	4.5	N/A
Stadler, et. al.	2020	N/A	3
Straathof, et. al.	2024	N/A	4.5
Swift, et. al.	2023	4.5	1.5
Swift, et. al.	2022	4.5	N/A
Terry, et.al.	2022	N/A	3
Thafkali, et. al.	2024	N/A	1.5
Vadde, et. al.	2022	4.5	3.5
Wartell, et. al.	2022	4.5	1.5
Wartell, et. al.	2022	4.5	N/A
Weidhaas, et. al.	2021	2.5	3
Welling, et. al.	2022	4.5	N/A
West, et. al.	2023	3	N/A
Wilder, et. al.	2021	4.5	3.5
Wolfe, et. al.	2021	2.5	1.5
Wolfe, et. al.	2021	N/A	1.75
Wright, et. al.	2022	4.5	3
Wu, et. al.	2021	N/A	3
Wu, et. al.	2021	4.5	1.5
Wu et. al.	2020	N/A	1.5
Yang, et. al.	2024	N/A	3
Zambrana, et. al.	2022	3.75	1.5
Zhan, et. al.	2022	N/A	1.5
Zhan, et. al.	2023	N/A	1.5
Zhao, et. al.	2023	3	N/A
Zhao, et. al.	2022	3	3
Zhao, et. al.	2023	3	3