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Inter-institutional laboratory standardization for SARS-CoV-2 surveillance through wastewater-based epidemiology applied to Mexico City

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ABSTRACT

Objectives: Wastewater-based surveillance applied to SARS-CoV-2 viral load quantification for COVID-19 has become one of the most relevant complementary tools in epidemiologic prevention programs worldwide. However, this valuable decision-making tool still requires fine-tuning to produce comparable results between laboratories, especially when applied to the surveillance of megacities.
Methods: Six laboratories across Mexico and one from the United States executed an interlaboratory study to set up a singular standardized protocol considering method cost, installed infrastructure, materials available, and supply availability for SARS-CoV-2 quantification from five Mexico City sampling sites across this megacity.
Results: Comparable data from processing outcomes in the Mexican laboratories and in the external international laboratory serve as a validating data source. The Bland–Altman comparison showed consistency, with cycle threshold values within ±1.96 SD of SARS-CoV-2 genetic copies for the standard curve quantification, with a mismatch of two laboratories. In addition, MS2 bacteriophage recovery rates varied between 35% and 67% among all participating laboratories. Finally, the efficiency of viral genetic material recovered from all participating laboratories varied between 65% and 93% for the participating laboratories.
Conclusion: This work lays the foundation for extensive and continuous wastewater-based surveillance application across independent Mexican laboratories in a time- and resource-effective manner.

Introduction

According to the World Health Organization, SARS-CoV-2 was responsible for almost 7 million deaths worldwide by June 2023 [1]. However, a significant underestimation of active cases has been reported during the COVID-19 pandemic, especially in low- to middle-income countries, as public health care systems became overwhelmed. Diagnostic tests remained unavailable to more than 50% of the world's population living in areas with limited access to health care services, were supplied in insufficient quantities, or were only administered to patients

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who had covered a list of indicative symptoms, which may only appear 2-14 days after viral infection [2,3]. Individualized testing can provide valuable insight into the status of the pandemic and the effectiveness of response strategies planned and executed by health care providers but does not always capture patients who remain asymptomatic, those who are still developing symptoms, or those who cannot seek medical testing.

Wastewater-based surveillance (WBS) is a tool for epidemiologic surveillance, which originated as early as the 1980s; the first attempts of a WBS-like system in earlier times can be mentioned in two application attempts: during the 1930s and early 1950s, with the isolation of poliovirus from feces and the detection of enterovirus in sewage in Russia, respectively [4]. In WBS, public health information is obtained from the characterization and quantification of biomarkers in wastewater, which includes urine and feces from those living in the sewage catchment [5]. WBS has been previously applied to the detection of SARS-CoV-2 in different countries such as México, Germany, Australia, Israel, and Spain, among other [3,5–14] and, recently, for additional viruses, including norovirus, hepatitis A virus, and poliovirus [8].

WBS has proved effective as an early warning system for increases in active cases, even in low-incidence settings, given that viral genetic materials present in water can be detected before symptomatic cases appear [15]. Furthermore, WBS can be used to estimate disease prevalence by quantifying viral load, regardless of whether infected people are symptomatic or have access to health care or individual tests [16].

WBS studies for surveillance of viral diseases use techniques consisting of five main steps: sample collection, storage, concentration, genetic material extraction, and quantification. Although the processes are already established, it is important to emphasize that there are many factors that may affect the quantitative results, including a laboratory's infrastructure and capacity (i.e. methods and equipment used to perform the test) and wastewater sample collection, storage, and processing (e.g. degradation during transport and storage, losses during concentration or extraction processes and incomplete reverse transcription–polymerase chain reaction [RT-PCR] inhibition) and losses of the viral RNA signal in the sewer before collection [17]. According to Lu et al. [18], due to the low concentrations of SARS-CoV-2 virus particles in some wastewater samples, overcoming sample concentration is the most crucial challenge for the correct application and quantification of active cases by WBS. To tackle these limitations, Ahmed et al. [19] made several methodologic recommendations focused on minimizing false-positive or false-negative errors in WBS, such as adequate sampling methods, efficient RNA extraction, and acceptable interpretation of the obtained data. Nevertheless, as previously mentioned, WBS has proved effective as an early warning system; however, the need for standardized protocols for detecting SARS-CoV-2 in wastewater and quality control measures remains.

In this study, a previously established collaboration between Universidad Nacional Autónoma de México (UNAM), Instituto Politécnico Nacional (IPN), Servicios de Agua de la Ciudad de México (SACMEX), Arizona State University (ASU), and Tecnológico de Monterrey (ITESM) was rapidly adapted in the first effort in Mexico to conduct an interlaboratory study for pathogen surveillance in wastewater samples, which is an important step toward obtaining reliable information for such a large population or a complete nation, as presented by Chik et al. [20]. The objective of the consortium work was to set up an internal, singular, standardized protocol for the characterization of samples, considering the cost, infrastructure, and availability of materials and supplies for SARS-CoV-2 quantification. The study was carried out using five different sampling sites (Figure 1), characterized by the institutions involved using the same techniques to compare obtained data variability and technique reliability. The aims of this study were to (i) determine the reproducibility of SARS-CoV-2 viral load detection and quantification in laboratories using different reverse transcription quantitative polymerase chain reaction (RT-qPCR) equipment, (ii) assess SARS-CoV-2 viral loads in wastewater samples originating from the five sampling sites within Mexico City (CDMX), and (iii) measure viral genetic material losses during sample pretreatment in all participating laboratories.

Methodology

Study area and participating laboratories

The study area covers part of CDMX, located in central Mexico. This study was developed using five samples from different sampling points across CDMX (Figure 1), including two hospitals (Hospital "A" and Hospital "B"), two wastewater treatment plants (WWTP) (WWTP Chapultepec and WWTP Deportiva) and one pumping station (BP Aculco).

The present study was developed through collaborative efforts by six national laboratories and institutions in different cities nationwide.

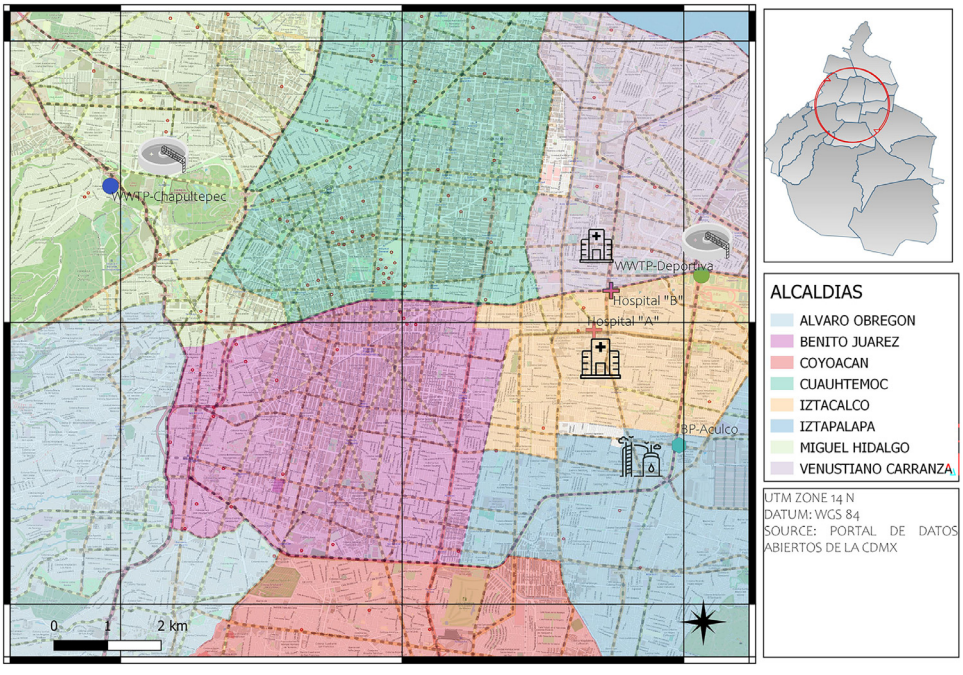

Figure 1. Map showing the sampling locations in Mexico City. Representative images indicate the type of sample site (WWTP, Hospital, pumping station [BP Aculco]), each colored shaded area in the map indicates the municipality or *alcaldia* around the sampling point in Mexico City.

Four of them are located in CDMX (Laboratorio Nacional de Ciencias de la Sostenibilidad [LANCIS], Instituto de Ecología at UNAM [UNAM LANCIS]; Laboratorio de Ingeniería Ambiental from the Instituto de Ingeniería at the same university [UNAM CU]; IPN, Campus Zacatenco; and SACMEX), which are in charge of the water distribution in CDMX. The other two participating laboratories were the Laboratorio de Investigación en Procesos Avanzados de Tratamiento de Aguas of the Instituto de Ingeniería, Unidad Académica Juriquilla, Querétaro, UNAM Juriquilla, and the Laboratorio de Monitoreo de Aguas Residuales TEC (MARTEC), affiliated to the ITESM in Monterrey, Nuevo León. In addition, an international collaborative network with laboratories at ASU, located in Tempe, Arizona, United States, was also included for benchmarking purposes from external collaborators.

Sample collection, storage, and shipment

Wastewater sampling procedures were adapted for each sampling site: at Hospital A, Hospital B, and BP Aculco; samples were collected directly from the sewage systems at specific discharge points, whereas at both WWTPs, samples were collected from the influent after the first screening step in the wastewater treatment process. In all cases, sampling was conducted by collecting a 12 L grab sample for each site at a determined time of the day. Afterward, the samples were thoroughly homogenized and divided into seven high-density polyethylene bottles and kept on ice at 4°C until stored in disposable insulation boxes, including ice and frozen gel bags to maintain appropriate temperature and prevent biomarker degradation during transport. Laboratories and institutions located at CDMX received samples within the same day, whereas shipment to UNAM Juriquilla, ITESM, and ASU were conducted through next-day courier services; each sample was collected in triplicate. No blank samples were delivered because each laboratory used its own blank controls (MilliQ water process in a parallel run with the wastewater samples).

RT-qPCR curves for viral load quantification

To obtain quantitative results using an RT-qPCR sample, a positive SARS-CoV-2 genome 2019-nCoV_NP control that was diluted to the concentration of 200,000 copies per microliter was obtained from Integrated DNA Technologies Acquires Arche (Coralville, Iowa, USA) and provided to all participating laboratories. Aliquots from the original control were serially diluted to obtain concentrations of 100,000, 10,000, 1000, 100, 10, and one copy per polymerase chain reaction.

For all dilutions, RT-qPCR assays were performed using the SARS-CoV-2 RT-qPCR Test kit for wastewater samples (IDEXX, Westbrook, Maine), which contains primers and probes targeting SARS-CoV-2 N gene. To perform RT-qPCR assay, the Tecnológico de Monterrey used a QuantStudio 5 system: UNAM CU, LANCIS, and SACMEX on a StepOne Plus equipment and UNAM Juriquilla and IPN on a StepOne equipment. All thermocyclers from the brand Applied Biosystems from ThermoFisher scientific (Waltham, Massachusetts). Reactions consisted of 5 µL of SARS-CoV-2 mix (containing N1 and N2 primers and probes), 5 µL of RNA Master Mix (reverse transcriptase, hot-start polymerase, and a reference dye), and 5 µL of extracted genetic material, and the programmed thermal cycle consisted of an initial hold for 15 minutes at 50°C and 1 minute at 95°C, followed by 45 cycles of 95°C for 15 seconds and 60°C for 30 seconds. All dilutions were analyzed in triplicate, including negative controls (RNAse-free water) and positive controls included in the kit (positive N1/N2 genes control and the parallel detection of ribonuclease P as internal sample control). Cycle thresholds (Cts) were recorded for each dilution and linearity indexes (R^2) were calculated for each laboratory. The IDEXX positive control provided in SARS-CoV-2 RT-qPCR Test kit (Westbrook, Maine) was used for the wastewater samples, and, for the no template control, elution buffer provided in Water DNA/RNA Magnetic Bead Kit from IDEXX was used.

Calibration curves were validated by assessing the level of agreement between all participating laboratories using Bland–Altman plots. Cts of detection for all RT-qPCR assays were loaded into a database, and results from ITESM and UNAM Juriquilla were taken as the standard to which all other laboratories were compared. All statistical analyses were performed using the MATLAB R2023a software (MathWorks, Natick, Massachusetts). ASU SARS-CoV-2 detection protocols were performed as outlined by Wright et al. [21]. These were used as a gold-standard comparison due to the expertise and experience and a proved SARS-CoV-2 detection protocol. The detection protocol of ASU was used as a point of comparison to confirm the homogeneity of the values obtained for all participating laboratories in Mexico. Calibration curves was development in Sigmaplot software (Systat software, San José, California) abscissa axis correspond to the log10 of SARS-CoV-2 genome 2019-nCoV_NP concentration and the ordinate axis correspond to Ct value obtained in RT-qPCR assay.

To determine the consistency between measurements obtained by all participating laboratories, a modified Bland–Altman chart was used. For this, the Ct values obtained for each concentration level by both reference laboratories (ITESM and UNAM Juriquilla) were averaged and SDs (or σ) were obtained. Then, the differences between each replicate from all other participants and the mean Ct value from the reference laboratories were calculated, expressed as SDs and plotted into the chart as a dot. Finally, two horizontal red lines were added to the plot at a distance from the mean of ± 1.96 SD to represent a 95% confidence interval in accordance with the data expected from a normal distribution. All data points that fell within the interval were considered congruent with the reference, whereas data points falling outside the interval or replicates where no amplification was observed were considered incongruent. The performance of each participant laboratory was measured by the proportion of data points congruent with the reference.

Sample processing and analysis

The SARS-CoV-2 viral load quantification methodologies in wastewater samples in all participating laboratories were identical. First, sample pretreatment for the concentration of viral genetic material was based on the method reported by Sapula et al. [22], with described modification; briefly, solids were precipitated by centrifuging three aliquots of 35 mL each at 5000 g for 5 minutes and supernatants were transferred into new tubes containing a 3-mL solution of polyethylene glycol 8000 (15%) and a NaCl (2%), homogenized thoroughly, and centrifuged at 12,000 g for 1 hour and 45 minutes. Pellets were serially resuspended in 300 µL of molecular biology grade water. Concentrated samples were stored at −20°C until further processing.

According to the supplier's instructions, nucleic acid extraction was performed using Water DNA/RNA Magnetic Bead Kit (IDEXX, Westbrook, Maine). Extractions were performed manually. Eluted genetic materials were recovered and stored at −20°C until analysis. For SARS-CoV-2 viral loads detection, the RT-qPCR assay was performed using the SARS-CoV-2 RT-qPCR Test kit for wastewater samples provided by IDEXX (Westbrook, Maine). Reactions consisted of 5 µL of SARS-CoV-2 mix (containing N1 and N2 primers and probes), as mentioned previously. The qPCR efficiency was determined by the formula: qPCR efficiency = $10^{(-1/\text{slope})} - 1$.

Assessment of genetic material loss

To evaluate the amount of viral genetic material lost during sample processing, an assay using MS2 bacteriophages (ATCC 15597-B1) as a process control was conducted in two of the five sampling sites chosen at random. Bacteriophages were propagated over an *Escherichia coli* (ATCC 15597) culture and harvested, which were used to spike one of the 35 mL aliquots of raw wastewater samples with a concentration of 2.3×10^{11} plaque-forming units per mL (PFU/mL) of MS2 bacteriophages.

MS2 bacteriophage recovery was determined using two parallel methodologies. First, inoculated samples were concentrated with the modified PEG/precipitation method previously presented, and, from the concentrated extract, the active bacteriophages were measured as PFU/mL, using the double agar layer assay, as reported by Cormier and Janes [23]. Second, the number of bacteriophage genome copies in each inoculated sample were quantified using RT-qPCR, following the method described by Miranda and Steward [24]. For quantitative purposes, calibration curves using dilutions at 1×10^5, 1×10^6, 1×10^7, 1×10^8, and 1×10^9 PFU/mL, analyzed in duplicate, were established. The slopes of the calibration curves were calculated from the plot of the log base 10 of PFU/mL value vs corresponding Cts. All participating laboratories reported the recovery rate in both assays as the percentage of total inoculated viral particles that were quantified after sample treatment.

Results and discussion

Calibration curves in all participating laboratories

All laboratories successfully set up calibration curves for quantifying SARS-CoV-2 viral loads in wastewater samples using RT-qPCR platforms. Quantification proved reliable between 10 and 100,000 copies per reaction in all cases because logarithmic tendency lines were established between viral load and Ct of detection, with R^2 values between 0.9644 (IPN) and 0.9995 (UNAM CU). Samples with a viral load of one copy per reaction could only be detected as positive at UNAM Juriquilla (one of three repetitions), IPN (three of three), and UNAM LANCIS (two of three). At the highest concentration measured, 100,000 copies per reaction, the average Ct values in participating laboratories varied between 18.81 (IPN) and 24.57 (SACMEX) for the same concentration of 100,000 copies per reaction, whereas in values for the lowest concentration that yielded consistent detection, the Ct values ranged from 32.68 (IPN) to 38.19 (UNAM CU). Variations between repetitions of a single assay were minimal, with average Ct values for each dilution showing less than one cycle of SD, mostly below one Ct, indicating the robustness and reproducibility of the RT-qPCR platforms used, as reported in previous studies [13,20]. The curves for each of the participating laboratories are presented in Supplementary Figure 1, whereas a direct comparison between all curves is presented in Figure 2. The qualitative polymerase chain reaction efficiency for each standard curve per laboratory were as follows: ITESM (0.97), UNAM Juriquilla (1.14), IPN (1.15), UNAM LANCIS (1.15), UNAM CU (0.94), and SACMEX (1.01). Acceptable efficiency values range from 0.9 to 1.10 [25]. Values outside this range may be due to several factors such as poor pipette calibration, a thermocycler used in non-optimal conditions, genetic material degradation or contamination. Variations were also presenting in the viral load, particularly, in IPN, UNAM Juriquilla, UNAM LANCIS, and SACMEX, and could be due to variation in equipment and laboratory infrastructure [26,27]. However, it should also be considered that the rest of the evaluations demonstrated the reproducibility of the Ct values obtained.

In all cases, curves showed similar behaviors to the one obtained by Stahl et al. [28] using standard samples with genetic material concentrations in the same order of magnitude as those used in this study. However, it should be noted that the targeting the N and E genes in a multiplex reaction using TaqMan probes for a specific and reliable detection by Stahl et al. [28]. Similarly, the protocol described for RT-qPCR detection of SARS-CoV-2 by Wilhelm et al. [29], which targets the M and RdRP regions of the viral genome using TaqMan probes and SYBR Green as a fluorophore, leads to a similar standard curve for absolute quantification, which shows the flexibility and reliability of the method for the detection of pathogens in complex matrixes, such as wastewater using different genome sequences. However, it must be noted that both papers reported detection in reactions using concentrations of less than 10 copies/L, indicating the possibility of obtaining lower detection limits than the one reported in this study if pretreatment procedures are properly optimized.

The high degree of agreement between the results from all laboratories was confirmed using Bland–Altman comparisons that had implemented similar protocols for SARS-CoV-2, (ITESM and UNAM Juriquilla) and those that received training as part of a technology transfer program, IPN, SACMEX, UNAM CU, and UNAM LANCIS (plot shown in Figure 3). Results indicate that variations between the Ct values presented by each laboratory were not statistically significant; however, it should be noted a tendency of increased variation with increased sample dilution (Supplementary Figure 2). Furthermore, the results show robustness and reproducibility of the analytical methods standardized across all members of the collaborative network, which are important features to ensure reliable SARS-CoV-2 detection in WBS systems as indicated by previous reports [20,30]. In contrast, it should be noted that

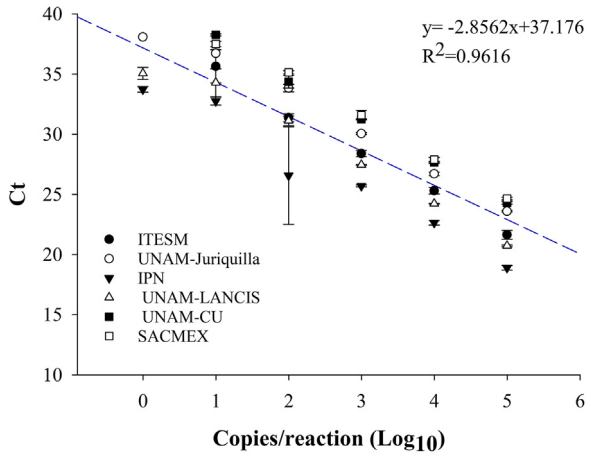

Figure 2. Comparison between the calibration curves obtained at all participating laboratories. The dotted blue line represents a curve obtained from the average Ct between laboratories for each number of copies per reaction. Ct, cycle threshold; IPN, Instituto Politécnico Nacional; LANCIS, Laboratorio Nacional de Ciencias de la Sostenibilidad; SACMEX, Servicios de Agua de la Ciudad de México; ITESM, Tecnológico de Monterrey; UNAM, Universidad Nacional Autónoma de México.

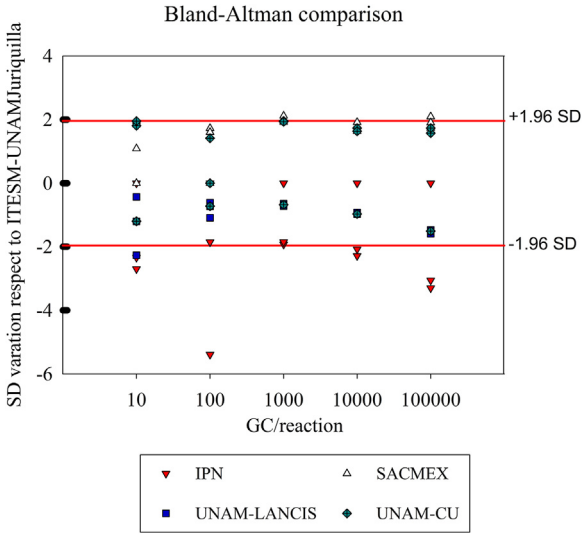

Figure 3. Modified Bland–Altman comparison between ITESM and UNAM Juriquilla laboratories compared with trained laboratories, IPN, SACMEX, UNAM-CU, and UNAM LANCIS, using cycle threshold value data for quantification standard curve comparison between ITESM and UNAM Juriquilla vs GC/reaction. Each replicate data set was compared separately.

Table 1
Viral load quantification results were reported by all participating laboratories in the five sampling sites selected. Quantitative results from ASU were not included because their quantification methodology is not comparable to the rest of the laboratories.

Sampling site	Gene N copies/L						
	ITESM	UNAM Juriquilla	IPN	UNAMLANCIS	UNAM CU	SACMEX	ASU
Hospital A	0.00	13,450.59 (SD ± 1801.12)	251.46 (SD ± 118.54)	1507.66 (SD ± 981.94)	5758.81 (SD ± 4072.09)	0.00	+
Hospital B	0.00	9726.80 (SD ± 3294.29)	0.00	0.00	0.00	0.00	-
WWTP Chapultepec	3916.68 (SD ± 1405.5)	32,437.89 (SD ± 18,384.08)	5647.95 (SD ± 501.10)	501.72 (SD ± 79.68)	9906.73 (SD ± 2099.25)	1048.08 (SD ± 741.1)	+
WWTP Ciudad deportiva	16,420.34 (SD ± 5320.53)	23,718.27 (SD ± 4687.65)	9163.05 (SD ± 3329.88)	8434.08 (SD ± 2164.83)	54,548.84 (SD ± 7987.25)	31,696.54 (SD ± 10,975.38)	+
BP Aculco	2590.80 (SD ± 320.6)	23,893.64 (SD ± 2789.97)	3482.34 (SD ± 94.29)	3048.08 (SD ± 1034.27)	15,911.95 (SD ± 15,761.43)	27,551.40 (SD ± 203.72)	+

ASU, Arizona State University; IPN, Instituto Politécnico Nacional; SACMEX, Servicios de Agua de la Ciudad de México; ITESM, Tecnológico de Monterrey; UNAM, Universidad Nacional Autónoma de México; WWTP, wastewater treatment plants.

the variations between IPN and SACMEX laboratories, those that present greater data variability than the rest of the laboratories, may be an effect of laboratory infrastructure variance and equipment calibration, variables that should be considered when carrying out a standardization process for epidemiologic and monitoring applications [26].

Reproducibility was confirmed by Bland–Altman comparison between laboratories ITESM and UNAM Juriquilla and trained laboratories IPN and SACMEX. In addition, this comparison was evaluated between UNAM CU and UNAM LANCIS laboratories. The comparison indicates the reproducibility of the GC/reaction data and shows a good concordance and nonsignificant difference between the laboratory results between UNAM CU and UNAM LANCIS. However, SACMEX and IPN showed a variation outside ±1.96 SD, indicating that these two laboratories should pass for standardization process to achieve alignment with specialized laboratory reproducibility. As mentioned in the discussion, there are factors that must be addressed, reflecting the importance of large-sample homogenization process and the development of an acceptable range, such as variations in samples and control preparation, reagents quality, and even the skills and abilities of the operators. On the other hand, UNAM CU and UNAM LANCIS data show their reproducibility and consistent values related to ITESM-UNAM Juriquilla values. This result is demonstrated by not observing any data outside the acceptable ranges of the Bland–Altman comparison. This statistical analysis has an acceptable range of variation established within ±1.96 SD, which confirms the reproducibility of the Cts obtained by trained laboratories compared with the Cts obtained by the ITESM and UNAM CU laboratories. Consistent with the experience of those laboratories, the Ct values were taken as control measures.

SARS-CoV-2 viral loads in five sampling sites in CDMX

The detection of SARS-CoV-2 genetic material in wastewater samples is generally consistent among laboratories, and the results obtained by ASU successfully validated the qualitative results (Table 1). All quantitative results, which varied between 251.46 and 54,548.84 copies/L fell between the range of linearity reported during calibration, and no unexpected data were reported. It must be noted that quantification results reported by ASU are not directly comparable to those from all other participating laboratories because the methodology used for sample preparation and viral load quantification differs from the one reported in the methodology section. The ASU protocol was performed as mentioned in the studies by Wright et al. [21] and Bowes et al. [31].

However, some discrepancies were observed. UNAM Juriquilla reported SARS-CoV-2 detection in the sample from Hospital B, whereas all other laboratories could not detect it. Given that the validation study conducted by ASU reported a negative result, this is likely due to contamination during the preparation of the RT-qPCR reaction. Similarly, SACMEX and ITESM reported the sample from Hospital A as negative, whereas all other laboratories (including ASU) reported it as positive. Therefore, this was likely the result of genetic material losses during large-sample homogenization.

Although the qualitative results mainly were consistent, a great degree of variability in the viral load for each sample was observed at all participating laboratories. The calibration curves did not vary significantly; this indicates that the viral genetic material recovery rate varied significantly between laboratories, negatively effecting the reproducibility of the study. Further research to improve sample pretreatment procedures among participating laboratories is still needed.

Previous WBS studies focusing on SARS-CoV-2 surveillance indicate that given the number of factors that may alter the amount of a given biomarker present on a sample, such as temperature, mixing, solids, biological charge, etc., high levels of variability are often found in measurements even if adequate statistical modeling of the distribution and the rates of degradation of the biomarker of interest and a standardized sampling and treatment protocols are appropriately considered, indicating a technical limitation for WBS applications that remains to be addressed [32]. In fact, a similar study by Amoah et al. [33] reported the average viral load from wastewater samples originating from a single sampling site to be 1441.01 copies/mL, with an SD of 1977.8 copies/mL. In addition, Zheng et al. [34] reported viral loads of 2770.06 ± 2847.69 copies/L on samples from a single sampling site. In both cases, variability was related directly to the complexity of wastewater sample because it usually contains a wide range of chemical substances at high concentrations, which may affect the accuracy of the measurements by degrading viral RNA, hindering RNA recovery, inhibiting RT-qPCR, or interfering with the measurement of fluorescence during quantification. These factors may have contributed to the variability of the viral load reported on samples used in this study; however, the extent of their contribution is still unclear.

Viral genetic material losses during sample pretreatment

The results from the double agar layer assay are presented in Figure 4a. The average concentration of active MS2 bacteriophages recovered from the assay conducted on wastewater samples from Hospital A was 7.65×10^4 PFU/mL, with an interquartile range (IQR) of 3.80×10^4 to 1.60×10^5 PFU/mL. For assays using samples from WWTP Chapultepec, the average concentration of bacteriophages recovered was 4.39×10^5 PFU/mL, with an IQR of 9.54×10^4 to 6.26×10^5 PFU/mL. Overall, the recovery rate of active bacteriophages in all participating laboratories varied between 35% and 67%.

The results from the RT-qPCR quantification of recovered MS2 bacteriophage genetic material are presented in Figure 4b. In Hospital A samples, the average concentration of viral genomes recovered was 3.81×10^{10} copies/L, with an IQR of 2.75×10^{10} to 7.04×10^{10} copies/L. The recovery rates in samples from WWTP Chapultepec were

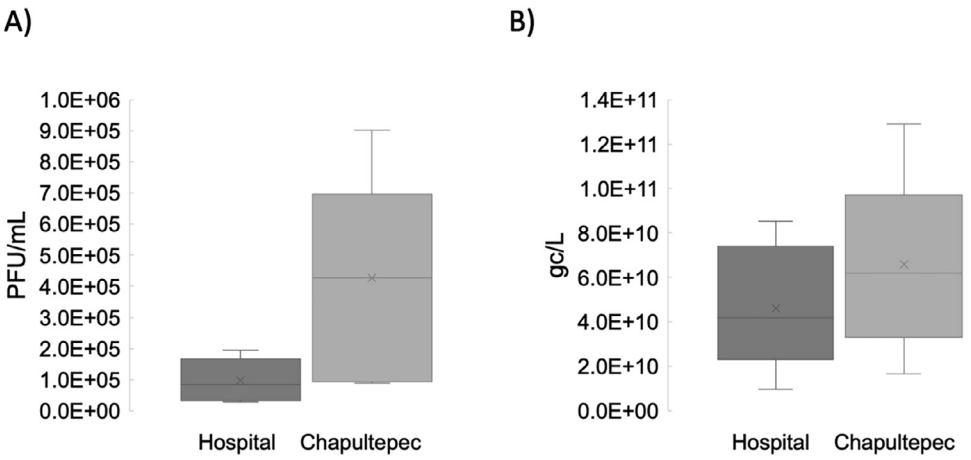

Figure 4. (a) Recovery of active MS2 bacteriophages from wastewater samples from hospital A and WWTP Chapultepec sampling sites quantified by the double agar layer method, (b) recovery of MS2 bacteriophage genetic material from wastewater samples from hospital A and WWTP Chapultepec sampling sites quantified by reverse transcription-polymerase chain reaction, whiskers represent the minimum and maximum bacteriophage plaque-forming units/mL determined in the assay.

also high in this assay, with an average of 5.83×10^{10} copies/L recovered and an IQR of 3.86×10^{10} to 8.64×10^{10} copies/L. Overall, the rate of viral genetic material recovery in all participating laboratories varied between 65% and 93%, which is consistent with previous reports made by Medema et al. [13], where the recovery rates were determined at 73 ± 50%, and noticeably higher than in reports made by Sherchan et al. [14], where the recovery of surrogate Pseudomonas bacteriophage Φ6 was determined at 53%. Furthermore, Pérez-Cataluña et al. [35] established the recovery rate of SARS-CoV-2 genetic material using a similar pretreatment process at 42.9 ± 9.5%, enough to detect the N1 and N2 genes in 91.2% and 85.3% of essays, respectively.

Overall, the recovery rate of viral genetic material in participating laboratories was acceptable in all cases, exceeding the recovery rates suggested by Miranda et al. [30], Stahl et al. [28], and Chik et al. [20], which led to the consistent detection of SARS-CoV-2 across participating laboratories in all five sampling sites. However, the variability across laboratories is still a challenge for implementing robust and reproducible surveillance networks that can be used across entire cities, such as CDMX, because it will require extensive, continued collaboration between institutions. This collaborative network can serve as an example for the implementation of similar WBS platforms in other cities in low- to middle–resource countries.

Conclusion

In this study, a collaborative network for surveillance of SARS-CoV-2 cases in CDMX was established through the detection and quantification of viral genetic materials in wastewater samples from five sampling sites distributed across the city. This work lays the foundation for extensive and continuous WBS application in a time- and resource-effective manner to evaluate the circulating pathogens in the population connected to the sampled sewer system. The network created by the Mexican and US universities in collaboration with the water and sewage system operators in CDMX can be used to organize an early warning system for new outbreaks and provide valuable insights for policymakers and health care providers that aids in the establishment of epidemiological monitoring and containment. Developing WBS methods for viral tracing based on RT-qPCR and standardizing this monitoring tool for consistent results by all participating laboratories is intended to be applied for future pathogen monitoring protocol in the whole metropolitan area of CDMX. Through the technology transfer program highlighted in this study, participating laboratories were able to detect the presence of SARS-CoV-2 genetic material consistently and reliably in wastewater samples from all sampling sites. Viral load quantification varied between laboratories, likely due to inconsistent recovery rates during sample pretreatment, impacting reproducibility. This limitation has previously been reported by similar studies and must be addressed in future research to build robust and flexible public health surveillance systems. To achieve this objective and build a larger network of surveillance, standardization of laboratory infrastructure/equipment and sample treatment and molecular detection protocols are required, as well as mandatory corrective action for laboratories with values outside of the acceptable range of quantification.

Declarations of competing interest

The authors have no competing interests to declare.

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Ethical approval

The present work does not require ethical approval.

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Author contributions

Conceptualization: MAOM, AAA, DCV, JESH; project administration: MAOM, AAA, DCV, HMNI, HV GB, AN, JESH, RPS; data curation: MAOM, AAA, DCV, JCR, ACEG, EC, EMD, AAC, OR, MBT, NVS, MASM, MZM, MMH, RUH; investigation: MAOM, AAA, DCV, JCR, ACEG, EC, SLLS, AAC, MBT, NVS, MASM, MZM, MMH; methodology: MAOM, DCV, JCR, EC, EMD, SLLS, RUH; writing – original draft: MAOM, AAA, JESH; review & editing: MAOM, AAA, DCV, JCR, ACEG, EC, EMD, SLLS, AAC, OR, MMR, MBT, NVS, MASM, MZM, HMNI, MMH, HV, GB, AN, RUH, JESH, RPS; formal Analysis: DCV, JCR, ACEG, MBT, NVS, MASM, MZM, MMH; funding acquisition: JESH, RPS; Supervision: HMNI, HV, GB, AN, RUH, RPS; visualization: AAA, ACEG, EMD, AAC, OR, RUH, NVS, JESH; validation: MMH, HV, GB, AN, RUH, JESH, RPS.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijregi.2024.100429.

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