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Isothermal amplification as a water safety tool: rapid detection of viruses in surface water and wastewater†

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This study introduces a simple and rapid multi-wavelength, semi-quantitative detection strategy for monitoring SARS-CoV-2 and MS2 bacteriophage in water and wastewater using reverse transcription loop-mediated isothermal amplification (RT-LAMP). By integrating microplate-based spectrophotometry, we enabled higher throughput monitoring through simple optical measurements, thereby reducing the complexity of sample processing. Our findings demonstrate that RT-LAMP can be performed at lower temperatures, such as 45 °C, with incubation times of ≤60 minutes, while maintaining assay accuracy. The RT-LAMP yielded a conservative positivity threshold of ≥0.25 $\Delta OD_{434-560nm}$ for both SARS-CoV-2 and MS2, with limits of detection (LOD) of ~180 copies per μL and 1000 PFU mL^{-1} for SARS-CoV-2 and MS2, respectively. Statistically significant agreement with RT-qPCR was observed above 100 copies per μL ($p < 0.001$), with strong inverse correlations between Cq values and $\Delta OD_{434-560nm}$ readings for both targets ($p < 0.001$). Variability was primarily confined to low-template samples (<100 copies per μL), where stochastic primer dynamics and matrix inhibitors likely broadened coefficient of variation percentages; however, precision tightened to <10% once targets exceeded 500 copies per μL. To assess real-world applicability, RT-LAMP was applied to raw wastewater and eluates from granular activated carbon (GAC)-based passive samplers in surface waters. In wastewater, RT-LAMP detected endogenous SARS-CoV-2 and MS2 with 100% and 85% positive predictive values, respectively, aligning with RT-qPCR benchmarks. In surface waters, SARS-CoV-2 was detected in 10% of RT-LAMP replicates, while MS2 remained undetected. These results support the use of isothermal amplification with spectrophotometry and scalable sampling for rapid, field-deployable viral detection.

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Water impact

Loop-mediated amplification technology is well-suited for water quality management, with applications in microbial source tracking and real-time monitoring of water quality. Its integration with portable, low-cost spectrophotometry will enable a rapid, field-deployable solution for low-resource settings, addressing challenges in underserved regions and advancing global water safety.

1. Introduction

Ensuring water safety demands analytical tools that can keep pace with the diversity and resilience of viral pathogens in water. Microbiological indicators, such as faecal coliforms, *Escherichia coli* (*E. coli*), and Enterococci, remain regulatory mainstays for water quality indication,[1,2] yet they are readily inactivated by chlorination and sunlight, survive for shorter periods than most enteric viruses, and correlate poorly with viral occurrence in freshwaters.[3–7] These limitations have motivated a shift toward direct detection of viruses to provide a more accurate and specific assessment of water safety, particularly for drinking and recreational waters.

Viruses pose unique challenges for water safety because many viruses exhibit prolonged persistence under a variety of environmental conditions, are more resistant to treatment methods compared to bacterial pathogens, and include genotypes that are not culturable.[8] Enteroviruses, adenoviruses, noroviruses, and rotaviruses are repeatedly implicated in water-related gastroenteritis outbreaks, yet culture-based assays overlook low-titer or unculturable strains, leaving significant infection risk from water being undetected and underreported.[9]

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Many culture-based methods are also labor-intensive and result in several days of processing steps prior to obtaining results.

The COVID-19 pandemic catalysed advancements in molecular tools for wastewater surveillance, leading to an expansion of available monitoring protocols and prompting national surveillance programmes and accelerated protocol harmonization.[10,11] Consequently, this has revealed a high prevalence of pathogenic viruses to be present in domestic wastewaters, highlighting the risks associated with inadequately treated wastewater and its implications for water safety.[12] Quantitative and digital polymerase chain reaction (qPCR and dPCR) methods remain the analytical "gold standard" for monitoring pathogens because of their high sensitivity and specificity in detecting and quantifying viral pathogens.[13] Nonetheless, they require complex workflows, including labour-intensive nucleic acid concentration and purification steps to remove inhibitory compounds that are not well suited for practical use in field-deployable or resource-limited settings.[14]

Loop-mediated isothermal amplification (LAMP) offers a practical alternative to PCR-based methods. Operating at a single temperature, LAMP platforms can deliver results within 30 to 45 minutes without thermal cyclers or extensive sample preparation. LAMP is also more tolerant to inhibitors, making it especially advantageous for use with complex sample matrices that often hinder qPCR methods.[15,16] Since its introduction, RT-LAMP methods have been reported for detecting SARS-CoV-2 in wastewater,[17–19] Zika virus,[20] and Astrovirus[21] in surface waters, MS2 bacteriophage in lakes and wastewater effluents,[22] and even antimicrobial resistance markers in pond water.[23] During the COVID-19 pandemic, isothermal methods were widely employed for the rapid detection of SARS-CoV-2 in clinical settings.[24,25] A handful of recent studies have also begun to explore the use of RT-LAMP for detecting SARS-CoV-2 in wastewater, demonstrating its potential as a rapid, resource-efficient monitoring tool.[26–29] However, the broader application of isothermal methods, particularly for surface water monitoring, remains underexplored.[30] Advancements in biosensors have accelerated the transition from laboratory qPCR workflows to portable, field-deployable colourimetric and spectrophotometric platforms. For example, Kim and Yeo (2016) developed a microfluidic paper-based analytical device for colourimetric detection of *E. coli* in environmental waters.[31] More recently, Nguyen *et al.* (2022) demonstrated a smartphone-based RT-LAMP platform that uses ambient light and a 3D-printed sample chamber to quantify SARS-CoV-2 amplification in wastewater through image-based colour analysis.[32] These studies highlight how portable, optical detection systems are evolving to meet pathogen surveillance demands, but these studies and others lack well-characterized, quantitative, or semi-quantitative thresholds for decision making in the field. To address this gap, our study aims to integrate dual-wavelength spectrophotometry with RT-LAMP to enable semi-quantitative detection of SARS-CoV-2 RNA and *Emesvirus zinderi* (MS2) bacteriophage in surface water and wastewater. This work seeks to inform future biosensor-enabled monitoring platforms by establishing empirical benchmarks for the application of spectrophotometry-based isothermal amplification for genomic pathogen detection.

2. Materials & methods

2.1. Wastewater and surface water samples

To investigate SARS-CoV-2 and MS2 in wastewater samples, 24 hour composite influent samples were collected from a local wastewater treatment facility in Nova Scotia, Canada. The raw wastewater sample was collected and stored in a 1 L high-density polyethylene bottle and transported back to the laboratory at 4 °C. Upon arrival, 40 mL of wastewater was directly extracted using the Promega Wizard® Enviro TNA Kit.

Additionally, passive samples were collected from surface waters in Nova Scotia, Canada, after 1-week deployment periods. Passive sampling was conducted using the 3D-printed passive sampler developed by Hayes *et al.* (2021).[33] For each deployment, 3 g of granular activated carbon (GAC) was placed in a heat-sealable nylon mesh sleeve. After deployment, GAC was removed from the passive sampler and eluted with 40 mL of a Tween20®-based buffer solution.[33] This 40 mL elution was subsequently extracted using the Promega Wizard® Enviro TNA Kit (ESI:† section 1.2).

2.2. Preparation of MS2 bacteriophage

MS2 bacteriophage was utilized as a viral surrogate to evaluate the detection performance of the MS2 RT-LAMP and RT-qPCR assays. The propagation and enumeration of the MS2 bacteriophage was based on the U.S EPA. Method 1601 double-layer agar technique using tryptic soy agar with American Type Culture Collection (ATCC) *Escherichia coli* bacteriophage MS2 virus (ATCC #15597-B1) as a host.[34] For propagation, 0.1 mL at ~10^7 plaque-forming units (PFU) per mL of MS2 was inoculated into 20 mL of exponentially growing *E. coli-3000* (ATCC #15597) host suspension and left to aerate overnight for ~16 h at 37 °C. The host-associated MS2 suspension was then centrifuged at ~3000*g* for 10 min to pellet the bacterial cells and debris. The supernatant, containing the MS2 virions, was further purified by a 0.45 μm Millex® syringe filter (Millipore, MA, USA), and the filtrate was diluted 1 : 1000 in 1× PBS (pH of ~7.5) and titrated using the double-agar-layer method to determine the stock concentration in PFU mL^{-1}.

2.3. Bacteriophage MS2 and SARS-CoV-2 RT-qPCR assays

A one-step RT-qPCR assay previously validated by Gendron *et al.* (2010) was carried out for the detection of bacteriophage MS2 gene copies.[35] Each 20-μL RT-qPCR reaction mixture included TaqMan Fast Virus 1-Step Master Mix, forward and reverse primers, a probe at the working concentrations listed in ESI:† Table S1, 5 mg mL^{-1} bovine serum albumin, and 3 μL of template RNA. Additionally, a multiplex RT-qPCR assay, designed by Hayes *et al.* (2022), was employed in this study to quantify SARS-CoV-2 gene copies.[36] For the SARS-CoV-2 multiplex reaction, a 20 μL mixture was prepared with 3 μL of

template RNA, 5 μL of TaqMan Fast Virus 1-Step Multiplex Master Mix (ThermoFisher, MA, USA), and primers and probes at the concentrations (ESI:† Table S1). The RT-qPCR reactions for both MS2 and SARS-CoV-2 were performed separately using a Gene Count Q-96 thermocycler (LuminUltra Technologies, NB, CA), with thermal cycling conditions specific to each assay as detailed in ESI:† Table S1.

The standard quantification curve for the SARS-CoV-2 assay was generated using SARS-CoV-2 RNA reference material (Twist Bioscience, CA, USA), prepared according to the manufacturer's instructions (ESI:† section 1.1). The reference material was then serially diluted to create known concentrations ranging from $\sim10^0$ to $\sim10^6$ copies per μL of RNA from an initial stock concentration of $\sim10^6$ copies per μL. For the MS2 positive control, gBlocks® gene fragments were obtained from Integrated DNA Technologies (Coralville, IA, USA). A standard curve was constructed by serially diluting the gBlocks® gene fragments 10-fold, from an initial stock concentration of $\sim1.28 \times 10^{12}$ copies per μL to final concentrations ranging from $\sim10^8$ to $\sim10^2$ copies per μL. Further details of the standard reference materials are provided in the ESI.† Each of the dilution points on the standard curves for both the MS2 and SARS-CoV-2 assays was analyzed in triplicate.

Quantification, precision, and linearity were assessed using the coefficient of correlation (R^2) obtained from the linear regression of each standard curve, with an R^2 value of ≥0.90 considered acceptable for validation. The amplification efficiency (ε) was further calculated from the slope of the standard curve using the formula $\varepsilon = 100 \times (10^{-1/\text{slope}} - 1)$.[37] For the MS2 standard curve, the R^2 value was 0.971, the amplification efficiency was ~110%, and the *Y*-intercept was 40.152. The SARS-CoV-2 standard curve exhibited an efficiency of ~95%, an R^2 value of 0.995, and a *Y*-intercept of 40.14.

2.4. Bacteriophage MS2 RT-LAMP assay

The MS2 RT-LAMP assay was developed using a pH-based WarmStart® Colorimetric LAMP 2× Master Mix with UDG (New England Biolabs, MA, USA). The final concentrations of the RT-LAMP primers were determined based on previous studies to be 0.2 μM each for the outer primers F3 and B3, 1.6 μM each for the inner primers FIP and BIP, and 0.8 μM each for the loop primers LF and LB.[22,38] Each reaction was prepared to a final volume of 25 μL, containing 12.5 μL of WarmStart® Colorimetric LAMP 2× Master Mix, 2.5 μL of a prepared 10× LAMP primer mix, 1 μL of the sample, and 9 μL of molecular biology grade nuclease-free water. Once prepared, each reaction was incubated at 45 °C for 60 minutes and quantified *via* spectrophotometry following incubation.

2.5. SARS-CoV-2 RT-LAMP assay

For the RT-LAMP analysis of SARS-CoV-2, the SARS-CoV-2 Rapid Colorimetric RT-LAMP Assay Kit (New England Biolabs, MA, USA) was used. The assay was prepared according to the manufacturer's specifications, with minor modifications to the incubation temperature and duration. The assay components included a 10× primer mix targeting the N_2 and E genes of SARS-CoV-2 (ESI:† Table S1), a 10× internal control primer mix (rActin), a WarmStart Colorimetric LAMP 2× Master Mix with UDG, and 10× guanidine hydrochloride. Each RT-LAMP reaction was prepared in a total volume of 25 μL, consisting of 12.5 μL of WarmStart Colorimetric LAMP 2× Master Mix, 2 μL of the SARS-CoV-2 primer mix, 2.5 μL of the rActin primer mix, 5.5 μL of nuclease-free water, 2.5 μL of guanidine hydrochloride, and 2 μL of sample or control. Each reaction was then incubated at a constant temperature of 45 °C for a pre-determined incubation period between 10, 20, 30, 40, 50, and 60 minutes, depending on the experiment. All replicates were quantified *via* spectrophotometry, as described in section 2.7.

2.6. Spectrophotometry

In this study, SARS-CoV-2 and MS2 targets were quantified using a BioTek H1 Synergy microplate spectrophotometer (BioTek, CA, USA). The RT-LAMP reactions were carried out in 96-well round-bottom sterile polystyrene microplates. The spectrophotometer reader's internal light source, a 20 W xenon flash bulb, was coupled with a series of monochromators to precisely control the wavelength directed into each well of the microplate. The Gen5 software, pre-installed on the spectrophotometer, was utilized to set the parameters necessary for quantifying the RT-LAMP reactions. This quantification was based on the absorbance (ΔOD) at 434 nm and 560 nm, where the colour change following incubation was defined as the difference in optical densities between these two wavelengths ($\Delta OD_{434-560nm}$).

2.7. Data analysis

Following the approach used by Akter *et al.* (2024), Cohen's Kappa (κ) statistic was calculated to assess the level of agreement between the RT-qPCR and RT-LAMP methods.[39] As well, Spearman's rank correlation coefficient was used to assess the relationship between RT-qPCR (copies per μL) and RT-LAMP ($\Delta OD_{434-560nm}$) values, and a logistic regression model was fitted to explore the predictive relationship between copies per μL and RT-LAMP detections.[40] Percent coefficient of variation (CV) was calculated to measure the relative variability of data points around the calculated mean for each detection method.[41] Negative and positive predictive values (NPV and PPV) of the RT-LAMP detections were calculated according to true- and false-negative and false-positive results compared to the RT-qPCR. As well, the RT-LAMP limit of detections (LOD) that yielded a positive detection in 95% of replicates was estimated for the RT-LAMP assays using a logistic regression model.[42] All statistical analyses and generation of figures were conducted using RStudio (Version 4.2.3), with packages such as tidyverse, ggtext, dplyr, psych, caret, pROC, Metrics, ggplot2, and viridis utilized.[43,44]

2.8. Quality assurance-quality control (QA-QC)

Nucleic acid extractions and RT-qPCR assay preparation were carried out in separate laboratories, maintaining a unidirectional workflow. All materials were either ordered sterilized or sterilized *via* autoclaving, and each workstation was outfitted with dedicated laboratory equipment, reagents, and personal protective gear. Work surfaces were decontaminated with 1% bleach for 30 minutes, rinsed with DNase/RNase-free water, and then exposed to UV light for 90 minutes.

Various controls were incorporated at each stage of sample processing and analysis, including blank extraction controls and both positive and negative RT-qPCR and RT-LAMP controls. DNase/RNase-free water was used for negative controls and blanks, while synthetic reference materials for each virus (ESI:† Table S3) served as positive template controls. Standards from the minimum information for publication of quantitative real-time PCR experiments guidelines[37] and environmental microbiology minimum information guidelines[45] were followed throughout the study (ESI:† Table S4). RT-qPCR results were reported based on a cycle quantification (Cq) threshold of <37 cycles, with values beyond this threshold considered non-detects. PCR inhibition in extracted samples was evaluated through serial dilutions. If Cq values from diluted samples differed by more than two cycles from the reference control, the sample was deemed inhibited. For any results to be valid, the negative and positive controls needed to pass, and if either control failed, the affected samples were re-analyzed.

3. Results & discussion

3.1. RT-LAMP assay design and validation for SARS-CoV-2 and MS2 detection

Minimum RT-LAMP incubation time for SARS-CoV-2 detection. In this work, we investigated the potential of spectrophotometry for real-time monitoring of RT-LAMP reactions at an operational temperature of 45 °C. To determine the minimum incubation time required to detect SARS-CoV-2 RNA across a range of concentrations (10^1 copies per μL to 10^5 copies per μL), the $\Delta OD_{434-560nm}$ was recorded, following incubation periods of 10, 20, 30, 40, 50, and 60 minutes (Fig. 1). The SARS-CoV-2 RT-LAMP assay demonstrated a time-dependent increase in viral detection as reflected by $\Delta OD_{434-560nm}$ across a range of RNA concentrations.

The maximum $\Delta OD_{434-560nm}$ readings were recorded between 40 to 60 minutes of incubation, depending on the viral load. Positive samples measured a $\Delta OD_{434-560nm}$ between 0.25 and 0.5, and samples that were considered to be not amplified had $\Delta OD_{434nm-560nm}$ values of zero or below. Samples with higher RNA concentrations ($\geq 10^3$ copies per μL) showed a rapid initial colour change in the reaction wells, with a peak colourimetric response being measured at $\sim 10^5$ copies per μL within 40 minutes of incubation, while lower concentrations (10^1–10^2 copies per μL) required up to 60 minutes. However, no change in optical density between the negative control and samples containing an RNA concentration of $\sim 10^1$ copies per μL.

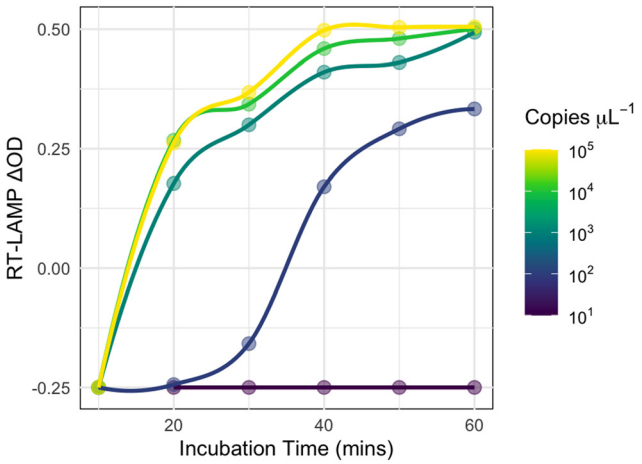

Fig. 1 Spectrophotometric $\Delta OD_{434-560nm}$ measurements 10, 20, 30, 40, 50, and 60 minutes at 45 °C across five SARS-CoV-2 RNA concentrations (10^1 to 10^5 copies per μL). Points represent the average $\Delta OD_{434-560nm}$ measured at each point (n = 10), and the lines are smoothed trends generated using LOESS smoothing function in RStudio.

These findings suggest that incubation time and temperature likely play an important role in ensuring adequate isothermal amplification. At viral concentrations $<10^4$ copies per μL, longer incubation times may be required to avoid the occurrence of false negatives at lower incubation temperatures. Previous research has indicated that a similar RT-LAMP assay can robustly detect SARS-CoV-2 infection in clinical samples with Cq values <30 within 20 to 30 minutes when incubated at 65 °C, with a particular focus on primer sets targeting the N gene.[46] However, our results showed a delayed response in colour change at 45 °C, requiring around 30 to 40 minutes. Nevertheless, conducting RT-LAMP reactions at lower temperatures for slightly longer incubation periods offers advantages for low-resource settings by allowing the use of less sophisticated equipment that does not require precise thermal control or high-temperature capacity.

The use of spectrophotometry for monitoring RT-LAMP reactions offers unique advantages over other detection methods by providing real-time, semi-quantitative data.[47] While turbidimetric methods rely on simple detection of turbidity changes, their lack of specificity increases the risk of false positives in environmental samples.[48] Similarly, fluorescent-based methods offer improved specificity through real-time monitoring and melt-curve analysis, but they require expensive equipment for precise thermal control, limiting their applications.[49] Colourimetric methods, while ideal for qualitative, on-site monitoring, are often susceptible to matrix effects such as pH shifts, salinity, and natural organic matter, which can complicate accurate quantification.[18] In contrast, spectrophotometric approaches, as demonstrated in this study, utilize accessible and less complex equipment like microplate readers, enabling real-time, semi-quantitative detection.

RT-LAMP *versus* RT-qPCR detection of synthetic SARS-CoV-2 RNA. To determine the minimum detectable concentration of SARS-CoV-2 RNA, we assessed amplification in reaction mixtures containing known synthetic RNA concentrations ranging from 10^0 to 10^5 copies per μL. After incubating the samples at 45 °C for 40 minutes, the change in optical density between 434 nm and 560 nm ($\Delta OD_{434-560nm}$) was measured. As shown in Fig. 2, we compared the detection of synthetic SARS-CoV-2 RNA using RT-qPCR and RT-LAMP methods. Logistic regression analysis confirmed that increasing concentrations of SARS-CoV-2 RNA were predictive of RT-LAMP detection, with an estimated $LOD_{95\%}$ of ~180 copies per μL. RT-LAMP ΔOD signals were significantly correlated with increasing SARS-CoV-2 RNA concentrations ($p < 0.0001$). However, at concentrations $\geq 10^2$ copies μL^{-1}, complete agreement was observed between RT-qPCR and RT-LAMP detection outcomes (Cohen's kappa, $\kappa = 1.0$). Notably, the RT-LAMP coefficient of variation percentages (%CV) at RNA concentrations $\leq 10^2$ copies per μL was calculated to be 88.36% compared to the RT-qPCR 20.47%. However, the RT-LAMP CV% reduced to ~6% for RNA concentrations >500 copies per μL.

Our findings align with previous studies demonstrating that while RT-LAMP is valuable for rapid detection, it exhibits lower sensitivity compared to RT-qPCR. For instance, Wang *et al.* (2023) found that qPCR analysis of *Bacteroidales* had a superior limit of detection compared to LAMP analysis.[50] Kaneko *et al.* (2007) also reported that RT-LAMP is less sensitive than RT-qPCR but is still advantageous for quick diagnostic applications.[51] However, Lalli *et al.* (2021) found that RT-LAMP had similar analytical sensitivity to RT-qPCR on crude saliva samples, detecting SARS-CoV-2 RNA in all samples down to ~10^1 particles per reaction.[52] Likewise, Hara-Kudo *et al.* (2005) found that the specificity of LAMP methods was similar to real-time PCR, but the sensitivity of LAMP was greater.[53] In contrast to our study findings, Amoah *et al.* (2021) reported that RT-LAMP analysis successfully amplified synthetic SARS-CoV-2 RNA with a $LOD_{95\%}$ between 4 and 40 copies per μL.[54] Moreover, Bivins *et al.* (2021) found that for RT-LAMP detection of SARS-CoV-2 in wastewater samples, the probability of detection exceeded 50% at RNA concentrations ≥253.5 copies per μL for the SARS-CoV-2 N1 gene and ≥60.8 copies per μL for the SARS-CoV-2 E_Sarbeco gene.[29] Akter *et al.* (2020) also evaluated the agreement between RT-LAMP and RT-qPCR qualitative detection of SARS-CoV-2 RNA in wastewater samples.[39] The authors observed 93% accuracy for RT-LAMP analysis when results were analyzed in triplicate and 83% when only a single reaction was run. Our results also support that increased replication is important for achieving reliable RT-LAMP detections, particularly at RNA concentrations less than 500 copies per μL.

Molecular *versus* plaque-based detection of bacteriophage MS2. To compare the sensitivity of RT-LAMP to plaque and RT-qPCR assays, a 10-fold dilution series of MS2 stock solution was prepared in PBS and analyzed in parallel using all three methods (Fig. 3). While diluted samples were directly analyzed by RT-LAMP, total nucleic acids were purified using a commercial kit described above for RT-qPCR analysis. RT-LAMP readings were consistent for both plaque and RT-qPCR assays, showing peak colourimetric response at MS2 concentrations above 10^5 PFU mL^{-1} and Cq values below 30. Spearman's rank correlation analysis revealed a significant inverse relationship between Cq values and MS2 plaque counts of ~10^3 to ~10^8 PFU mL^{-1} ($\rho = -0.98$, $p < 0.001$) and RT-LAMP $\Delta OD_{434-560nm}$ readings ($\rho = -0.88$, $p < 0.001$). However, at MS2 counts of ~10^3 PFU mL^{-1}, RT-qPCR replicates exhibited a %CV of 5.7%, while RT-LAMP replicates varied by 37.6%. However, at plaque counts >10^4 PFU mL^{-1}, the %CV of RT-LAMP replicates decreased significantly to <3.5% ($p < 0.001$).

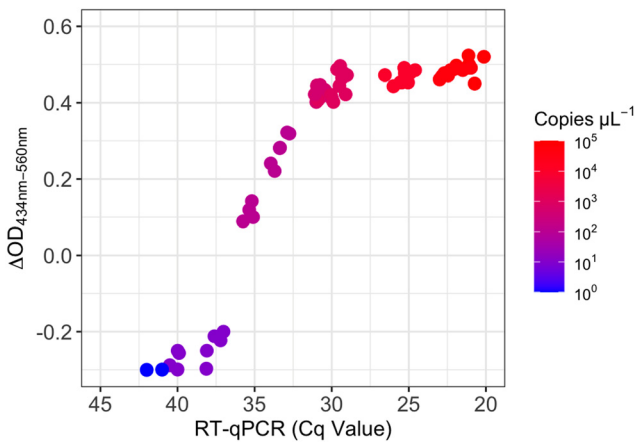

Fig. 2 RT-LAMP detections ($\Delta OD_{434-560nm}$) *versus* paired RT-qPCR cycle quantification (Cq) values of synthetic SARS-CoV-2 RNA. Each point represents an individual sample, with $\Delta OD_{434-560nm}$ on the *y*-axis and Cq on the *x*-axis. The colour gradient indicates RNA concentrations of the synthetic reference material.

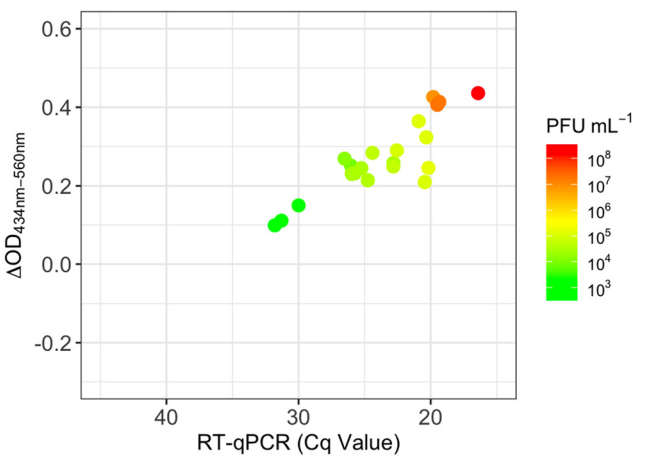

Fig. 3 Positive MS2 detections, by RT-qPCR cycle quantification (Cq) values plotted against the corresponding RT-LAMP $\Delta OD_{434-560nm}$ measurements. Data points are colour-coded according to corresponding PFU mL^{-1} concentrations measured in parallel.

Studies are beginning to show clear correlations between RT-qPCR Cq values and cell-culture positivity, demonstrating that molecular tools can indeed serve as a reliable proxy for viral risks when appropriate thresholds are applied. For instance, recent research suggested that SARS-CoV-2 Cq values below 32 correlated with positive viral cultures.[55] Traditional plaque assays, although definitive for infectivity, are time-consuming and resource-intensive, often taking days to yield results, and require strict laboratory biosafety requirements. Both RT-qPCR and RT-LAMP shorten this turnaround to hours and can be performed in laboratories with standard molecular-biology infrastructure, making them practical for routine water-quality surveillance.

In this work, we observed that at higher RNA template levels, the RT-LAMP multistep amplification was almost instantaneous, whereas at ≤180 copies per μL stochastic variations in primer annealing became more pronounced, leading to delays in amplification initiation or failed reactions. Matrix components common to water and wastewater samples, like humic acids, polysaccharides, and divalent metals, can chelate Mg^{+2} or adsorb polymerase, slowing strand displacement and accentuating stochastic effects.[56,57] Previous studies have described positive LAMP signals appearing in only a subset of RT-LAMP replicates when template levels were ≤50 to 100 genome copies per reaction in wastewater and animal feces.[16] The inclusion of multiplex LAMP primers, internal amplification controls, and greater numbers of replicates per sample can tighten confidence intervals and lower the practical limit of detection.[58]

3.2. Extraction-free detection of SARS-CoV-2 and MS2 in surface water and wastewater samples

Ensuring water safety requires rapid, field-deployable methods capable of detecting viral contamination across diverse water matrices. In this work, we assessed the potential of RT-LAMP for real-world water safety applications by evaluating its ability to directly detect SARS-CoV-2 and MS2 in composite wastewater samples and passive surface water samplers, bypassing the nucleic acid purification step typically required for molecular analysis. For comparison, paired RT-qPCR was performed on lysed and purified nucleic acids extracted from the same sample sets (Table 1).

In wastewater samples, all replicates tested positive for both SARS-CoV-2 and MS2 using RT-qPCR. In surface water samples, RT-qPCR detected SARS-CoV-2 in 50% of replicates and MS2 in 20% of replicates. The RT-LAMP assay showed a PPV of 100% for SARS-CoV-2 detection in wastewater, correctly identifying 85% of positive replicates, compared to RT-qPCR detections. For the surface water samples, the RT-LAMP assay detected SARS-CoV-2 in 10% of replicates, with a PPV of 100% and an NPV of 50%. For MS2, RT-LAMP achieved 100% detection in wastewater but did not detect MS2 in surface water, likely due to dilution effects common in surface waters, which can lower viral concentrations below detection thresholds.[59]

These findings align with previous studies that evaluated isothermal amplification methods for detecting viruses in wastewater. Akter *et al.* (2024) observed that out of 30 wastewater samples, 96.7% tested positive for SARS-CoV-2 with RT-qPCR, and 90% tested positive using RT-LAMP, with 66.7% positivity across all RT-LAMP replicates tested.[39] Similarly, human adenovirus and norovirus have been detected in 93.8% (15/16) and 42% (5/12) of wastewater samples tested with LAMP methods, respectively.[60,61] The quantification of MS2 directly from raw wastewater has also been described in previous work using a custom digital LAMP system configured on a membrane.[62] Huang *et al.* (2018) also detected MS2 in lake and pond water samples using a custom hydrogel-based RT-LAMP assay.[22] To our knowledge, these are the only studies that have investigated the application of LAMP technology for the detection of MS2 in surface waters.

Viral monitoring in surface water is notoriously challenging due to the low concentrations of viruses commonly detected in rivers and lakes.[63,64] While these low concentrations are often difficult to detect, they are significant enough to cause human disease and therefore a concern for water safety.[65,66] In attempts to overcome the challenges of detecting low viral signals in surface waters, current guidance and methodologies recommend filtering at least "a few hundred liters" of water intended for drinking water, at least 1500 L for groundwaters, and up to 1000 L for recreational water.[67,68] However, collection and concentration methods for such large volumes are labor-intensive, time-consuming, and require substantial resources, making it impractical for routine or emergency monitoring. In recent years, passive sampling methods have emerged as a potentially viable, simple, and cost-effective alternative for the *in situ* concentration of a wide range of enteric and non-enteric viruses in freshwater, groundwater, and wastewater.[10,69,70] The advantage of concentrating viruses directly in the environment

Table 1 Detection of SARS-CoV-2 and MS2 using RT-LAMP and RT-qPCR assays

		RT-qPCR		RT-LAMP	
Target	Sample type	Total samples	Total positive replicates/total replicates	Total samples	Total positive replicates/total replicates
SARS-CoV-2	Wastewater	10	20/20	10	17/20
SARS-CoV-2	Surface water	10	10/20	10	2/20
MS2	Wastewater	10	20/20	10	20/20
MS2	Surface water	10	4/20	10	0/20

offered by passive samplers eliminates the need for large volumes of water to be collected and transported for analysis. If combined with isothermal amplification technologies, passive sampling could provide a highly accessible, field-deployable solution for water safety monitoring.

3.3. Future applications of LAMP technology

The continued emergence of waterborne pathogens and limitations in current laboratory-based monitoring frameworks highlight the need for robust, accessible, and cost-effective detection platforms. Isothermal amplification methods, like LAMP, are well suited to address this need due to their operation at a single temperature, high tolerance to common environmental inhibitors, and minimal equipment requirements compared to RT-qPCR.[22,71–74] LAMP has already shown promise in applications including microbial source tracking (MST), diagnostics in engineered water systems, and emergency microbial assessments during flooding or overflow events. For instance, LAMP assays have been successfully applied to detect *Enterococcus* spp. in surface waters with performance comparable to EPA-recommended qPCR methods, but with reduced infrastructure and faster turnaround times.[75] Emerging sensing platforms coupled with simplified sample preparation, isothermal amplification, and spectrophotometric detection are positioned to support near-real-time microbial surveillance in the field, potentially supplementing more laborious qPCR or sequencing-based workflows.

While commercial benchtop spectrophotometers are often cost-prohibitive and are not often feasible for *in situ* applications, recent progress in sensor miniaturization and low-power electronics is rapidly closing the performance gap between laboratory instruments and *in situ* detection platforms.[76] Chip-scale spectrometers have been reduced to the size of a grain of rice, offering a practical pathway toward compact, *in situ* optical sensing for real-time water quality monitoring.[77] Recent innovations have already begun integrating isothermal amplification with smartphone interfaces, LED-photodiode readers, and paper-based microfluidics, showing promising results for virus detection without reliance on complex optics infrastructure.[78,79] Smartphone-based spectrophotometers built with 3D-printed housings and open-source electronics have demonstrated reliable absorbance measurements (450–750 nm) and have been assembled for approximately USD $250.[80] Low-cost electrochemical DNA biosensors on a printed circuit board electrode have shown promise for wastewater surveillance, with manufacturing costing as low as USD $0.55 per electrode for quantities of 100.[81] While these examples highlight the many foundational components that are being designed for compact, low-cost molecular detection, the translation of this technology into robust, field-ready platforms for water quality monitoring still lacks well-characterized workflows. Integration, reliability, and validation of this kind of technology remain active challenges, especially in decentralized water monitoring contexts.

Using a microplate spectrophotometer, we showed that a simple two-wavelength read-out ($\Delta OD_{434-560nm}$) gives reproducible, semi-quantitative detection of viral RNA across a range of concentrations, even in turbid water and wastewater matrices. This work also establishes empirical benchmarks that can be used to inform future handheld LAMP platforms: (i) a conservative positivity benchmark ($\Delta OD_{434-560nm} \geq 0.25$), (ii) an effective incubation window of 40–60 min at 45 °C that is compatible with low-power heaters, and (iii) recommended sample replication to maintain detection precision below 10% CV. Despite limitations at low RNA concentrations, spectrophotometry-coupled RT-LAMP offers a rapid and resource-efficient method for semi-quantitative pathogen detection.

Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts of interest

There are no conflicts to declare.

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