

Comparative evaluation of heating instruments for detecting *Bacteroides* HF183, SARS-CoV-2, and Aichi virus A in untreated wastewater using colorimetric LAMP

Mariah C. A. do Nascimento^{a,b}, Wendy J. M. Smith^a, Yawen Liu^{a,c}, Vivaldo Gomes da Costa^b, Stuart L. Simpson^a, Aaron Bivins^d, Paula Rahal^b and Warish Ahmed^{a,*}

^a CSIRO Environment, Ecosciences Precinct, 41 Boggo Road, Brisbane, QLD 4102, Australia

^b Department of Biology, São Paulo State University – UNESP, São José do Rio Preto, São Paulo 15054-000, Brazil

^c State Key Laboratory of Marine Environmental Science, College of the Environment & Ecology, Xiamen University, Xiamen 361102, China

^d Department of Civil & Environmental Engineering, Louisiana State University, Baton Rouge, LA 70803, USA

*Corresponding author. E-mail: warish.ahmed@csiro.au

ABSTRACT

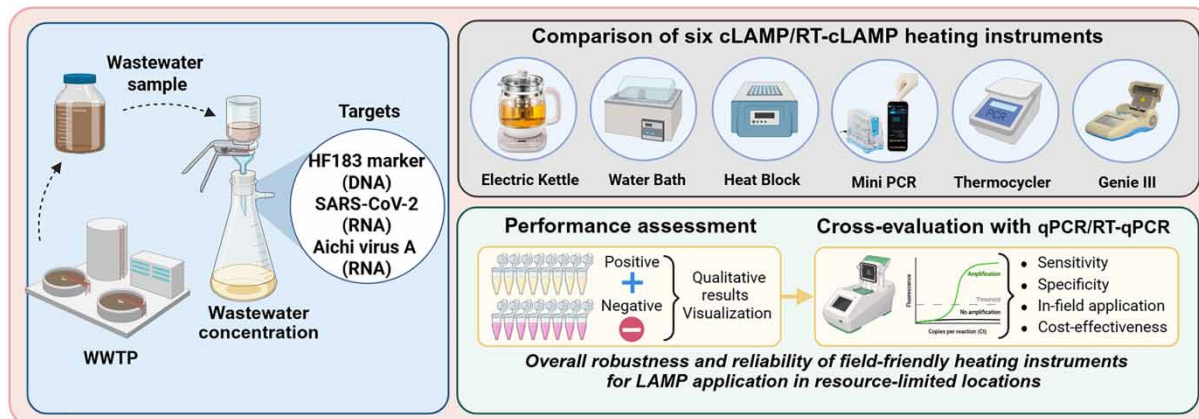
This study assessed the accuracy, field suitability, and cost-effectiveness of colorimetric LAMP (c-LAMP) assays using six heating instruments: Electric Kettle, Water Bath, Heating Block, miniPCR, Thermocycler, and Genie® III. *Bacteroides* HF183 (HF183), SARS-CoV-2, and Aichi virus A (Aiv-A) were tested using control materials and wastewater samples, comparing cLAMP with qPCR/RT-qPCR detection. qPCR/RT-qPCR reliably detected HF183 and Aiv-A genetic fragments at dilutions as low as 1×10^{-5} pg/μL, corresponding to 1.24 and 1.49 log₁₀ GC/reaction. cLAMP/RT-cLAMP detected these fragments at 1×10^{-4} pg/μL (2.28 and 2.63 log₁₀ GC/reaction), though Electric Kettle, miniPCR, and Genie® III occasionally produced false negatives. RT-qPCR reliably detected SARS-CoV-2 fragments at 1×10^0 GC/μL (1.86 log₁₀ GC/reaction), with cLAMP/RT-cLAMP performing similarly across most instruments except Thermocycler. No significant differences ($p > 0.05$) were observed among instruments, though inconsistencies appeared at lower concentrations. The findings offer valuable insights into heating instrument performance for cLAMP/RT-cLAMP assays, guiding their application in wastewater-based pathogen detection.

Key words: cost-effectiveness, field-performance, loop-mediated isothermal amplification, specificity, sensitivity, virus detection

HIGHLIGHTS

- Six heating instruments were compared for cLAMP sensitivity, specificity, and field performance.
- cLAMP detected HF183, SARS-CoV-2, and Aiv-A at 1×10^4 pg/μL, but some instruments yielded false negatives.
- No significant differences were found among the heating instruments for detecting HF183, SARS-CoV-2, and Aiv-A.
- Discrepancies noted in cLAMP/RT-cLAMP results at lower concentrations with different instruments.

GRAPHICAL ABSTRACT



INTRODUCTION

Tracking sewage pollution in environmental waters using human fecal markers is essential for safeguarding public health and managing environmental risks (Harwood *et al.* 2014). Host-associated fecal markers allow for precise identification of fecal pollution sources, distinguishing between human and animal origin (Harwood *et al.* 2014; Ahmed *et al.* 2023a). This enables targeted remediation efforts to ensure water bodies remain safe for recreational activities and drinking water sources, while also preventing waterborne diseases, supporting compliance with water quality regulations, and promoting sustainable water management practices. Ultimately this contributes to the well-being of both human populations and natural environments (Thompson *et al.* 2020; Wear *et al.* 2021).

The most widely recognized human fecal markers for sewage pollution tracking are *Bacteroides* HF183 (Green *et al.* 2014), *Carjivirus* (Stachler *et al.* 2017), and pepper mild mottle virus (PMMoV) (Kitajima *et al.* 2018). The HF183 gene sequence, used as human fecal pollution maker, is highly specific to *Bacteroides* in human feces. The performance of the HF183 marker has been widely validated in diverse geographic regions and is known for its high host sensitivity and specificity in detecting human fecal pollution in environmental waters (Ahmed *et al.* 2016). In addition to general sewage pollution tracking, wastewater surveillance has gained significant traction in recent years for monitoring pathogens like severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), enabling the early detection of COVID-19 outbreaks, often before clinical cases are reported, providing valuable lead time for public health responses (McClary-Gutierrez *et al.* 2021; Kumar *et al.* 2024). Another important pathogen, Aichi virus (AiV), is a waterborne pathogen that spreads through fecal contamination, posing a public health risk (Lodder *et al.* 2013). Although infections are often asymptomatic, symptomatic cases can lead to diarrhea, abdominal pain, nausea, vomiting, and fever, particularly in children, elders, and immunocompromised individuals (Rivadulla & Romalde 2020).

Polymerase chain reaction (PCR) and quantitative PCR (qPCR) are widely recognized for their specificity and sensitivity in nucleic acid-based molecular diagnostics. However, they have several limitations that can hinder their application, particularly in resource-limited settings. These limitations include the significant capital investment required for equipment such as Thermocyclers for precise temperature cycling and gel documentation for target detection and quantification. Additionally, PCR/qPCR requires trained personnel, and expensive reagents, further limiting their accessibility (Garg *et al.* 2022).

As an alternative, isothermal amplification methods such as loop-mediated isothermal amplification (LAMP) have emerged as a viable option, particularly where rapid and cost-effective analysis is needed. LAMP is considered a leading isothermal amplification method due to its high sensitivity and tolerance to PCR inhibitors (Zhang *et al.* 2023). It has been demonstrated that LAMP can amplify target nucleic acid to as many as a billion copies within an hour, making it highly suitable for pathogen detection in both clinical and environmental samples (Ongerth & Danielson 2021; Zhang *et al.* 2023). The LAMP product can be confirmed using simple methods, the most common being the observation of a color change in a colorimetric master mix containing a visible pH indicator, enabling rapid and straightforward detection (Donia *et al.* 2022). More importantly, this method operates at a constant temperature, allowing thermocycling and gel documenting equipment to be replaced with simpler, often readily available apparatus such as Water Baths or Heating Blocks, and portable fluorometers (Das *et al.* 2022; Tran *et al.* 2022).

Various studies developed and evaluated different types of instruments for monitoring SARS-CoV-2 in wastewater using LAMP. For instance, Boza *et al.* (2024) assessed a field portable device named MINI, Alvarez-Serna *et al.* (2023) introduced a portable field-effect transistor (FET)-based sensor for detecting N and ORF1ab genes of the SARS-CoV-2 genome, and Donia *et al.* (2022) described a lab-on-a-chip (LOC) system that integrates reverse-transcription LAMP (RT-LAMP) and microfluidic technology. However, to the best of our knowledge, no study has yet compared the performance of heating instruments for detecting fecal markers and pathogens in wastewater using colorimetric LAMP (cLAMP), which is primarily employed in fieldwork for rapid visual result interpretation. Therefore, the objectives of this study were to (i) assess the performance of six heating instruments (Electric Kettle, Water Bath, Heating Block, miniPCR, Thermocycler, and Genie® III) using cLAMP and RT-cLAMP assays to detect the HF183 marker, SARS-CoV-2, and AiV-A from control materials and 12 untreated wastewater samples compared to qPCR/RT-qPCR, and (ii) evaluate their sensitivity, specificity, and cost-effectiveness compared to qPCR/RT-qPCR, as well as their suitability for field use. This study provides valuable guidance for selecting appropriate heating instruments for cLAMP/RT-cLAMP assays, enhancing the detection of fecal marker and viral pathogens in wastewater.

MATERIALS AND METHODS

Detection sensitivity assessment using serially diluted control materials

Control materials in the form of gBlocks gene fragments were purchased from Integrated DNA technologies for HF183 cLAMP and AiV-A RT-cLAMP (IDT; Coralville, IA, USA). The primers and gBlocks sequences are shown in Supplementary material, Table S1. These control materials were 10-fold serially diluted with the concentration ranging from 1 pg to 1×10^{-5} pg/ μ L for HF183 and AiV-A. For SARS-CoV-2, a gamma-irradiated control material was obtained from the Australian Centre for Disease Preparedness (ACDP), CSIRO. RNA was extracted from the gamma-irradiated control material using QIAamp Viral RNA MINI kit based on the manufacturer's instructions. These control materials were used to assess the detection sensitivity of HF183, SARS-CoV-2, and AiV-A using cLAMP/RT-cLAMP assays using six heating instruments compared to qPCR/RT-qPCR.

Detection agreement assessment using untreated wastewater samples

Archived wastewater samples were used in this study, which were obtained from the Queensland Health Wastewater Surveillance program (<https://www.data.qld.gov.au/dataset/queensland-wastewater-surveillance-for-sars-cov-2>) between 23/06/2022 and 08/06/2023. All untreated wastewater samples were collected from wastewater treatment plants (WWTPs) across Queensland, Australia, using sterile 1-L plastic bottles, and subsequently stored at -20°C until processing. A total of 12 24-h (one sample was taken in every hour) composite wastewater samples were collected from 12 different WWTPs in Queensland, Australia, using sterile 1-L plastic bottles. These wastewater samples were used to assess the detection agreement of HF183, SARS-CoV-2, and AiV-A using cLAMP/RT-cLAMP assays with six heating instruments, compared to qPCR/RT-qPCR.

Wastewater sample concentration and nucleic acid extraction

A 50 mL aliquot of each wastewater sample was concentrated using an adsorption-extraction (AE) method (Ahmed *et al.* 2021) for the detection and quantification of HF183, SARS-CoV-2, and AiV-A. Each sample was filtered using a 0.45- μ m pore size (47-mm diameter) HA electronegative membrane (Merck Millipore, Tokyo, Japan). Following filtration, the membrane was immediately removed, rolled, and placed into a 5-mL bead-beating tube (Qiagen, Hilden, Germany) for nucleic acid extraction. Nucleic acid extraction from the HA membranes was performed using the RNeasy PowerWater Kit (Cat. No. 14700–50-NF; Qiagen). Lysis buffer PM1 (990 μ L) and 2-mercaptoethanol (10 μ L; Sigma Aldrich, St. Louise, MO, USA) were added to each bead-beating tube before homogenization. Homogenization was carried out using a Precellys 24 tissue homogenizer (Bertin Technologies, Montigny-le Bretonneux, FR), set for three cycles of 15 s at 10,000 rpm with a 10 s interval between cycles. After homogenization, the tubes were centrifuged at 4,000 g for 4 min to pellet the filter debris and beads. Sample lysate supernatant, ranging from 600 to 800 μ L, was then used for nucleic acid extraction following the manufacturer's protocol. The DNase treatment step was omitted from the extraction protocol to obtain both DNA and RNA from each sample, which was then eluted in 100 μ L of RNase-Free Water (Qiagen).

cLAMP and RT-cLAMP assays

Previously published cLAMP and RT-cLAMP assays were used for the detection of HF183 and AiV-A, respectively (do Nascimento *et al.* 2024a, 2024b). For SARS-CoV-2, a commercial RT-cLAMP assay kit was purchased from New England Biolabs (Ipswich, MA, USA). Both cLAMP and RT-cLAMP reactions were prepared using the following components: 12.5 μ L of WarmStart Colorimetric LAMP 2 $\times$ Master Mix with UDG (New England Biolabs), 2.75 μ L of LAMP primer mix (10 $\times$) from the stock primer mix, 2.75 μ L of Guanidine Hydrochloride, 5 μ L of Nuclease-free water, and 2 μ L of nucleic acid. gBlocks gene fragments (IDT, USA) or gamma-irradiated viruses were used as control materials for each LAMP assay. For the no-template control (NTC), 2 μ L of ultrapure water was used.

The cLAMP and RT-cLAMP reactions were performed in 0.2-mL strip tubes from SSIBio (Cat. No. 3245-00; Lodi, CA, USA) for all heating instruments, except for the Genie[®] III (OptiGene, Horsham, UK), which used 0.2-mL tube strips (Cat. No. OP-0008-500; OptiGene). The heating instruments used in this study were: an Electric Kettle (Lyeef, Sydney, Australia), Precision[™] GP 10 Water Bath (ThermoFisher Scientific, Waltham, MA, USA), a Heating Block (Eppendorf[®] Thermomixer[®] C, Hamburg, Germany), including its SmartBlock PCR 96 and a ThermoTop[®] (Eppendorf), a miniPCR (mini16; miniPCR bio, Cambridge, MA, USA), Mastercycler[®] Nexus Gradient (PCR/Thermal Cycler; Eppendorf, Hamburg, Germany) and Genie[®] III (OptiGene).

To ensure consistency and accuracy in the experiment, several precautions were taken. For example, Parafilm (Sigma Aldrich) was applied around the lids of the strip tubes to prevent evaporation during the Heating Block experiment, and a Thermotop[®] lid was used to maintain the programmed temperature. Samples were quickly placed into the Water Bath to avoid temperature fluctuations due to heat dissipation. A 96-well support plate was used to accommodate the strip tubes within both the Electric Kettle and Water Bath and the temperature of the Mastercycler[®] Nexus Gradient lid was set to 70 °C (5 °C above the annealing temperature) to match that of the miniPCR, since the Mastercycler[®] is the only device equipped with an adjustable temperature lid. The strips were then gently vortexed, briefly centrifuged, and placed directly into the heating instruments. All instruments were set at a temperature of 65 °C for 40 min for HF183 marker and SARS-CoV-2 and at 65 °C for 50 min for AiV-A.

To minimize expenses and account for supply constraints, all cLAMP and RT-cLAMP detection for control materials using heating instruments were performed in duplicate. RT-cLAMP detection of SARS-CoV-2 in wastewater using different heating instruments was conducted in duplicate, while cLAMP detection of HF183 and RT-cLAMP detection of AiV-A were performed in triplicate. Following amplification, the observed color changes were interpreted as positive (yellow), presumptive positive (orange), or negative (pink) for each reaction. To verify the presumptive-positive results (i.e., orange color), 1 µL of the amplified product was analyzed using the E-Gel[™] Power Snap Plus Electrophoresis System with E-Gel[™] Sizing DNA Ladder Green features (Cat. No. 10488100) and E-Gel[™] SizeSelect[™] II Agarose Gels, 2% (Cat. No. G661012) from Invitrogen (ThermoFisher Scientific, USA). The results of the colorimetric methods post amplification and electrophoresis on 2% agarose gel are presented in Supplementary material, Figure S1.

qPCR/RT-qPCR assays

Previously published qPCR/RT-qPCR assays were used to quantify HF183, SARS-CoV-2 N1 gene and AiV-A 3CD junction in control materials and wastewater samples (Green *et al.* 2014; CDC 2020; do Nascimento *et al.* 2024b). The HF183 and AiV-A standard curves (ranging from 1×10^6 to 1×10^0 GC/µL) were prepared from the gBlocks gene fragments, while the SARS-CoV-2 standard curve (ranging from 1×10^5 to 1×10^0 GC/µL) was prepared from the extracted RNA samples of gamma-irradiated viruses. The primers and probe sequences and gBlocks fragment sequences, as well as cycling parameters, are presented in Supplementary material, Table S1. All qPCR and RT-qPCR reactions were performed in 25 µL reaction volumes. For HF183 marker, the qPCR mixture was composed of 12.5 µL of 2× QuantiNova Probe PCR Master Mix (Qiagen, Hilden, Germany), 1,000 nM each of forward and reverse primers, 100 nM of probe, and 7.87 µL of nuclease-free water. For SARS-CoV-2, the RT-qPCR mixture included 6.25 µL of 4× TaqMan Fast Virus 1-step Master Mix (Applied Biosystems, Waltham, MA, USA) 800 nM each of forward and reverse primers, 200 nM of probe, and 14.1 µL of nuclease-free water. For AiV-A, the RT-qPCR reaction mixture consisted of 6.25 µL of 4× TaqMan Fast Virus 1-step Master Mix (Applied Biosystems), 800 nM each of forward and reverse primers, 300 nM of probe, and 14.3 µL of nuclease-free water. In all reactions, 2 µL of eluted nucleic acid was added. The assays were performed using a Bio-Rad CFX96 thermal cycler (Bio-Rad Laboratories, Richmond, CA, USA) with manual settings for threshold and baseline (i.e., 100 RFU for HF183 and SARS-CoV-2, and 80 RFU for AiV-A). All reactions were performed in triplicate, and samples were analyzed alongside three negative controls.

PCR inhibition

A test was performed to assess PCR inhibition in wastewater nucleic acid samples using a murine hepatitis virus (MHV) RT-PCR assay. Samples exhibiting a two-cycle quantification delay (C_q) were considered to potentially contain PCR inhibitors (do Nascimento *et al.* 2024b). None of the wastewater nucleic acid samples showed the evidence of inhibitors as determined by the MHV inhibition control RT-PCR assay.

Quality control

To minimize cLAMP/RT-cLAMP and qPCR/RT-qPCR contamination, nucleic acid extraction and LAMP/PCR setup were performed in separate laboratories. For each cLAMP/RT-cLAMP run, identical master mixtures and nucleic acids were combined, evenly distributed, and simultaneously assessed across all six heating instruments.

Data interpretation and statistical analysis

For HF183, SARS-CoV-2, and AiV-A control materials, a sample was considered cLAMP- and RT-cLAMP-positive if amplification was detected in at least one of the two replicates. For HF183 and AiV-A detection in wastewater samples, a sample was considered positive if amplification was detected in at least two out of the three replicates, while for SARS-CoV-2,

positivity required detection in one out of two replicates. No amplification in two replicates (or one out of three) was considered not detected (ND). A positive cLAMP/RT-cLAMP outcome was confirmed by observing the desired color change (yellow = positive) or, for presumptive positive result (orange), confirmation through gel electrophoresis.

For HF183, SARS-CoV-2, and AiV-A, a sample was classified qPCR- and RT-qPCR-positive if amplification was detected in at least two out of three replicates. A sample was considered qPCR/RT-qPCR non-quantifiable (NQ) if the quantification value was <2 GC/reaction. If all replicates in qPCR and RT-qPCR were negative, the sample was classified as not detected (ND). False-positive and false-negative cLAMP/RT-cLAMP results were determined by comparing with qPCR/RT-qPCR results.

The detection results of cLAMP/RT-cLAMP for wastewater samples and control materials were aggregated for statistical comparisons of the performance of six heating instruments. Chi-Square Test and Cochran's Q Test were used to determine if there were significant differences in detection results of cLAMP/RT-cLAMP among six instruments for HF183 marker, SARS-CoV-2 and AiV-A, respectively. Differences among instruments' performance were considered significant when $p < 0.05$.

RESULTS

RT-qPCR/qPCR assays performance

The qPCR assay targeting the HF183 marker exhibited an efficiency of 99.2% and an R^2 of 0.99. The standard curve had a slope of -3.34 and a Y-intercept of -39.2 . The RT-qPCR assay targeting the N1 gene of SARS-CoV-2 demonstrated an efficiency of 93.4% and a linearity (R^2) of 0.99. The slope of its standard curve was -3.49 , with a Y-intercept of 40.5 . For the AiV-A RT-qPCR assay targeting the 3CD junction, the efficiency was 97.1% with an R^2 of 0.99. The slope of the standard curve was -3.39 and the Y-intercept was 37.6 .

Comparison of assay detection sensitivity for HF183, SARS-CoV-2 and AiV-A using PCR and LAMP across control material dilution levels and heating instruments

The detection sensitivity of the cLAMP/RT-cLAMP assays (HF183, SARS-CoV-2 and AiV-A) using six heating instruments were compared to qPCR/RT-qPCR results. For HF183, qPCR produced quantifiable data across all six levels of the dilution series ranging from 6.23 ($1 \text{ pg}/\mu\text{L}$) to 1.24 ($1 \times 10^{-5} \text{ pg}/\mu\text{L}$) $\log_{10}$ GC/reaction (Table 1). cLAMP yielded positive amplifications at a dilution of $1 \times 10^{-3} \text{ pg}/\mu\text{L}$ of control materials, which corresponded to a concentration of $3.40 \log_{10}$ GC/reaction by qPCR across all six heating instruments. At the $1 \times 10^{-3} \text{ pg}/\mu\text{L}$ dilution, all heating instruments yielded positive results in 2/2 replicates except for the miniPCR, where only one out of two replicates showed a positive result. At the $1 \times 10^{-4} \text{ pg}/\mu\text{L}$ dilution (qPCR concentration $2.28 \log_{10}$ GC/reaction), the Water Bath, Heating Block, miniPCR, and Thermocycler returned one positive result out of two replicates, while the Genie® III and Electric Kettle failed to produce amplification in any of the replicates. At the $1 \times 10^{-5} \text{ pg}/\mu\text{L}$ dilution (qPCR concentration $1.24 \log_{10}$ GC/reaction), HF183 was not detected using any heating instruments.

Table 1 | Comparison of assay detection sensitivity for HF183 qPCR and cLAMP across control material dilution levels and heating instruments

Control material dilution series (pg/ μL)	HF183 qPCR No. of positive/no. of replicates tested ($\log_{10}$ GC/reaction $\pm$ SD)	HF183 cLAMP No. of positive/no. of replicates tested (sample results designation)					
		Electric Kettle	Water Bath	Heating Block	miniPCR	Thermocycler	Genie® III
1	3/3 (6.23 ± 0.04)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)
1×10^{-1}	3/3 (5.30 ± 0.03)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)
1×10^{-2}	3/3 (4.36 ± 0.03)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)
1×10^{-3}	3/3 (3.40 ± 0.03)	2/2 (+)	2/2 (+)	2/2 (+)	1/2 (+)	2/2 (+)	2/2 (+)
1×10^{-4}	3/3 (2.28 ± 0.06)	0/2 (ND)	1/2 (+)	1/2 (+)	1/2 (+)	1/2 (+)	0/2 (ND)
1×10^{-5}	3/3 (1.24 ± 0.11)	0/2 (ND)	0/2 (ND)	0/2 (ND)	0/2 (ND)	0/2 (ND)	0/2 (ND)

SD, standard deviation; ND, not detected.

+ indicates positive amplification.

For SARS-CoV-2, RT-qPCR generated quantifiable results across all six levels of the dilution series, with values ranging from 6.51 (corresponding to 1×10^5 GC/ μ L) to 1.86 (corresponding to 1×10^0 GC/ μ L) $\log_{10}$ GC/reaction (Table 2). RT-cLAMP yielded positive amplification down to a dilution 1×10^1 GC of control material, with a concentration of 2.69 $\log_{10}$ GC/reaction by RT-qPCR across all six heating instruments. At the 1×10^0 dilution (corresponding to 1.86 $\log_{10}$ GC/reaction), the Water Bath, Heating Block, miniPCR, and Genie® III showed amplification in one out of two replicates, while the Electric Kettle and Thermocycler did not produce amplification in any replicates.

Similarly, for AiV-A, RT-qPCR yielded quantifiable data across all six-dilution levels, ranging from 6.70 (1 pg/ μ L) to 1.49 $\log_{10}$ GC/reaction (1×10^{-5} pg/ μ L) (Table 3). RT-cLAMP showed positive amplification down to a dilution 1×10^{-3} pg/ μ L of control material, with a concentration of 3.65 $\log_{10}$ GC/reaction by RT-qPCR across all six heating instruments. At the 1×10^{-4} pg/ μ L dilution (corresponding to 2.63 $\log_{10}$ GC/reaction), the Water Bath and Thermocycler continued to show both replicates with amplification, while the Electric Kettle and Heating Block showed in one out of two replicates. Both the miniPCR and Genie® III did not produce any amplification in any replicates at this concentration. At the dilution 1×10^{-5} pg/ μ L dilution (RT-qPCR concentration of 1.49 $\log_{10}$ GC/reaction), no amplification was observed with any instruments in both replicates.

Comparison of endogenous HF183, SARS-CoV-2 and AiV-A detection in wastewater samples using PCR and LAMP across six heating instruments

For HF183, qPCR produced quantifiable data across all 12 wastewater samples, with concentrations ranging from 5.03 (WW12) to 7.76 (WW5) $\log_{10}$ GC/50 mL (Table 4). In cLAMP, all 12 samples targeting HF183 showed amplification in all three replicates using all six heating instruments.

Table 2 | Comparison of assay detection sensitivity for SARS-CoV-2 RT-qPCR and RT-cLAMP across control material dilution levels and heating instruments

Control material dilution series (GC/ μ L)	SARS-CoV-2 RT-qPCR No. of positive/no. of replicates tested ($\log_{10}$ GC/reaction $\pm$ SD)	SARS-CoV-2 RT-cLAMP No. of positive/no. of replicates tested (sample results designation)					
		Electric Kettle	Water Bath	Heating Block	miniPCR	Thermocycler	Genie® III
1×10^5	3/3 (6.51 $\pm$ 0.02)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)
1×10^4	3/3 (5.56 $\pm$ 0.01)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)
1×10^3	3/3 (4.61 $\pm$ 0.01)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)
1×10^2	3/3 (3.68 $\pm$ 0.00)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)
1×10^1	3/3 (2.69 $\pm$ 0.02)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)
1×10^0	3/3 (1.86 $\pm$ 0.08)	0/2 (ND)	1/2 (+)	1/2 (+)	1/2 (+)	0/2 (ND)	1/2 (+)

SD, standard deviation; ND, not detected.

+ indicates positive amplification.

Table 3 | Comparison of assay detection sensitivity for AiV-A RT-qPCR and RT-cLAMP across control material dilution levels and heating instruments

Control material dilution series (pg/ μ L)	AiV-A RT-qPCR No. of positive/no. of replicates tested ($\log_{10}$ GC/reaction $\pm$ SD)	AiV-A RT-cLAMP No. of positive/no. of replicates tested (sample results designation)					
		Electric Kettle	Water Bath	Heating Block	miniPCR	Thermocycler	Genie® III
1	3/3 (6.70 $\pm$ 0.03)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)
1×10^{-1}	3/3 (5.71 $\pm$ 0.03)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)
1×10^{-2}	3/3 (4.74 $\pm$ 0.06)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)
1×10^{-3}	3/3 (3.65 $\pm$ 0.03)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)
1×10^{-4}	3/3 (2.63 $\pm$ 0.03)	1/2 (+)	2/2 (+)	1/2 (+)	0/2 (ND)	2/2 (+)	0/2 (ND)
1×10^{-5}	3/3 (1.49 $\pm$ 0.07)	0/2 (ND)	0/2 (ND)	0/2 (ND)	0/2 (ND)	0/2 (ND)	0/2 (ND)

SD, standard deviation; ND, not detected.

+ indicates positive amplification.

Table 4 | Comparison of endogenous HF183 detection in wastewater samples using qPCR and cLAMP across six heating instruments

Wastewater samples	HF183 qPCR No. of positive/ no. of replicates tested (log ₁₀ GC/50 mL ± SD)	HF183 cLAMP No. of positive/no. of replicates tested (sample results designation)					
		Electric Kettle	Water Bath	Heating Block	miniPCR	Thermocycler	Genie® III
WW1	3/3 (5.21 ± 0.03)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
WW2	3/3 (5.25 ± 0.01)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
WW3	3/3 (6.11 ± 0.02)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
WW4	3/3 (6.54 ± 0.01)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
WW5	3/3 (7.76 ± 0.05)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
WW6	3/3 (5.81 ± 0.02)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
WW7	3/3 (6.76 ± 0.02)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
WW8	3/3 (5.99 ± 0.06)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
WW9	3/3 (6.95 ± 0.02)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
WW10	3/3 (6.32 ± 0.02)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
WW11	3/3 (7.44 ± 0.05)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
WW12	3/3 (5.03 ± 0.01)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)

SD, standard deviation.

+ indicates positive amplification.

For SARS-CoV-2, RT-qPCR also generated quantifiable data across all 12 wastewater samples, with mean concentrations ranging from 3.02 (WW7) to 4.77 (WW4) log₁₀ GC/50 mL (Table 5). In RT-cLAMP, SARS-CoV-2 was detected in 11/12 (91.7%), 9/12 (75.0%), 11/12 (91.7%), 10/12 (83.3%), 11/12 (91.7%), and 9/12 (75.0%) wastewater samples using Electric Kettle, Water Bath, Heating Block, miniPCR, Thermocycler, and Genie® III, respectively. Overall, RT-cLAMP detection was less consistent when RT-qPCR-measured concentration fell below 3.92 log₁₀ GC/50 mL, with most heating instruments failing to produce amplification in both replicates. However, an exception was observed for sample WW2, which had an

Table 5 | Comparison of endogenous SARS-CoV-2 detection in wastewater samples using RT-qPCR and RT-cLAMP across six heating instruments

Wastewater samples	SARS-CoV-2 RT-qPCR No. of positive/no. of replicates tested (log ₁₀ GC/50 mL ± SD)	SARS-CoV-2 RT-cLAMP No. of positive/no. of replicates tested (sample results designation)					
		Electric Kettle	Water Bath	Heating Block	miniPCR	Thermocycler	Genie® III
WW1	3/3 (4.67 ± 0.05)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)
WW2	3/3 (3.87 ± 0.02)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)
WW3	3/3 (4.01 ± 0.18)	1/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)
WW4	3/3 (4.77 ± 0.02)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)
WW5	3/3 (4.09 ± 0.06)	2/2 (+)	2/2 (+)	1/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)
WW6	3/3 (4.63 ± 0.06)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)
WW7	3/3 (3.02 ± 0.25)	1/2 (+)	0/2 (ND)	0/2 (ND)	1/2 (+)	0/2 (ND)	0/2 (ND)
WW8	3/3 (4.32 ± 0.02)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)
WW9	3/3 (3.62 ± 0.10)	2/2 (+)	0/2 (ND)	1/2 (+)	1/2 (+)	2/2 (+)	0/2 (ND)
WW10	3/3 (3.68 ± 0.16)	0/2 (ND)	1/2 (+)	2/2 (+)	0/2 (ND)	1/2 (+)	0/2 (ND)
WW11	3/3 (4.13 ± 0.04)	1/2 (+)	1/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)	2/2 (+)
WW12	3/3 (3.92 ± 0.06)	2/2 (+)	0/2 (ND)	1/2 (+)	0/2 (ND)	1/2 (+)	1/2 (+)

SD, standard deviation; ND, not detected.

+ indicates positive amplification.

RT-qPCR concentration below 3.92 log₁₀ GC/50 mL but still yielded positive results in both replicates across all heating instruments. Among the heating instruments, the Electric Kettle, Heating Block, and Thermocycler returned higher detection rates when SARS-CoV-2 concentrations ranged between 3.02 (WW7) and 3.92 (WW12) log₁₀ GC/50 mL of wastewater, compared to other instruments.

For AiV-A, nine out of 12 wastewater samples (75.0%) were quantifiable in RT-qPCR, with concentrations ranging from 3.42 (WW9) to 4.56 (WW7) log₁₀ GC/50 mL (Table 6) in quantifiable samples. RT-cLAMP detected AiV-A in 7/12 (58.3%) samples using the Electric Kettle, 7/12 (58.3%) using the Water Bath, 6/12 (50.0%) using the Heating Block, 8/12 (66.7%) using miniPCR, 9/12 (75.0%) using the Thermocycler, and 8/12 (66.7%) using Genie® III. In general, when the RT-qPCR-measured concentration of AiV-A was below 4.07 log₁₀ GC/50 mL, detection by RT-cLAMP was either inconsistent across replicates or not achieved for certain heating instruments.

Comparison of HF183 cLAMP, SARS-CoV-2, and AiV-A RT-cLAMP detection performance in combined control materials and wastewater samples across heating instruments

Across various heating instruments, the Thermocycler, Heating Block, and Water Bath demonstrated the highest performance, each yielding positive detections in 93.8% replicates (45/48 positive) (Table 7). However, the presumptive-positive rates varied, with the Thermocycler at 8.88%, the Heating Block at 4.44%, and the Water Bath at 2.22%. Similarly, the Heating Block and Thermocycler exhibited the highest detection rates for SARS-CoV-2, with amplification observed in 83.3% of replicates (30/36 positive) and a presumptive-positive rate of 3.33% for both. For AiV-A, the Thermocycler demonstrated the best performance, with 72.9% of replicates (35/48 positive) yielding amplification and a 5.71% presumptive-positive rate. HF183 cLAMP and SARS-CoV-2 RT-cLAMP showed no false-positive results across six heating instruments compared to qPCR/RT-qPCR results. However, AiV-A RT-cLAMP produced false-positive results in 1/48 replicates (2.10%) using both the Water Bath and Electric Kettle. False-negative results were observed for HF183, SARS-CoV-2 and AiV-A across heating instruments when compared to qPCR/RT-qPCR. The false-negative rates ranged from 6.25 to 8.33% for HF183, 16.7 to 25.0% for SARS-CoV-2, and from 4.21 to 18.8% for AiV-A. The Chi-Square Test and Cochran's Q Test revealed no significant differences ($p > 0.05$) in the cLAMP/RT-cLAMP qualitative results obtained among the six heating instruments for the HF183 marker, SARS-CoV-2, and AiV-A, respectively.

DISCUSSION

While PCR is widely regarded as the gold standard for pathogen detection in water and wastewater, it may not be ideal for use in remote or resource-limited settings (Jiménez-Rodríguez *et al.* 2022). This limitation arises from its reliance on sophisticated

Table 6 | Comparison of endogenous AiV-A detection in wastewater samples using RT-qPCR and RT-cLAMP across six heating instruments

Wastewater samples	AiV-A RT-qPCR No. of positive/no. of replicates tested (log ₁₀ GC/50 mL ± SD)	AiV-A RT-cLAMP No. of positive/no. of replicates tested (sample results designation)					
		Electric Kettle	Water Bath	Heating Block	miniPCR	Thermocycler	Genie® III
WW1	0/3 (ND)	0/3 (ND)	0/3 (ND)	0/3 (ND)	0/3 (ND)	0/3 (ND)	0/3 (ND)
WW2	3/3 (3.65 ± 0.05)	0/3 (ND)	1/3 (ND)	1/3 (ND)	0/3 (ND)	3/3 (+)	3/3 (+)
WW3	3/3 (4.34 ± 0.16)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
WW4	3/3 (3.91 ± 0.04)	3/3 (+)	3/3 (+)	2/3 (+)	3/3 (+)	2/3 (+)	2/3 (+)
WW5	0/3 (ND)	0/3 (ND)	1/3 (ND)	0/3 (ND)	0/3 (ND)	0/3 (ND)	0/3 (ND)
WW6	3/3 (4.46 ± 0.04)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
WW7	3/3 (4.56 ± 0.06)	2/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
WW8	0/3 (ND)	1/3 (ND)	0/3 (ND)	0/3 (ND)	0/3 (ND)	0/3 (ND)	0/3 (ND)
WW9	3/3 (3.42 ± 0.14)	0/3 (ND)	2/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
WW10	3/3 (4.07 ± 0.11)	3/3 (+)	3/3 (+)	2/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
WW11	3/3 (4.07 ± 0.25)	2/3 (+)	2/3 (+)	0/3 (ND)	2/3 (+)	2/3 (+)	1/3 (ND)
WW12	3/3 (3.69 ± 0.06)	2/3 (+)	1/3 (ND)	1/3 (ND)	2/3 (+)	3/3 (+)	3/3 (+)

SD, standard deviation; ND, not detected.

+ indicates positive amplification.

Table 7 | Comparative detection performance of HF183 cLAMP, SARS-CoV-2, and AiV-A RT-cLAMP for combined control materials and wastewater samples across heating instruments

cLAMP/RT-cLAMP results	HF183 marker (No. of replicates showing positive/negative amplification/no. of replicates tested) (%)					
	Electric Kettle	Water Bath	Heating Block	miniPCR	Thermocycler	Genie® III
Positive ^a	44/48 (91.6%)	45/48 (93.8%)	45/48 (93.8%)	44/48 (91.7%)	45/48 (93.8%)	44/48 (91.7%)
Negative ^b	4/48 (8.33%)	3/48 (6.25%)	3/48 (6.25%)	4/48 (8.33%)	3/48 (6.25%)	4/48 (8.33%)
True-presumptive positive ^c	1/44 (2.27%)	1/45 (2.22%)	2/45 (4.44%)	1/44 (2.27%)	4/45 (8.88%)	1/44 (2.27%)
False-presumptive positive ^d	1/4 (25.0%)	0/3 (0.00%)	0/3 (0.00%)	1/4 (25.0%)	0/3 (0.00%)	0/4 (0.00%)
False positive ^e	0/48 (0.00%)	0/48 (0.00%)	0/48 (0.00%)	0/48 (0.00%)	0/48 (0.00%)	0/48 (0.00%)
False negative ^f	4/48 (8.33%)	3/48 (6.25%)	3/48 (6.25%)	4/48 (8.33%)	3/48 (6.25%)	4/48 (8.33%)
SARS-CoV-2 (No. of replicates showing positive/negative amplification/no. of replicates tested) (%)						
Positive ^a	29/36 (80.6%)	27/36 (75.0%)	30/36 (83.3%)	29/36 (80.6%)	30/36 (83.3%)	28/36 (77.8%)
Negative ^b	7/36 (19.4%)	9/36 (25.0%)	6/36 (16.7%)	7/36 (19.4%)	6/36 (16.7%)	8/36 (22.2%)
True-presumptive positive ^c	0/29 (0.00%)	1/27 (3.70%)	1/30 (3.33%)	1/29 (3.44%)	1/30 (3.33%)	1/28 (3.57%)
False-presumptive positive ^d	0/7 (0.00%)	0/9 (0.00%)	0/6 (0.00%)	0/7 (0.00%)	0/6 (0.00%)	0/8 (0.00%)
False positive ^e	0/36 (0.00%)	0/36 (0.00%)	0/36 (0.00%)	0/36 (0.00%)	0/36 (0.00%)	0/36 (0.00%)
False negative ^f	7/36 (19.4%)	9/36 (25.0%)	6/36 (16.7%)	7/36 (19.4%)	6/36 (16.7%)	8/36 (22.2%)
AiV-A (No. of replicates showing positive/negative amplification/no. of replicates tested) (%)						
Positive ^a	29/48 (60.4%)	32/48 (66.7%)	27/48 (56.3%)	30/48 (62.5%)	35/48 (72.9%)	32/48 (66.7%)
Negative ^b	19/48 (39.6%)	16/48 (33.3%)	21/48 (43.8%)	18/48 (37.5%)	13/48 (27.1%)	16/48 (33.3%)
True-presumptive positive ^c	2/29 (6.89%)	0/32 (0.00%)	3/27 (11.1%)	2/30 (6.66%)	2/35 (5.71%)	2/32 (6.25%)
False-presumptive positive ^d	2/19 (10.5%)	1/16 (6.25%)	6/21 (28.6%)	0/18 (0.00%)	0/13 (0.00%)	1/16 (6.25%)
False positive ^e	1/48 (2.08%)	1/48 (2.08%)	0/48 (0.00%)	0/48 (0.00%)	0/48 (0.00%)	0/48 (0.00%)
False negative ^f	9/48 (18.8%)	6/48 (12.5%)	9/48 (18.8%)	5/48 (10.4%)	2/48 (4.16%)	3/48 (6.25%)

^aThe results were either visually identified as positive (i.e., yellow color) or confirmed as such.^bThe results were either visually identified as negative (i.e., pink color) or confirmed as such.^cAmong the positive results obtained, the number of results that were visually identified as presumptive positive (i.e. orange color) and subsequently confirmed by electrophoresis analysis is as follows (positive).^dAmong the negative results obtained, the number of results that were visually identified as presumptive positive (i.e., orange color) and subsequently confirmed by electrophoresis analysis is as follows (negative).^eFalse positive was determined by comparing results with qPCR/RT-qPCR.^fFalse negative was determined by comparing results with qPCR/RT-qPCR.

laboratory equipment, highly trained personnel, expensive reagents, and the extended analysis time required to obtain reliable results (Schrader *et al.* 2012; de Oliveira Coelho *et al.* 2021). In contrast, LAMP offers several advantages as a point-of-care (POC) or point-of-exposure (POE) diagnostic platform (de Oliveira Coelho *et al.* 2021) over PCR. For example, cLAMP provides rapid results, typically within 30–40 min, and can be performed in minimalistic settings using a Water Bath or Heating Block (Mu *et al.* 2016; Das *et al.* 2022; Tran *et al.* 2022). While the use of various instruments for cLAMP has been well-documented (Nzulu *et al.* 2016; Rubinfien *et al.* 2020; Wang *et al.* 2020; Peltzer *et al.* 2021), this study compares the performance and effectiveness of cLAMP/RT-cLAMP in detecting HF183, SARS-CoV-2, and AiV-A in wastewater samples using different heating instruments, in comparison to qPCR/RT-qPCR.

HF183, SARS-CoV-2, and AiV-A detection using qPCR/RT-qPCR was consistent across control material dilution series. Unlike qPCR/RT-qPCR, the detection of HF183, SARS-CoV-2, and AiV-A using cLAMP/RT-cLAMP across all heating instruments showed variability at lower dilution levels, with either all replicates being negative or inconsistent amplification observed. The discrepancy between qPCR/RT-qPCR and cLAMP/RT-cLAMP-based assays may be due to several factors, such as qPCR's higher amplification efficiency, which relies on thermal cycling parameters to create numerous copies of the target nucleic acid, thereby enhancing detection sensitivity (Smith & Osborn 2009). On the other hand, LAMP relies on isothermal conditions, which may result in less efficiency of the target, particularly at very low concentrations (do Nascimento *et al.* 2024b). While qPCR typically uses fluorescent dyes or probes to monitor amplification in real-time,

providing greater sensitivity. LAMP usually relies on visual indicators, such as color changes, or specific dyes, which may not offer the same level of sensitivity as qPCR (Smith & Osborn 2009; de Oliveira Coelho *et al.* 2021). Finally, the enzymes used in qPCR (Taq polymerase) and LAMP (Bst polymerase) have distinct properties. Taq polymerase in qPCR is highly specific and efficient at amplifying targets over multiple cycles, while Bst polymerase used in this cLAMP may be less effective at amplifying low amounts of target nucleic acid (Smith & Osborn 2009; do Nascimento *et al.* 2024b).

It would have been useful to test the sensitivity of the cLAMP/RT-cLAMP assay in ambient wastewater samples by seeding HF183, SARS-CoV-2 and AiV-A, followed by serial dilutions (Ahmed *et al.* 2015, 2022). However, due to the lack of access to AiV-A culture, we were unable to conduct the necessary seeding experiment. Additionally, targets selected in this study are naturally occurring in wastewater at high to moderate concentrations. Therefore, rather than seeding, we compared the detection agreement of endogenous HF183, SARS-CoV-2 and AiV-A in archived wastewater samples using qPCR/RT-qPCR and cLAMP/RT-cLAMP across six heating instruments.

Comparing the detection agreement of cLAMP and RT-cLAMP across different heating instruments with qPCR/RT-qPCR enabled us to assess their performance at various target concentrations typically found in wastewater samples. HF183 detection in wastewater samples was consistent across all heating instruments using cLAMP, with 100% positivity across all replicates. This demonstrates reliable agreement for HF183-positive detection by cLAMP, comparable to qPCR. However, it is important to note that such absolute positivity (100% detection) is expected given the high concentrations of the endogenous HF183 marker in wastewater samples (Ahmed *et al.* 2015; Chettleburgh *et al.* 2023; Curtis *et al.* 2024).

For SARS-CoV-2, RT-qPCR consistently achieved high detection rates across all wastewater samples. RT-cLAMP also showed greater detection of SARS-CoV-2 in most samples, although some wastewater samples were negative when the concentration was below 3.92 log₁₀ GC/50 mL. At lower concentrations of SARS-CoV-2 in wastewater, the Genie® III and Water Bath exhibited slightly reduced performance compared to other heating instruments. AiV-A detection also varied across instruments, with some (e.g., Thermocycler and Genie® III) providing consistent results for most samples, while the Heating Block and Water Bath showed reduced sensitivity. The reason for such discrepancy is not fully understood, but sub-sampling error may contribute, in addition to the varying thermal regimes of each instrument. Overall, no significant differences ($p > 0.05$) were observed in the cLAMP/RT-cLAMP detection results across the six heating instruments for the HF183 marker, SARS-CoV-2, and AiV-A.

The performance of various heating instruments was generally comparable, with the Water Bath, Heating Block, Thermocycler, and Genie® III showing consistently high detection for cLAMP and RT-cLAMP methods. However, the Electric Kettle and miniPCR exhibited more variability, with occasional loss of amplification signal. Thermocycler and Genie® III consistently delivered high sensitivity across all three targets, matching or approximating the performance of the Thermocycler in both control materials and wastewater samples. Both the Heating Block and Water Bath provided reliable detection for most targets, although some loss of positive amplifications was observed, particularly for AiV-A. The Electric Kettle, while innovative for its low-cost and accessibility, demonstrated reduced positivity for targets like AiV-A and SARS-CoV-2. Nonetheless, the Electric Kettle may still be desirable in field settings for detecting sewage markers such as HF183, *Carjivirus* and human adenovirus (HAdV).

Comparative detection performance of HF183 cLAMP, SARS-CoV-2 and AiV-A RT-cLAMP across heating instruments showed good concordance with the established qPCR/RT-qPCR methods. Positive LAMP amplification was consistently achieved for 91–94% of replicates, with no false-positives recorded for HF183 and SARS-CoV-2. Presumptive-positive results were minimal and primarily observed at higher dilutions or with instruments like the miniPCR and Electric Kettle, suggesting that, while cLAMP is a highly reliable technique, some variability exists at lower target concentrations. The false-negative rates for HF183 were as high as 8.33%, which were lower than those for SARS-CoV-2 (up to 25.0%) and AiV-A (up to 18.8%). These results suggest that LAMP may not be sensitive enough for trace detection in wastewater samples, especially when disease prevalence is low in the community. However, this may not be an issue for smaller communities such as indigenous communities, or dormitories with on-site sewage systems, where less dilution of viruses is expected to occur. Detection sensitivity can be further enhanced by implementing more effective concentration and nucleic acid extraction methods (Ahmed *et al.* 2023b).

In addition to detection performance evaluation, considering cost is also important for laboratories with limited resources or use in field settings (Table 8). Considering weight, cost, and potential portability, the adjustable Electric Kettle emerges as a viable option, although its performance has yet to be compared further with other heating instruments. Another accessible option, with the second-lowest cost and the lightest weight, is the miniPCR. It features advanced technology suitable for

Table 8 | Heating instruments used in this study and their corresponding updated values in 06/24

Heating instruments	Manufacturing Company	Numbers of samples/ run	Dimensions (cm)	Weight (kg)	Type	Cost
Electric Kettle	Lyeeef, Australia	96 wells (plate support)	23 × 18 × 40.6	2.04	Requires electricity or charger. Can be make portable	A\$ ~80
Water Bath (Precision™ GP 10)	Thermo Scientific, USA	Up to 2× 96 wells (plate support)	39.3 × 38.3 × 23.3	9	Non-portable	A\$ 2,961 ^a
Heating Block (Thermomixer® C, 96 Well Adaptor & ThermoTop®)	Eppendorf, Germany	96 wells	20.6 × 30.4 × 13.6	6.3	Non-portable	A\$ 7,182 (5,066 + 1,212 + 904)
MiniPCR	MiniPCR bio, USA	16 wells	15 × 8.5 × 10.5	0.38	Portable with the versatility of being used with or without an electricity (battery operated)	A\$ ~1,200 ^a
Mastercycler® Nexus Gradient Genie® III	Eppendorf, Germany	96 wells	27.5 × 43 × 33	11.5	Non-portable	A\$ 14,157
	OptiGene, UK	8 wells	25.4 × 16.5 × 8.9	1.75	Portable with the versatility of being used with or without an electricity (battery operated)	A\$ 17,696
Accessories						Cost
Genie® tubes large pack size – 500 strips of eight tubes (Cat. No. OP-0008-500)	OptiGene, UK	NA	NA	NA	NA	A\$ 1,038 (A\$ 2.08/strip)
0.2 mL tube strips 120 strips of eight tubes (Cat. No. 3245-00)	SSIbio, USA	NA	NA	NA	NA	A\$ 171 (A\$ 1.42/strip)

^aValues are shown in Australian Dollars, with amounts converted from US Dollars using the exchange rate of the day (1 USD = 1.50 AUD).

The Eppendorf Mastercycler® Nexus Gradient (PCR/Thermocycler) is no longer available for sale in Australia, prompting us to explore alternatives with similar, next-generation configurations. NA, not applicable.

both laboratory and field use, with control available via a smartphone or laptop. However, it can process only 16 samples/run, whereas the Electric Kettle can accommodate a 96-well plate. When considering cost alone, the Water Bath emerges as the third option. While it is not portable and is relatively heavy, it allows for the highest number of samples to be analyzed at once. In fourth and fifth places, respectively, are the Heating Block and the Thermocycler. Both have higher costs and weight when compared to the other instruments, but they can process 96 samples. Finally, the Genie[®] III ranks last as the most expensive instrument. It requires the exclusive use of its strip tubes and processes the fewest samples (i.e., eight samples/run), but is portable and lightweight. While it may not be the best option for cLAMP in the field, given the availability of other instruments, it is highly valuable for real-time fluorescent detection in the field (Khodaparast *et al.* 2022) and for the optimization of new assays (do Nascimento *et al.* 2024a, 2024b).

CONCLUSIONS

- This study offers important insights into the effectiveness of various heating instruments for LAMP-based assays in detecting fecal markers and pathogens in wastewater. Overall, there were no significant differences ($p > 0.05$) in the cLAMP/RT-cLAMP detection across six heating instruments for the HF183 marker, SARS-CoV-2, and AiV-A, respectively.
- The Heating Block and Thermocycler performed better than other heating instruments, particularly in detecting SARS-CoV-2 and HF183 marker. More accessible options like the Electric Kettle and Water Bath also demonstrated potential for field use, although they had some limitations.
- Future studies should focus on fine-tuning the LAMP assay protocol to minimize false-positives and negatives, particularly for low level targets like AiV-A. Additionally, exploring the integration of LAMP assays with innovative technologies, such as LOC devices and portable sensors, could expand their utility in various settings, from urban areas to remote rural regions.
- By enhancing the accessibility, affordability, and accuracy of LAMP-based diagnostics, researchers can support more effective public health measures and environmental protection efforts globally.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary material.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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