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WASTEWATER-BASED EPIDEMIOLOGY AS A TOOL FOR MONITORING EMERGING AND RE-EMERGING VIRAL DISEASES

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ABSTRACT: *Wastewater-based epidemiology (WBE) has emerged as a powerful and cost-effective approach for monitoring the spread of viral diseases within populations. By analyzing wastewater for viral genetic material shed in human excreta, WBE enables the early detection and surveillance of pathogens such as Rotavirus, Astrovirus, Adenovirus, Sapovirus, poliovirus, and noroviruses. This method provides a non-invasive, community-level assessment of infection trends, capturing data from both symptomatic and asymptomatic individuals. Despite its advantages, challenges such as variability in wastewater composition, environmental degradation of viral RNA, and the need for standardized protocols remain. Nevertheless, WBE holds significant promise as a complementary surveillance tool, particularly in low-resource settings, enabling timely responses to emerging and re-emerging viral threats. WBE gained global prominence during the COVID-19 pandemic, where it was successfully used as an early warning system to predict outbreaks and guide public health interventions. Advances in molecular techniques, including PCR and genomic sequencing, have enhanced the sensitivity and specificity of viral detection in wastewater samples. Wastewater-based epidemiology represents an innovative and scalable strategy for public health monitoring, offering valuable insights into population-level disease dynamics and supporting proactive disease control measures.*

KEYWORDS: Wastewater-based epidemiology, Wastewater, PCR, Rotavirus, Sampling.

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INTRODUCTION

Water, an integral component of life on earth, is being hampered or exploited to a great extent owing to both natural and anthropogenic activities. The definition of wastewater is "Used water from any combination of domestic, industrial, commercial or agricultural activities, surface runoff /storm water, and any sewer infiltration or sewer inflow" (Tilley *et al.,* 2014). The applicability of wastewater as a diagnostic tool referred to as wastewater-based epidemiology (WBE), began in late 1990s when the presence of illicit drugs was detected and correlated with the drug consumption in the associated community. Since then, the technology has been exploited for various purposes such as poliovirus in 2003 and SARS-CoV-2 detection as the recent one (Masachessi *et al.,* 2018; Aakanksha *et al.,* 2022).

The proportion of the population with access to safely managed sanitation increased from 47 % to 54 % between 2015 and 2020, despite the global recognition of efforts to improve drinking water facilities (United Nations, 2023). However, current estimates suggest that over 2 billion people lack improved water sources, and more than 3.5 billion lack access to proper sanitation (WHO; UNICEF, 2023) Socioeconomic and environmental factors exacerbate this crisis, contributing to the degradation of water and sanitation infrastructure, especially in developing regions, exposing billions to water, sanitation, and hygiene (WaSH)-related diseases (Naidoo & Olaniran, 2013). The global prevalence of viral diseases in wastewater are as follows; rotavirus (44%), norovirus (34-61%), enterovirus (22%) (Chacon *et al*., 2021).

Wastewater-Based Epidemiology (WBE) serves as a complementary surveillance tool, particularly for monitoring community-level infectious diseases caused by viruses and other microorganisms (Aguiar-Oliveira *et al.,* 2020). WBE serves as a valuable tool for the timely identification of potential outbreaks, monitoring temporal and geographical trends in infectious diseases, gathering comprehensive epidemiological data in specific regions, especially within socially vulnerable areas, and providing real-time insights into the situation (Prado *et al*., 2023). It is distinguished by its heterogeneous composition of biological materials, including wound materials and various bodily fluids, such as respiratory and nasal secretions, saliva, urine, feces, and semen from infected individuals (symptomatic, asymptomatic, presymptomatic, and post-symptomatic) (Tiwari *et al.,* 2023).

Conventional public health surveillance strategies rely on the confirmation of suspected cases through clinical, epidemiological, or laboratory outcomes, which limits the identification of asymptomatic and pre-symptomatic individuals. (Aguiar-Oliveira *et al.,* 2020). Conversely, WBE provides a methodology to analyze the composition, detect waste, and quantify pathogens in wastewater Choi *et al*., 2018]. Initially, utilized for poliovirus surveillance (Paul & Task, 1979) and detection of illicit drugs and various chemicals (Singer et al., 2023), researchers have since applied WBE to screen a wide range of microorganisms, further expanding its scope during the COVID-19 pandemic (Zehadi *et al.,* 2021).

Wastewater-Based Epidemiology (WBE) facilitates a real-time assessment of community health status, exposure levels to different substances and microorganisms, and tracking of pathogen sources by tracing pipelines and delimiting geographic areas (Mao *et al.,* 2020). Additionally, it is a valuable tool for issuing early warnings about infectious disease outbreaks through wastewater monitoring, providing epidemiological data for precise interventions by governments and health entities (Mao *et al.,* 2020).

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Wastewater-Based Epidemiology (WBE) is a scientific approach that analyzes wastewater and sewage to gather insights into public health trends within communities. By detecting and quantifying biomarkers such as pathogens, chemicals, and metabolites in wastewater, WBE provides a non-invasive method to monitor community-wide health indicators. It enables researchers to track the prevalence of diseases, assess drug use patterns, evaluate environmental exposure, and detect emerging health threats early.

Wastewater-based epidemiology (WBE), also known as wastewater-based surveillance, is a rapidly evolving scientific discipline that leverages community wastewater to assess population health in near real-time (Daughton, 2018). This is accomplished by measuring captured human excreted biomarkers in composited wastewater that are indicative of various aspects of human health, behavior, exposure, and activity. Since the early 2000s, WBE has been used as a beneficial tool for monitoring population-level substance use, and has most recently gained widespread public exposure during the COVID-19 global pandemic, where SARS-CoV-2 monitoring reaffirmed early-warning capabilities and the potential to reveal hotspots of infection not otherwise detected by individualized clinical surveillance (Bowes *et al.,* 2023).

Wastewater surveillance, also called sewer monitoring or wastewater-based epidemiology, is an aggregate and anonymous method of monitoring for population-level infection trends and the spread of viral variants by testing samples of communal wastewater. Viruses that are shed in feces or urine can be detected using wastewater testing (Donia, 2021). Wastewater-based epidemiology has also been adapted to measure the load of pathogens such as SARS-CoV-2 in a community (Madema *et al.,* 2020).

Testing wastewater for the presence of specific pathogens allows for the monitoring of a large population at the same time that can include subclinical, asymptomatic, and symptomatic cases, thus providing a more complete picture of population-level infection than clinical testing alone. This is particularly important when clinical confirmation of infection is difficult or resource intensive (Donia, 2021). Systematic and continuous monitoring of wastewater samples for communicable pathogens can be used for early warning alerts for the emergence of new variants during an ongoing outbreak or monitoring for future outbreaks (Donia, 2021). Viruses are known to be high-risk pathogens, posing a significant threat due to their propensity for mutation and adaptation to new hosts, particularly RNA viruses (Sims, 2020; Xagoraraki and Brien, 2020).

REVIEW

Concept of wastewater-based epidemiology

The concept dates to the 1920s and gained significant attention during the Covid-19 pandemic, with global efforts and initiatives to monitor the virus in wastewater. This approach along with data modelling offers real-time tracking of viral presence in the community, serving as an early warning system for potential outbreaks and enabling proactive public health measures. This presentation will examine the methodological framework of WBE systems through the following three core components:

i. sampling strategies that address spatial–temporal variability in wastewater systems,
ii. comparative performance of different platforms in pathogen detection,

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iii. Predictive modeling integrating machine learning approaches.

Emerging and Re-Emerging Diseases

Emergence and re-emergence factors are all the elements or conditions that often act synergistically and contribute to situations that favour the emergence of emerging and re-emerging diseases. The emergence factors are classified into factors related to the infectious agent, human and animal activities, and environmental changes. Wastewater surveillance, also called sewer monitoring or wastewater-based epidemiology, is an aggregate and anonymous method of monitoring for population-level infection trends and the spread of viral variants by testing samples of communal wastewater. Systematic and continuous monitoring of wastewater samples for communicable pathogens can be used for early warning alerts for the emergence of new variants during an ongoing outbreak or monitoring for future outbreak. (Donia, 2021). Viruses are known to be high-risk pathogens, posing a significant threat due to their propensity for mutation and adaptation to new hosts, particularly RNA viruses. (Xagoraraki & Brien, 2020).

Viruses that are shed in feces or urine can be detected using wastewater testing (Donia, 2021). Viral surveillance in wastewater entails a series of systematic procedures, commencing with the sampling of a specific site within a wastewater collection system and subsequently proceeding to the steps of viral concentration, extraction, and detection (Alygizakis *et al.,* 2021).

This practice, known as Wastewater-Based Epidemiology (WBE), involves the sampling, concentration, and systematic analysis of wastewater markers, enabling the provision of population-scale information regarding activity and exposure in each sewage system-connected area. *(Choi* et *al.,* 2019*)*. The extreme diversity of emerging and re-emerging viral pathogens, the change of human lifestyle, the globalization of travel, business exchanges, and tourism potentiate the risk of emergence of highly pathogenic zoonotic diseases. The cross-cutting ecological and health data at the national and global scales are effective means for sustaining good health in human, animal, and ecosystem (in particular the viral ecology) (Carmo dos Santos *et al*., 2024).

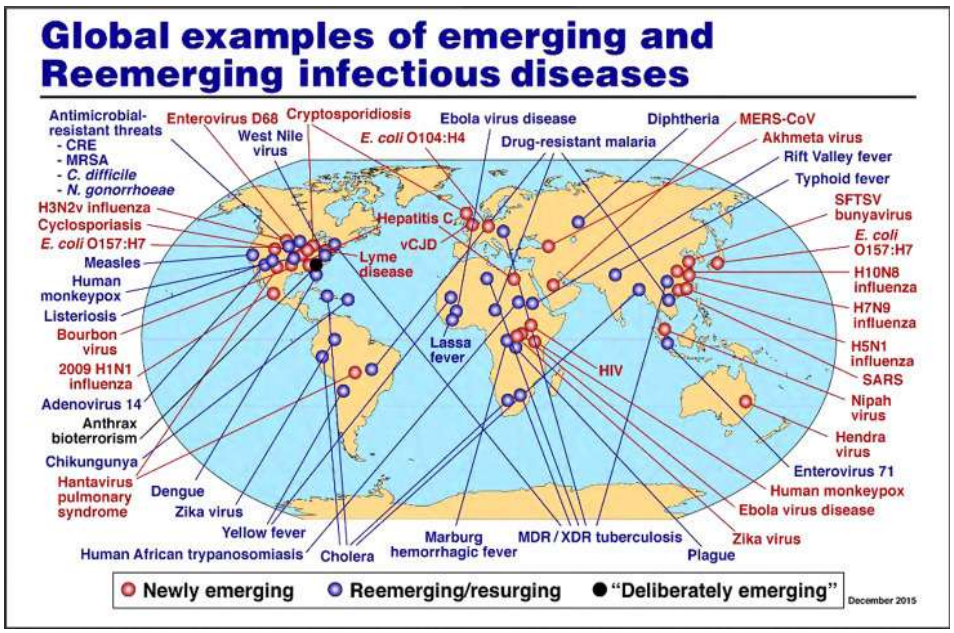

Figure 1: Image showing emerging and re-emerging diseases. (Adapted from National institute of health, 2018).

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Effective wastewater sampling emerges as a crucial tool to address the uncertainties associated with analyzing the spread of the virus (Ahmed *et al*., 2021). Poorly designed sampling techniques can yield unrepresentative samples, leading to potential false-negative errors (Ort *et al*., 2010).

Viral sampling

Several techniques have been employed for the concentration of viral particles in wastewater samples. Among these, three primary concentration methods have emerged as the most widely used: vacuum filtration utilizing an electronegative membrane; a precipitation method employing PEG 8000; and ultrafiltration (Fonseca *et al*., 2022).

Nucleic acid isolation (DNA/RNA)

In PCR (polymerase chain reaction)-based detection methods, the viral genome's target sequence is amplified. However, this necessitates the isolation of genetic material (DNA/RNA), typically involving stages of lysis, purification, and recovery (Barbosa *et al*., 2016) a process commonly performed on human specimens for diagnostic purposes.

Viral detection

For many years, cell culture-based methods have served as the gold standard for detecting infectious viruses in environmental samples (Mackul'ak *et al*., 2021). However, these techniques are limited to the ability to cultivate viruses of interest in vitro. Consequently, PCR and Quantitative (QPCR) PCR methods have become crucial for identifying viruses in water samples due to their rapid detection, high sensitivity, and specificity, enabling test adaptation based on the desired specificity, whether to cover broad viral groups such as enteroviruses or target more specific viral strains based on selected target DNA/RNA sequences (Haramoto *et al*., 2018).

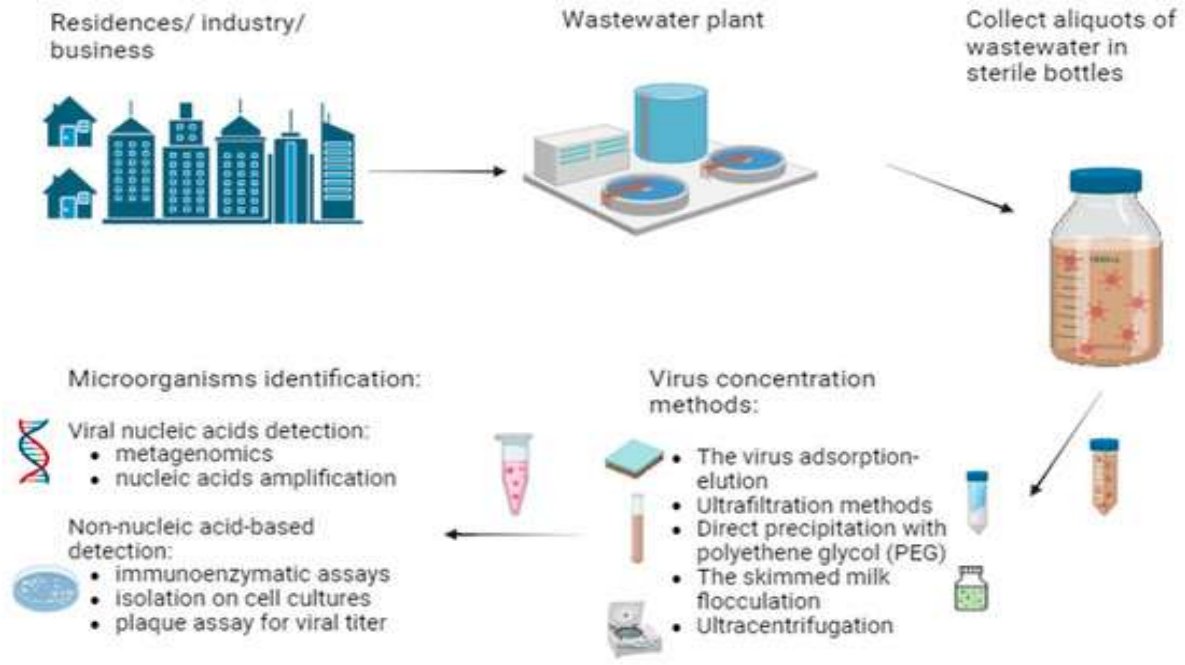

Figure 2: Workflow for virus detection in wastewater (adapted from Bleotu *et al.,* 2024

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Monitoring Tool/Methodology

Wastewater-based epidemiology (WBE) utilizes tools like Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR) and genomic sequencing to detect viral pathogens (e.g., SARS-CoV-2) in sewage (Barbosa *et al*., 2016). This practice, known as Wastewater-Based Epidemiology (WBE), involves the sampling, concentration, and systematic analysis of wastewater markers, enabling the provision of population-scale information regarding activity and exposure in each sewage system-connected area (Choi *et al*., 2019)

Sample Collection Methods: Active sampling (automated, time-proportional or flow-proportional) is used to capture representative, 24-hour composite sewage samples.

Concentration Techniques: Methods such as ultrafiltration, polyethylene glycol (PEG) precipitation, or electronegative membrane filtration are used to concentrate viruses from large volumes of sewage before analysis. Quantitative reverse transcription polymerase chain reaction, also called RT-qPCR, is used to detect and quantify RNA. Total RNA or mRNA is first transcribed into complementary DNA (cDNA). The cDNA is then used as the template for the quantitative PCR or real-time PCR reaction (qPCR).

The working procedure can be divided into two steps:

A. Amplification

Denaturation

High temperature incubation is used to "melt" double- stranded DNA into single strands and loosen secondary structure in single-stranded DNA. The highest temperature that the DNA polymerase can withstand is typically used (usually 95°C). The denaturation time can be increased if template GC content is high (Duran-Jara *et al*., 2025).

Annealing

During annealing, complementary sequences have an opportunity to hybridize, so an appropriate temperature is used that is based on the calculated melting temperature (Tm) of the primers (5°C below the Tm of the primer).

Extension

At 70-72°C, the activity of the DNA polymerase is optimal, and primer extension occurs at rates of up to 100 bases per second. When an amplicon in real-time PCR is small, this step is often combined with the annealing step using 60°C as the temperature.

Detection

The detection is based on fluorescence technology. The specimen is first kept in proper well and subjected to thermal cycle like in the normal PCR. The machine, however, in the Real Time PCR is subjected to tungsten or halogen source that led to fluoresce the marker added to the sample and the signal is amplified with the amplification of copy number of sample DNA. The emitted signal is detected by a detector and sent to computer after conversion into digital signal that is displayed on screen (Duran-Jara *et al*., 2025).

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The signal can be detected when it comes up the threshold level (lowest detection level of the detector). Reverse transcription quantitative PCR (RT-qPCR) is a sensitive method for quantifying gene expression (Bustin *et al*., 2009).

B. Detection

The detection is based on fluorescence technology. The specimen is first kept in proper well and subjected to thermal-cycle like in the normal PCR. The machine, however, in the Real Time PCR is subjected to tungstenorhalogen source that led to fluoresce the marker added to the sample and the signal is amplified with the amplification of copy number of sample DNA. The emitted signal is detected by a detector and sent to computer after conversion into digital signal that is displayed on screen (Duran-Jaraetal., 2025).

The signal can be detected when it comes up the threshold level (lowest detection level of the detector). Reverse transcription quantitative PCR (RT-q PCR) is a sensitive method for quantifying gene expression (Bustinetal., 2009).

KEY MONITORING TECHNOLOGIES AND APPROACHES

Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR): The primary, most common tool used to quantify the viral load (such as SARS-CoV-2) in wastewater samples. Consequently, PCR and qPCR methods have become crucial for identifying viruses in water samples due to their rapid detection, high sensitivity, and specificity, enabling test adaptation based on the desired specificity, whether to cover broad viral groups such as enteroviruses or target more specific viral strains based on selected target DNA/RNA sequences (Haramoto, 2018).

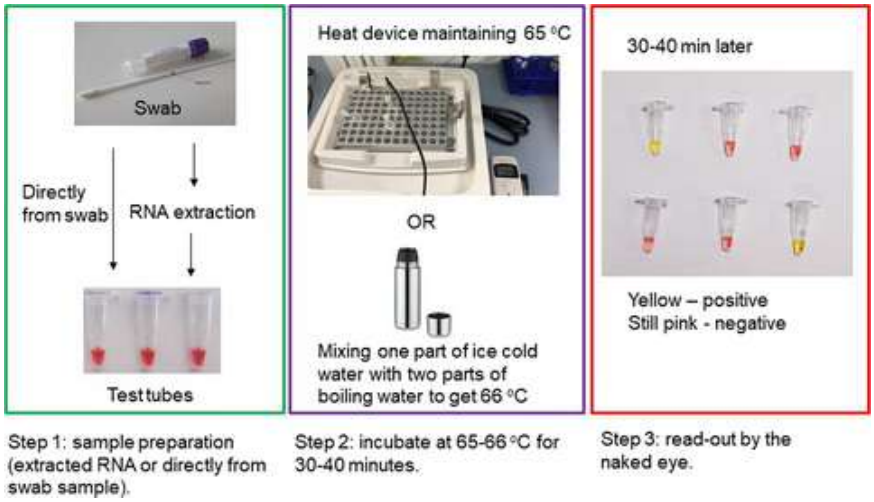

Figure 3: Schematic representation of RT-LAMP procedure (adapted Huang *et al*., 2020)

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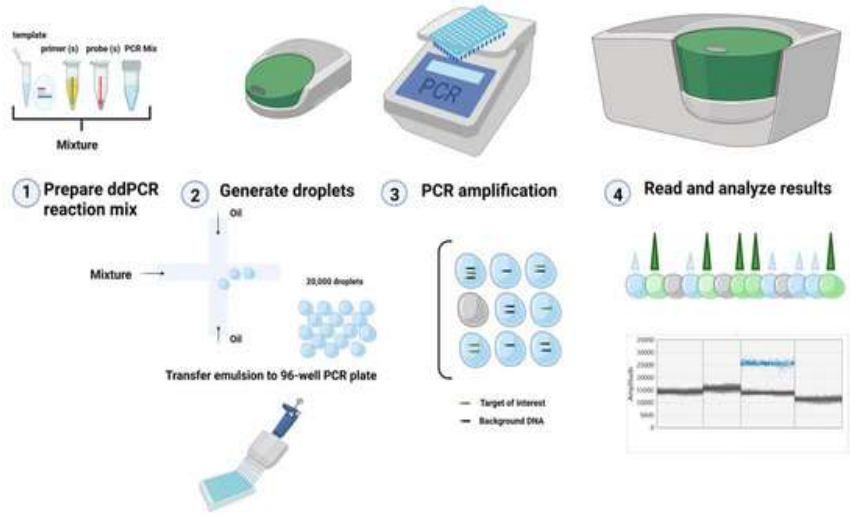

Figure 4: Schematic representation of DDPCR procedure (adapted from Lambrescu *et al*., 2022)

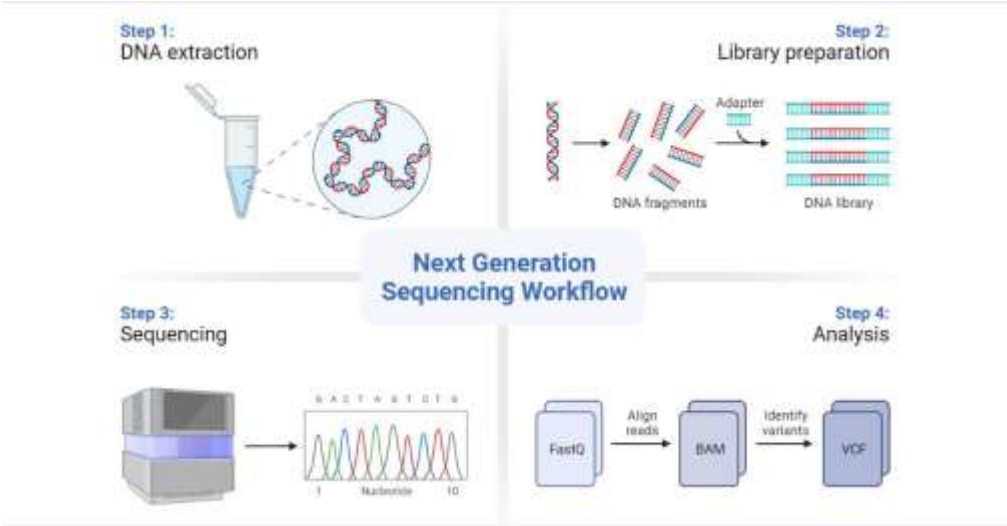

Figure 5: Schematic representation of NGS procedure (adapted from Duran-Jara *et al*., 2025)

Next-Generation Sequencing (NGS): Used for genomic sequencing to identify, track, and monitor variants of viruses circulating within a community. Genome sequencing has emerged as a pivotal tool for the discovery and characterization of viral variants in wastewater-based epidemiology (Gupta, 2023). Studies have underscored the significance of employing virome sequencing and next-generation sequencing approaches to unveil insights into viral evolution, dynamics between different viral species or variants, and the prevalence of emerging variants within communities (Biancononi, 2023).

Dumbbell Structure: The process uses 4 to 6 specifically designed primers that recognize 6 to 8 distinct regions on the target sequence, forming a "dumbbell-like" structure that serves as a foundation for rapid, exponential amplification.

Speed: The reaction is highly efficient, often yielding results in 15 to 60 minutes, which is significantly faster than traditional PCR (Marianna *et al*., 2021).

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Steps in NGS Workflow

1. DNA/RNA Extraction – Isolate genetic material from a sample.
2. Library Preparation – Fragment DNA and attach adapters.
3. Amplification – Use PCR or bridge amplification for signal detection.
4. Sequencing – Detect bases via fluorescence, pH, or electrical signals.
5. Data Analysis – Align sequences to a reference genome and interpret results.

Reverse transcription loop-mediated isothermal amplification (RT-LAMP)

The RT-LAMP can be defined as the technology where the nucleic acid is amplified that leads to the displacement of a strand and its response also leads the stem-loop structure to rapidly amplify the target in isothermal conditions while maintaining high precision and selectivity.

Reverse transcription loop-mediated isothermal amplification (RT-LAMP) is a fast and convenient method to amplify and identify the transcripts of a targeted pathogen. However, the sensitivity and specificity of RT-LAMP were generally regarded as inferior when compared with the gold standard RT-qPCR.

As the template is RNA sample, in addition to the reagents of DNA amplification (primers, DNA polymerase with strand displacement activity, substrates, etc.), reverse transcriptase is added to the reaction mixture. After mixing and incubating at a constant temperature between 60-65°C, amplification and detection can be carried out in a single step. (Huang *et al*., 2022)

Mechanism of Action

i. RT-LAMP works by combining reverse transcription and DNA amplification in a single-step process.

ii. Reverse Transcription: The enzyme reverse transcriptase synthesizes complementary DNA (cDNA) from a target RNA template.

iii. Isothermal Amplification: A DNA polymerase with high strand displacement activity (such as Bst polymerase) amplifies the cDNA at a constant temperature, typically between 60°C and 65°C (Wei *et al.*, 2020).

Procedure for RT-LAMP (One-Step)

i. Sample Preparation (Optional but Recommended)

ii. Direct Sample Method: Lysis of viral samples is carried out (e.g., using a commercial lysis buffer) at room temperature or 65°C for 10 min.

iii. Purified RNA Method: RNA is extracted from the sample using a commercial kit.

iv. Assemble Reaction Mixture (on ice).

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v. Prepare a master mix containing: Isothermal Buffer (1X), MgSO4 (6-8 mM), dNTPs (1.4 mM), Primer Mix (FIP/BIP 1.6 µM, F3/B3 0.2 µM, LoopF/B 0.4 µM), Bst 2.0 DNA Polymerase, WarmStart RTx Reverse Transcriptase, and dye.

vi. Add the RNA template to the reaction mix. (**Note**: Using a separate, non-template control (NTC) is required).

vii. Amplification (Isothermal Incubation)

viii. Incubate the mixture at a constant temperature, typically 63°C–65°C, for 30–60 minutes. (Note: For maximum sensitivity, transfer the sample directly from ice to a preheated heat block).

ix. Termination

x. Heat to 80°C–95°C for 2–5 minutes to inactivate the enzymes.

DETECTION AND ANALYSIS

Colorimetric: A change from pink to yellow (phenol red) or purple to sky-blue (HNB) indicates a positive result.

Fluorescence: Visible under UV light if using SYBR Green.

Turbidity: A white precipitate (magnesium pyrophosphate) indicates a positive result. (Wei *et al*., 2020).

Reverse transcription digital droplet PCR (RT-ddPCR): Combines reverse transcription and ddPCR to quantify RNA with high precision, sensitivity, and absolute quantification without a standard curve. It involves one-step (reverse transcription and amplification in one reaction) or two-step protocols for detecting viral RNA, gene expression, or low-copy transcripts.

Droplet digital polymerase chain reaction (ddPCR) is a third generation of PCR that was recently developed to overcome the limitation of direct quantification observed in real-time quantification PCR (qPCR). Recent studies have shown that ddPCR is more sensitive than the gold standard reverse transcription real-time quantitative polymerase chain reaction (RT-qPCR) in detecting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) samples. In combination with multiplexing, multiple RT-ddPCR assays can be developed to directly quantify different SARS-CoV-2 nucleic acid targets within a single sample, significantly saving on cost and time. Since ddPCR is tolerant to a number of inhibitors unlike qPCR, it can be used to detect and quantify samples from complex environments like wastewater (Nyaruaba, 2022).

Data Analysis and Normalization for Monitoring Wastewater

Data analysis and normalization for wastewater monitoring are crucial components of wastewater-based epidemiology (WBE), enabling public health officials to translate raw, variable data from sewage into actionable insights regarding disease prevalence and community health. Normalization corrects for variations in wastewater, such as dilution from

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storm water, industrial inputs, and fluctuating population sizes, which ensures that measured concentrations accurately reflect community trends (Ioana *et al*., 2022)

Data normalization is used as a measurement correction to address variations in wastewater, such as fecal strength and dilution in the watershed that can have an impact on the SARS-CoV-2 concentration in wastewater. Estimating the concentration of any biological or chemical marker in wastewater is complicated by flow changes and dilution events, the dynamic size of the population served, and the stability and transport mechanics of the biomarker being monitored in the sewer shed (Straathof & Hull, 2024).

CONCLUSION

Wastewater-Based Epidemiology (WBE) is an indispensable tool for modern public health, whose methodological refinements and cross-disciplinary integration are critical for transforming pandemic surveillance from reactive containment to proactive population health management. Wastewater-Based Epidemiology has emerged as a powerful, non-invasive, and cost-effective tool for monitoring emerging and re-emerging viral diseases, providing early warnings (often 7–14 days before clinical reporting) by detecting pathogens in sewage, and those shed by asymptomatic carriers. Wastewater surveillance systems can detect emerging infectious diseases or new viral variants in a community earlier than hospital-based clinical surveillance systems, thereby preventing the spread of infections. It is a valuable tool in public health management to respond to infectious diseases which the monitoring of infectious disease spread and pathogen mutation trends.

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