

**A PORTABLE DEVICE FOR DETECTING AND/OR QUANTIFYING A
TARGET MOLECULE AND METHOD OF USE THEREOF**

FIELD OF THE INVENTION

5 [0001] The present invention belongs to the field of analytical instrumentation, molecular biology device, and optics, particularly in relation to a device for rapid and multiplexed target molecule detection and/or quantification. The present invention also relates to a method of using said device for detecting and/or quantifying a molecule.

BACKGROUND

10 [0002] With regard to a device for CRISPR-Cas point-of-care detection, there have been several mobile based devices to measure fluorescence signals from the cleavage of probes using CRISPR-enzyme activated by the presence of target. However, these conventional devices have limitations such as low sensitivity, limited multiplexing
15 capability, low throughput (the number of samples to run simultaneously), as well as low quantification accuracy.

[0003] To address above mentioned challenge, it is herein disclosed a red, green, blue (RGB) sensor-based device for accurate, high-throughput, sensitive, low cost, quantitative, robust, and user-friendly CRISPR-Cas based detection. The device has been
20 named **qPOCT** and **mqPOCT**. In addition, CRISPR-Cas based detections mainly rely on preamplifications using isothermal enzymes such as recombinase polymerase amplification (RPA), and loop-mediated isothermal amplification (LAMP). However, these enzymes are relatively expensive. By integrating a thermal cycling mechanism into the device, the need for isothermal enzymes have been eliminated, opting for the
25 amplification of the target using cost-effective Taq polymerase enzymes. The precision of qPOCT was validated through the CRISPR-Cas13-based detection of different concentration of COVID-19 using fluorogenic probe which resulted in reproducible quantitative measurement of target. The performance of the device for the amplification and detection of DNA was also assessed in the context of CRISPR-Cas12a-based
30 detection of Brucella.

SUMMARY

[0004] In one aspect, the present disclosure refers to a portable device for detecting and/or quantifying a target molecule in a sample, the device comprising: a sample chamber comprising an outer shell and a rotor for receiving the sample comprising the target molecule; a plurality of red, green, blue (RGB) LEDs for generating illumination and inducing an optical signal of the target molecule; a plurality of RGB sensors for detecting the optical signal of the target molecule; and a microcontroller communicatively coupled to the RGB sensors, for connecting to an external device and transferring data; wherein integrated LED of the RGB sensor has been removed.

[0005] In another aspect, the present disclosure refers to a method of detecting and/or quantifying a target molecule in a sample, using the device as disclosed herein, the method comprising: providing the sample comprising the target molecule in the rotor; generating illumination and inducing the optical signal of the target molecule by the plurality of RGB LEDs; detecting the optical signal of the target molecule by the plurality of RGB sensors; and transferring the data to the external device by the microcontroller.

[0006] In another aspect, the present disclosure refers to a method of monitoring viral or bacterial disease outbreaks and/or evolution, comprising performing a method as disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0008] **Figure 1** shows (A) a perspective view and (B) an exploded view of the qPOCT. This diagram shows a 3D visualization of the device with components separated to illustrate their spatial arrangement. Each part is labeled for clarity, and the exploded format provides an overview of the device's structure. The components are: 1) Main rotor, 2) touch and LCD, 3) external housing, 4) temperature sensor (DS18B20), 5) RGB LED (WS2812), 6) RGB sensor (tcs230), 7) heat sink, 8) noise filter, 9) motor driver, 10) main board (Arduino 2560 R3), 11) base part, 12) Bluetooth module, 13) stepper motor, 14) motor coupling, 15) shaft, 16) internal compartment, 17) power supply, 18) power switch, 19) fan, 20) heater(40w-12v), 21) fan driver, 22) heater driver, 23) lid.

- [0009] **Figure 2** shows a flowchart representing each step and process involved in the qPOCT 's operation.
- [0010] **Figure 3** is a dot plot showing output light intensity to input light intensity ratio (I/I_0) calculated for red color across varying concentrations of target.
- 5 [0011] **Figure 4** is a dot plot showing output light intensity to input light intensity ratio (I/I_0) calculated for green color across varying concentrations of target.
- [0012] **Figure 5** is a dot plot showing output light intensity to input light intensity ratio (I/I_0) calculated for blue color across varying concentrations of target.
- [0013] **Figure 6** is a dot plot showing output light intensity to input light intensity ratio
- 10 (I/I_0) calculated for red, green and blue colors (three colors) across varying concentrations of target.
- [0014] **Figure 7** is linear curve showing linear regression equations.
- [0015] **Figure 8** is a bar graph showing results of probe concentration assay using CRISPR-Cas12 following amplification in qPOCT.
- 15 [0016] **Figure 9** are bar graphs showing a Multiplex detection of (A) FAM and (B) HEX (right) probes.
- [0017] **Figure 10** shows output-to-input light intensity ratio (I/I_0) for each red, green, and blue color across varying concentrations of xylose.
- [0018] **Figure 11** shows linear regression equations for RGB readout.
- 20 [0019] **Figure 12** shows linear regression equations of plate reader readout.
- [0020] **Figure 13** shows A) Standard curve for TSH quantification generated using plate reader Epoch (BioTek Instruments, Inc). The curve demonstrates a high degree of linearity ($R^2 = 0.986$) across the tested TSH concentrations (0, 0.5, 2.5, 5, 10, and 25 $\mu\text{IU/mL}$). The red circle represents the internal control, which align precisely within the
- 25 expected range on the standard curve, confirming the machine's accuracy and reliability. B) Standard curve for TSH quantification generated using the qPOCT. The curve also shows excellent linearity ($R^2 = 0.977$) across the same concentration range. The red circle indicates the internal control, which fall exactly within their predicted positions on the curve, reflecting the qPOCT's comparable precision and reliability to the conventional
- 30 system.
- [0021] **Figure 14** is a schematic representation of the experimental setup for testing individual probes. Probes, designed to diagnose viruses and bacteria associated with

Bovine Respiratory Disease (BRD), were tested with fluorophores and corresponding quenchers at concentrations of 0, 12.5, 25, and 50 nM. The aim was to simulate multiplex conditions and evaluate the qPOCT's sensitivity and precision in detecting different levels of target biomarkers relevant to BRD diagnostics.

5 [0022] **Figure 15** is a schematic representation of the multiplex experiment setup. Probes containing fluorophores with and without quenchers, as well as mixed forms combining fluorophore-only and fluorophore-quencher probes, were tested together in a single reaction tube at concentrations of 0, 12.5, 25, and 50 nM. This experiment aimed to simulate multiplex conditions and evaluate the device's ability to detect and quantify
10 multiple fluorophores accurately and simultaneously.

[0023] **Figure 16** shows experimental results demonstrating the performance of the RGB LED and sensors integrated in qPOCT device in detecting probes with (F-Q) and without (F) quenchers, as well as mixed forms of fluorophore-only (MIX-F) and fluorophore-quencher (MIX-F-Q) probes. Probes at concentrations of 0, 12.5, 25, and 50
15 nM were tested in both single-tube and mixed-probe formats to simulate multiplex conditions. Out of the 12 fluorophores tested (A-L), 10 (CY3.5, TET, Alexa Fluor 350, VIC, Texas-Red, CY5.5, FAM, TAMRA, ATTO 647N, and Alexa 750 NHS) showed excellent linearity ($R^2 > 0.9$) in both single-tube and mixed formats, highlighting the device's ability to accurately and simultaneously detect multiple fluorophores. These
20 results underscore the high precision and versatility of the qPOCT with built-in RGB LED and sensor, demonstrating its capability for advanced multiplexing applications.

[0024] **Figure 17** shows that the amplification curve corresponds to the genes amplified in the qPOCT device. The peaks for FAM, HEX, Texas-Red, and Cy5 show the level of specific gene amplification in the 4-plex.

25 [0025] **Figure 18** shows Agarose gel electrophoresis (1%) of chain polymerase reaction. (1) Product replicated in the Roche LightCycler® 96 Real-Time PCR device, (2, and 3) Products replicated in the qPOCT device. samples 2 and 3 relate to the repetition of the same reaction in a qPOCT device. (4) Negative control.

[0026] **Figure 19** shows a standard curve of Log (copy number/ml) of target versus
30 Ct value. All experiments used reagents with identical concentrations, with only the target copy number varying.

[0027] **Figure 20** shows Agarose gel electrophoresis (1%) of chain polymerase reaction. The amplified products in the reaction correspond to numbers 1, 2, 3, 4, and 5, with targets of 28×10^2 , 14×10^2 , 7×10^2 , 3.5×10^2 and 1.75×10^2 copies/ml, respectively. The negative control is represented by number 6.

- 5 [0028] **Figure 21** shows mqPOCT prototype (A) and its exploded view (B). This diagram shows a 3D visualization of the device with components separated to illustrate their spatial arrangement. Each part is labeled for clarity, and the exploded format provides an overview of the device's structure. The components are: 1) touch and LCD, 2) power supply input, 3) power Key, 4) external housing, 5) cooling fan, 6) fan driver,
- 10 7) element driver, 8) rear access door, 9) noise filter, 10) Bluetooth module, 11) main board (Arduino 2560 R3), 12) heat sink-1, 13) RGB sensor-1 (tcs230), 14) RGB LED-1 (WS2812), 15) temperature sensor (DS18B20), 16) element (40w-12v), 17) RGB LED-2 (WS2812), 18) heat transfer copper block, 19) RGB sensor-2 (tcs230), 20) heat sink-2, 21) test chamber lid retaining pin, 22) heating pad, 23) test chamber lid.
- 15 [0029] **Figure 22** is a flowchart representing each step and process involved in the qPOCT's operation.

DETAILED DESCRIPTION

- [0030] Red, green, blue (RGB) sensor-based portable devices, so called qPOCT and
- 20 mqPOCT for accurate, sensitive, low cost, quantitative multiplex analysis, are described in this disclosure. Both qPOCT and mqPOCT are able to carry out detection and quantification of biological (such as DNA, RNA, or protein) and chemical molecules in a liquid sample and with wide applications in various scientific and research fields. The performance and versatility of the qPOCT and mqPOCT stem from its innovative
- 25 integration of RGB LED and sensors for multiplex analysis of fluorophores. The key components of the devices, highlighting their individual functions and contributions to the system's ability to deliver precise, rapid, and multiplexed detection in various diagnostic applications, are described herein. Considering the combination of RGB LED and RGB sensors in the disclosed device, it can be a significant expansion of Multiplexing
- 30 by detecting over 10 nucleic acid targets in a single sample for a variety of molecular analyses such as Real-Time PCR, immunoassay, and spectrophotometry.

[0031] Current multiplexed RT-PCR is capable of detecting four to six nucleic acid targets in a single sample. Advantageously, qPOCT is capable of analysing 24-100 samples simultaneously, whereas mqPOCT has the capacity of two samples, retaining the advanced quantitative and multiplexing capabilities of the qPOCT while offering enhanced portability for field diagnostics and home use. The integration of RGB LED and RGB sensor with thermal cycling enabled precise quantitative detection of nucleic acids in real-time, supporting highly sensitive and multiplexed diagnostic applications. This capability demonstrates its potential for rapid, accurate, and very affordable detection of biomacromolecules and chemicals such as those derived from a pathogen, and genetic analysis across diverse settings.

[0032] Both devices are highly affordable, with a cost significantly lower than conventional real-time PCR machines, making them accessible for a wide range of diagnostic applications. Additionally, these devices can be adapted for immunodiagnostic purposes, broadening their potential use in diverse healthcare settings.

[0033] In this invention, a low-cost new portable and POC device, so called qPOCT, to carry out detection and quantification of biological (such as DNA, RNA, or protein) and chemical molecules in a liquid sample and with wide applications in various scientific and research fields has been developed.

[0034] The performance and versatility of the qPOCT stem from its innovative integration of RGB LED and sensors for multiplex analysis of fluorophores. This section details the key components of the device, highlighting their individual functions and contributions to the system's ability to deliver precise, rapid, and multiplexed detection in various diagnostic applications.

[0035] In one aspect, the present disclosure refers to a portable device for detecting and/or quantifying a target molecule in a sample, the device comprising: a sample chamber comprising an outer shell and a rotor for receiving the sample comprising the target molecule; a plurality of red, green, blue (RGB) LEDs for generating illumination and inducing an optical signal of the target molecule; a plurality of RGB sensors for detecting the optical signal of the target molecule; and a microcontroller communicatively coupled to the RGB sensors, for connecting to an external device and transferring data; wherein integrated LED of the RGB sensor has been removed.

[0036] The device as disclosed herein comprises the following components (the reference signs are from Fig. 1B, a representative figure of the device of the present invention, which do not limit the scope of the embodiments of the invention. The invention is not restricted to the specific embodiments represented by the reference signs illustrated in the figures, and other embodiments are possible.):

[0037] 1. **RGB LED (5)**: A single advanced RGB LED is used to produce light with precise wavelengths. This LED can generate a wide spectrum of light (red, green, blue) and its intensity is adjustable based on RGB codes. This capability ensures that the device can accurately and reliably analyze the fluorescence of samples.

[0038] 2. **RGB Color Sensor (TCS230) (6)**: For precise fluorescence analysis, a TCS230 color sensor is utilized. This sensor can detect fluorescence intensity and differentiate between various wavelengths with high accuracy. It enables rapid and reliable fluorescence measurement of samples, enhancing the efficiency of the experiments. Technically, the integrated LED in RGB sensors has been removed. Then RGB LED was integrated into device itself as component 1 above (separate from the RGB color sensors) to provide light.

[0039] 3. **Noise Filter (8)**: An advanced noise filter is integrated into the design to reduce electromagnetic interference and fluctuations. This filter ensures the sensors, LED, and other components operate without being affected by unwanted electrical disturbances or noise.

[0040] 4. **Motor Driver (9)**: The device includes a high-precision stepper motor driver that enables precise control of motor position and speed. This feature ensures samples are placed in the optimal position for accurate reading.

[0041] 5. **Main board (Arduino MEGA 2560) (10)**: The Arduino MEGA 2560 microcontroller serves as the device's brain, coordinating all components such as fans, heaters, sensors, and the Bluetooth module. This microcontroller executes complex algorithms to precisely control chamber temperature, LED intensity, and sensor data processing.

[0042] 6. **Stepper Motor (13)**: The device uses a highly precise stepper motor controlled by PWM. This motor ensures smooth and vibration-free sample movement, guaranteeing high accuracy during the experiment.

- [0043] 7. **Motor Coupling (14)**: A motor coupling is used to connect the motor to the main shaft, ensuring precise and vibration-free operation.
- [0044] 8. **Shaft (15)**: The metal shaft connects the motor to the device's main rotor and is designed to ensure durability and precision during long-term operation.
- 5 [0045] 9. **Power Supply Input (17)**: The device is equipped with a standard power supply input port that provides sufficient energy to run high-consumption components such as heaters, fans, and the LED. The design protects against voltage fluctuations, ensuring stable operation.
- [0046] 10. **Power Switch (18)**: A high-current power switch enables safe and secure
- 10 turning on and off of the device.
- [0047] 11. **Fan (19)**: A powerful cooling fan is included to manage the internal temperature of the components. The fan, with adjustable speed, prevents overheating and maintains ideal temperatures for sensitive experiments.
- [0048] 12. **Heater (20)**: The heater utilizes advanced thermal elements to precisely
- 15 regulate the chamber temperature. These elements heat rapidly and are fully adjustable, playing a critical role in the device's performance.
- [0049] 13. **Fan Driver (21)**: The fan driver uses PWM to control fan speed, reducing noise and optimizing energy efficiency.
- [0050] 14. **Heater Driver (22)**: The heater driver enables precise control of the
- 20 chamber temperature using the PID algorithm. It adjusts temperature changes in real-time to suit various experimental cycles.
- [0051] 15. **Heat Sink (7)**: A specially designed aluminium heat sink is used to dissipate heat from sensitive components like sensors and the microcontroller. It prevents damage from high temperatures and extends the device's lifespan.
- 25 [0052] 16. **Temperature Sensor (16)**: The device uses a highly precise digital temperature sensor to continuously measure the temperature of the chamber. The sensor sends collected data to the microcontroller, which uses a PID algorithm to control the chamber temperature with high accuracy. This advanced sensor technology ensures consistent calibration and accurate temperature measurements, even during long PCR
- 30 cycles.
- [0053] 17. **Lid (17)**: which houses the fan, heater and heating equipment, and through this, heat enters the chamber.

[0054] 18. **Main rotor (1)**: which houses the test samples.

[0055] 19. **External housing (3)**: which houses the LCD and contributes to the device's aesthetic appearance.

[0056] 20. **Internal compartment (16)**: is used as a heating chamber, and the temperature of this chamber is increased and decreased through the door of the device. It is also the place where the stepper motor is installed.

[0057] 21. **Bluetooth Module (12)**: The Bluetooth module allows wireless communication with mobile and computer software. Key features of the module include:

- Live monitoring of experimental data
- Remote adjustment of experiment settings
- Saving and sharing results for further analysis

[0058] 22. **Touchscreen and LCD (2)**: The device features a high-resolution 3.5-inch LCD TFT touchscreen that serves as the main user interface. This display allows users to view and adjust all the essential information and parameters related to PCR experiments.

Key features of the touchscreen include:

- Displaying critical information such as temperature, cycle count, experiment type, remaining time, and fluorescence and temperature graphs.
 - Precise parameter adjustments, including:
 - Number of cycles
 - Setting different temperatures for each stage of PCR
 - Selecting the experiment type (SYBR Green, Probe-based, or other methods)
 - Displaying warning messages in case of errors
 - Defining the number of samples
 - Saving custom settings for frequently repeated experiments.
- The user interface is designed to be fully user-friendly, while meeting the needs of laboratory professionals.

[0059] qPOCT can work under control temperature with or without thermal cycling-based amplification step. A software was designed to receive the data collected by the sensors directly via Bluetooth or USB and convert it to the desired chart in less than a minute. Also, this software receives and records information related to the location so that a person can easily identify the sample. Also, this software can connect to the cloud by

connecting to the internet and transfer the relevant information to the cloud space. It can also display reports and analyzes globally. This software has high processing power using AI and as the last case, and this software shows it as an error if there is a problem in the device so that the consumer can easily identify the error.

- 5 [0060] The device as disclosed herein comprises a sample chamber comprising an outer shell and a rotor for receiving the sample comprising the target molecule. The sample chamber is capable of receiving 1-100, 10-90, 20-80, 30-70, 40-60, 50-110, 60-120, 70-130, 80-140, 90-150, 1-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-150, or 150-200, 1-200, or more samples. In one example, the capacity
- 10 of qPOCT is to handle a wide variety of samples sizes, ranging from a single sample, to be scaled up to process over 100 samples, all achieved within a very modest budget. This highlights the qPOCT's efficiency, adaptability, and dedication to making advanced analytical capabilities accessible to a broader audience. In another example, qPOCT and mqPOCT offer versatility in its construction materials, allowing the outer shell to be
- 15 crafted from a material selected from the group comprising aluminum, plastic, polycarbonate, steel, and more. In another example, the outer shell has a body made of a material selected from the group comprising aluminum, plastic, polycarbonate, steel, ABS, PLA, PET, PETT, Nylon, PVA, HIPS, PA, HDPE, POM, PP, TPU, TPE, PMMA, PVC, and PEEK.
- 20 [0061] The device as disclosed herein comprises a plurality of red, green, blue (RGB) LEDs for generating illumination and inducing an optical signal of the target molecule. RGB LEDs are used to identify different fluorophores which allows a wide spectra for the best performance. These RGB LEDs have a spectrum equivalent to R(255), B(255), C(255), which is the full light spectrum. In another example, the device may comprise
- 25 white LED, but this will sacrifice the capability for multiplexing. In one example, the RGB LED can be located in different positions around sample tubes placed in rotors. In another example, the RGB LED is selected from the group comprising gallium nitride (GaN), indium gallium nitride (InGaN), gallium phosphide (GaP), and gallium arsenic (GaAs). In another example, the RGB LED generates illumination with a wavelength of
- 30 300–1100 nm. qPOCT and mqPOCT are versatile and can work with any RGB LED, regardless of its size or brand. Common types of RGB LED include but are not limited to

Common Anode RGB LED, Common Cathode RGB LED, RGB LED strips, RGB LED panels, RGB LED rings, and so on.

[0062] In another example, the RGB LED is positioned without a filter between the RGB LED and the sample. In another example, the RGB LED is positioned with a filter between the RGB LED and the sample. The filter can be selected from the group consisting of Bandpass Filters, Neutral Density Filters, Color Filters, and Polarizing Filters. Bandpass Filters allow only a specific range of wavelengths to pass through, blocking all others, and are useful if one wants to isolate a particular color or wavelength from the RGB LED. Neutral Density Filters reduce the intensity of all wavelengths equally, which can help control the brightness of the light reaching the sample without altering its color. Color Filters are designed to pass only certain colors (e.g., red, green, or blue) and can be used to selectively filter out other colors from the RGB LED. Polarizing Filters can reduce glare and reflections.

[0063] In another example, the device as disclosed herein comprises a plurality of RGB LEDs, ranging from 2-10, 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 4-12, 8-16, 12-20, or 16-20. In another example, the device as disclosed herein comprises more than 20 RGB LEDs.

[0064] The device as disclosed herein comprises a plurality of RGB sensors for detecting the optical signal of the target molecule. The RGB sensor has been removed its integrated LED. The RGB LED was integrated into device (separate from RGB sensors) to provide light, as described above. In some examples, the RGB sensor is selected from the group comprising TCS230, AS73211, TCS3200, TCS3400, TCS34715, TCS34727, colorPAL, SEN-11195, EV3, GY-33, VCNL4010, VCNL4040, MAX44009, ISL29125, BH1745, APDS-9960, AS7262, and AS7263. In some examples, the device as disclosed herein comprises 2-10 RGB sensors, or more than 10 RGB sensors through the incorporation of a transistor switch. In another example, the device comprises 2-10, 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, or more than 10 RGB sensors. qPOCT and mqPOCT incorporate one and two sensors initially, but with the integration of transistor switch, the capacity for sensors can be expanded to exceed 10. In another example, the ratio of the number of the RGB sensor and the RGB LED is 1:1 and 1:2. In other words, for each RGB sensor, 1 or maximum 2 RGB LEDs can be used.

[0065] In one example, the RGB sensor detects an optical signal selected from the group consisting of a fluorescent signal, a colorimetric signal, an absorbance signal, a luminescence signal, a bioluminescence signal, and a chemiluminescence signal. In one example, the optical signal is within the visible light range. The visible light spectrum is the portion of the electromagnetic spectrum that can be detected by the human eye. It typically ranges from 380 to 750 nanometers (nm) in wavelength. This range includes all the colors from violet (around 380 nm) to red (around 750 nm). In another example, the optical signal is within the invisible light range. Invisible light refers to parts of the electromagnetic spectrum that are not visible to the human eye. The invisible light can be selected from the group consisting of Ultraviolet (UV) light (Wavelengths from about 10 nm to 400 nm), and Infrared (IR) light (Wavelengths from about 700 nm to 1 mm).

[0066] The device as disclosed herein comprises a microcontroller communicatively coupled to the RGB sensors, for connecting to an external device and transferring data. the qPOT and mqPOCT are compatible with various types of microcontroller, including but not limited to single-board Arduino Uno microcontroller (Arduino), ARM, AVR, 8051, PIC, AVR, XMEGA, SAM, MSP430, AT MEGA, NXP, STM, LPC, GD, STM8 and STM32.

[0067] In some examples, the microcontroller comprises a Bluetooth module selected from the group comprising SPP-C, SPP2-0, CC2541, BT04-A, BK3231, BC417, CH-05, CH-08, CH-06, CH-10C, CH-07, CH-09, NRF52832, NRF51822, JDY-09, CC2541, HM-19, and HM-17 for wireless communication with the external device, or a portal for cable connection with the external device. In some examples, the external device is selected from the group comprising a smartphone, a tablet, a computer, a server or a cloud platform. In some examples, data transfer is possible through various means, including USB cable types B and C, as well as Bluetooth. Different modules, such as SPP-C, BK3231, and others, support these sensors for transmission.

[0068] In another example, any GPS module, including GY-NEO-6M and Ublox7, can be integrated into the device, to record the exact location where each sample is collected or analyzed, as well as to enhance traceability.

[0069] In one example, the device as disclosed herein may comprise a thermal cycler thermally coupled to the sample chamber. qPOCT and mqPOCT's heating system provide flexibility by accommodating various heating elements, including but not limited to

models like ceramic, carbon, iron, or any other suitable type. The thermal cycler comprises a heating element selected from the group comprising ceramic, carbon, iron, steel, glass, strip, silicon rubber, and belt. In addition, qPOCT and mqPOCT 's fan offer versatility, supporting various types such as axial, centrifugal, tangential, composite, and other suitable options. To regulate the environmental temperature, any heating element is suitable, and for transfer and circulation, and any type of fan motor, whether axial, centrifugal, tangential, composite, or others, can be employed. In this example, the microcontroller comprises a temperature sensor and a microcontroller program, being associated with the thermal cycler and configured to control a temperature profile of the sample chamber to perform a reaction. For the temperature sensor, any similar sensor within a working range of 1 to 100, 10-100, 10-90 degrees can be utilized, encompassing both mechanical and electronic options. In one example, the temperature profile is 1-100 °C, preferably 10- 90 °C. In another example, the temperature profile is 1-100 °C, 1-90 °C, 1-80 °C, 1-70 °C, 1-60 °C, 1-50 °C, or other ranges. In another example, the thermal cycler is suitable for use with a polymerase selected from the group comprising Taq polymerase, Pfu polymerase, DNA polymerase I, DNA polymerase III, DNA Polymerase α , DNA Polymerase δ , DNA Polymerase γ , Q5 DNA Polymerase, Phusion DNA Polymerase, and a specialized DNA Polymerase such as Terminal Transferase TdT. In another example, the thermal cycler is suitable for use with an isothermal enzymes suitable for use in a reaction selected from the group comprising recombinase polymerase amplification (RPA), loop-mediated isothermal amplification (LAMP), Rolling Circle Amplification (RCA), Multiple Displacement Amplification (MDA), Transcription-mediated Amplification (TMA), Nucleic Acid Sequence-Based Amplification (NASBA), Ligase Chain Reaction (LCR), and Helicase-dependent Amplification (HDA). In another example, the isothermal enzyme is selected from the group consisting of Bst DNA Polymerase, Phi29 DNA Polymerase, Klenow Fragment (exo-), T7 RNA Polymerase, T4 DNA Ligase, Recombinase Enzymes (e.g., UvsX, UvsY), Helicase Enzymes, RNase H, and T3 RNA Polymerase. In another example, the microcontroller is programmed for fault detection to automatically monitor and identify faults.

[0070] In one example, the rotor of the device as disclosed herein has a capacity to receive 1-200 samples, preferably 1-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80,

80-90, 90-100, 100-150, or 150-200 samples. In another example, the device has a capacity of 1, 2, 3, or 4 rotors.

[0071] In another example, the rotor has a body made of a material selected from the group comprising aluminum, plastic, polycarbonate, and steel, ABS, PLA, PET, PETT, Nylon, PVA, and HIPS.

[0072] In one example, the device as disclosed herein comprises a motor for rotating the rotors, and a speed control for rotating the rotor. The number of motors can be 1-10. In another example, the motor is a countable motor. In another example, the motor is a stepper motors which moves in discrete steps, ideal for applications requiring precise positioning and control. In another example, the motor is a servo motor which uses feedback systems which continuously adjusts the motor's position based on input from an encoder, for high precision and control.

[0073] In one example, the device as disclosed herein is operatable with a battery selected from the group comprising cadmium battery, Lithium polymer battery, Lithium ion battery, Nickel-metal hydride battery, Nickel-cadmium battery, Mercury battery, Zinc-carbon battery, and Silver oxide battery. qPOCt and mqPOCT accommodate a diverse range of battery types, including but not limited to lithium, acid, cadmium, and more, providing users with extensive options for their specific power needs.

[0074] In one example, the device as disclosed herein comprises an LCD screen for displaying results and errors, the LCD screen being selected from the group comprising character, graphic, TFT, OLED LCD, PLS, LTPS, IGZO, AMOLED, and RETINA.

[0075] In one example, the device as disclosed herein can be used to detect a target molecule in a sample selected from the group consisting of a biological, chemical or environmental sample. In another example, the biological sample is obtained from a tissue sample, saliva, blood, plasma, sera, stool, urine, sputum, mucous, lymph, synovial fluid, cerebrospinal fluid, ascites, pleural effusion, seroma, pus, or swab of skin or a mucosal membrane surface or a combination thereof. In another example, the chemical sample is obtained from a pharmaceutical substance, a chemical reagent, a cosmetic product, an industrial effluent, a pollutant, a hazardous material, a polymer, an adhesive, a solvent, an oil, a gas, a metal alloy, a nanomaterial, a fuel, a cleaning agent, a dye, or a combination thereof. In another example, the environmental sample is obtained from a food, a beverage, a paper surface, a fabric surface, a metal surface, a wood surface, a plastic

surface, a soil sample, a freshwater sample, a waste water sample, a saline water sample, exposure to atmospheric air or other gas sample, or a combination thereof. In another example, the biological, chemical or environmental sample is a crude sample, i.e. the target molecule is not purified or amplified. In another example, the target molecule is purified or amplified from the biological, chemical or environmental sample as disclosed herein before being detected or quantified by the device as disclosed herein.

[0076] In one example, the sample as disclosed herein contains a microbe selected from a bacteria, a virus, an archaea, a protozoa, a fungi, an algae, a slime mode, a lichen, and a prion.

- 10 [0077] In another example, the virus is selected from a group consisting of a double-stranded DNA virus, a single-stranded DNA virus, a double-stranded RNA virus, a positive sense RNA virus, a negative sense RNA virus, and a retrovirus. In another example, the virus is selected from a group consisting of a coronavirus, an Ebola virus, measles, SARS, Chikungunya virus, Marburg, MERS, Dengue, Lassa, influenza, rhabdovirus, HIV, a hepatitis virus (including hepatitis A, B, C, D, or E), an influenza virus (including an influenza A or influenza B), a human respiratory syncytial virus, Sudan ebola virus, Bundibugyo virus, Tai Forest ebola virus, Reston ebola virus, Achimota virus, Aedes flavivirus, Aguacate virus, Akabane virus, Alethinophid reptarenavirus, Allpahuayo mammarenavirus, Amapari mmarenavirus, Andes virus, Apoi virus, Aravan virus, Aroa virus, Arumwot virus, Atlantic salmon paramyxovirus, Australian bat lyssavirus, Avian bomavirus, Avian metapneumovirus, Avian paramyxoviruses, penguin or Falkland Islands virus, BK polyomavirus, Bagaza virus, Banna virus, Bat herpesvirus, Bat sapovirus, Bear Canon mammarenavirus, Beilong virus, Betacoronavirus, Betapapillomavirus 1-6, Bhanja virus, Bokeloh bat lyssavirus, Boma disease virus, Bourbon virus, Bovine hepacivirus, Bovine parainfluenza virus 3, Bovine respiratory syncytial virus, Brazoran virus, Bunyamwera virus, Caliciviridae virus, California encephalitis virus, Candiru virus, Canine distemper virus, Canine pneumovirus, Cedar virus, Cell fusing agent virus, Cetacean morbillivirus, Chandipura virus, Chaoyang virus, Chapare mammarenavirus, Colobus monkey papillomavirus, Colorado tick fever virus, Cowpox virus, Crimean-Congo hemorrhagic fever virus, Culex flavivirus, Cupixi mammarenavirus, Dengue virus, Dobrava-Belgrade virus, Donggang virus, Dugbe virus, Duvenhage virus, Eastern equine encephalitis virus, Entebbe bat

- virus, Enterovirus A-D, European bat lyssavirus 1-2, Eyach virus, Feline morbillivirus, Fer-de- Lance paramyxovirus, Fitzroy River virus, Flaviviridae virus, Flexal mammarynavirus, GB virus C, Gairo virus, Gemycircularvirus, Goose paramyxovirus SF02, Great Island virus, Guanarito mammarynavirus, Hantaan virus, Hantavirus Z10,
- 5 Heartland virus, Hendra virus, Hepatitis A/B/C/E, Hepatitis delta virus, Human bocavirus, Human coronavirus, Human endogenous retrovirus K, Human enteric coronavirus, Human genital-associated circular DNA virus- 1, Human herpesvirus 1-8, Human mastadenovirus A-G, Human papillomavirus, Human parainfluenza virus 1-4, Human paraechovirus, Human picornavirus, Human smacovirus, Ikoma lyssavirus, Ilheus
- 10 virus, Influenza A-C, Ippy mammarynavirus, Irkut virus, J-virus, JC polyomavirus, Japanese encephalitis virus, Junin mammarynavirus, KI polyomavirus, Kadipiro virus, Kamiti River virus, Kedougou virus, Khujand virus, Kokobera virus, Kyasanur forest disease virus, Lagos bat virus, Langat virus, Lassa mammarynavirus, Latino mammarynavirus, Leopards Hill virus, Liao ning virus, Ljungan virus, Lloviu virus,
- 15 Louping ill virus, Lujo mammarynavirus, Luna mammarynavirus, Lunk virus, Lymphocytic choriomeningitis mammarynavirus, Lyssavirus Ozemoe, MSS12Y225 virus, Machupo mammarynavirus, Mamastrovirus 1, Manzanilla virus, Mapuera virus, Marburg virus, Mayaro virus, Measles virus, Menangle virus, Mercadeo virus, Merkel cell polyomavirus, Middle East respiratory syndrome coronavirus, Mobala
- 20 mammarynavirus, Modoc virus, Moijang virus, Mokolo virus, Monkeypox virus, Montana myotis leukoencephalitis virus, Mopeia lassa virus reassortant 29, Mopeia mammarynavirus, Morogoro virus, Mossman virus, Mumps virus, Murine pneumonia virus, Murray Valley encephalitis virus, Nariva virus, Newcastle disease virus, Nipah virus, Norwalk virus, Norway rat hepacivirus, Ntaya virus, O'nyong-nyong virus,
- 25 Oliveros mammarynavirus, Omsk hemorrhagic fever virus, Oropouche virus, Parainfluenza virus 5, Parana mammarynavirus, Parramatta River virus, Peste-des-petits-ruminants virus, Pichande mammarynavirus, Picomaviridae virus, Pirital mammarynavirus, Piscihepevirus A, Porcine parainfluenza virus 1, porcine rubulavirus, Powassan virus, Primate T-lymphotropic virus 1-2, Primate erythroparvovirus 1, Punta
- 30 Toro virus, Puumala virus, Quang Binh virus, Rabies virus, Razdan virus, Reptile bomavirus 1, Rhinovirus A-B, Rift Valley fever virus, Rinderpest virus, Rio Bravo virus, Rodent Torque Teno virus, Rodent hepacivirus, Ross River virus, Rotavirus A-I, Royal

- Farm virus, Rubella virus, Sabia mammarenavirus, Salem virus, Sandfly fever Naples virus, Sandfly fever Sicilian virus, Sapporo virus, Sathuperi virus, Seal anellovirus, Semliki Forest virus, Sendai virus, Seoul virus, Sepik virus, Severe acute respiratory syndrome-related coronavirus, Severe fever with thrombocytopenia syndrome virus,
- 5 Shamonda virus, Shimoni bat virus, Shuni virus, Simbu virus, Simian torque teno virus, Simian virus 40-41, Sin Nombre virus, Sindbis virus, Small anellovirus, Sosuga virus, Spanish goat encephalitis virus, Spondweni virus, St. Louis encephalitis virus, Sunshine virus, TTV-like mini virus, Tacaribe mammarenavirus, Taila virus, Tamana bat virus, Tamiami mammarenavirus, Tembusu virus, Thogoto virus, Thottapalayam virus, Tick-
- 10 borne encephalitis virus, Tioman virus, Togaviridae virus, Torque teno canis virus, Torque teno douroucouli virus, Torque teno felis virus, Torque teno midi virus, Torque teno sus virus, Torque teno tamarin virus, Torque teno virus, Torque teno zalophus virus, Tuhoko virus, Tula virus, Tupaia paramyxovirus, Usutu virus, Uukuniemi virus, Vaccinia virus, Variola virus, Venezuelan Vesicular stomatitis Indiana virus, WU Polyomavirus,
- 15 Wesselsbron virus, West Caucasian bat virus, West Nile virus, Western equine encephalitis virus, Whitewater Arroyo mammarenavirus, Yellow fever virus, Yokose virus, Yug Bogdanovac virus, Zaire ebolavirus, Zika virus, or *Zygosaccharomyces bailii* virus Z viral sequence, Canine Parvovirus, Canine Distemper Virus, Canine Coronavirus, Canine Influenza Virus, Feline Herpesvirus, Feline Calicivirus, Feline Leukemia Virus,
- 20 Feline Immunodeficiency Virus, Canine Adenovirus - Type 1 and Type 2, Canine Herpesvirus, Tobacco Mosaic Virus, Potato Virus Y, Tomato Spotted Wilt Virus, Cucumber Mosaic Virus, Potato Virus X, Barley Yellow Dwarf Virus, Bean Common Mosaic Virus, Maize Dwarf Mosaic Virus, Soybean Mosaic Virus, Citrus Tristeza Virus, Rice Yellow Mottle Virus, Plum Pox Virus, Turnip Mosaic Virus, Aphid-Transmitted
- 25 Yellow Dwarf Viruses, Cotton Leaf Curl Virus, Papaya Ringspot Virus, Cassava Mosaic Virus, Rice Tungro Virus, Apple Mosaic Virus, Sugarcane Mosaic Virus or a combination thereof. In another example, the virus is a coronavirus. In another example, the virus is a coronavirus such as SARS-CoV-2.
- [0078] In another example, the bacteria is a Gram-positive bacterium or a Gram-
- 30 negative bacterium selected from the group consisting of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Enterococcus faecalis*, *Enterococcus*

faecium, *Bacillus anthracis*, *Bacillus cereus*, *Clostridium botulinum*, *Clostridium perfringens*, *Clostridium difficile* and *Clostridium tetani*, *Corynebacterium diphtheria*, *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Chlamydia trachomatis*, *Yersinia pestis*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Moraxella catarrhalis*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Proteus mirabilis*, *Enterobacter cloacae*, *Serratia marcescens*, *Helicobacter pylori*, *Salmonella enteritidis*, *Salmonella typhi*, and *Vibrio cholera*, *Brucella abortus*, *Brucella melitensis*, *Brucella suis*, *Brucella canis*, *Brucella ovis*, *Brucella neotomae*, *Mycoplasma mycoides*, *Clostridium perfringens*, *Mycobacterium avium* *Salmonella* spp., *Pasteurella multocida*, *Haemophilus parasuis*, *Actinobacillus pleuropneumoniae*, *Yersinia pestis*, *Francisella tularensis*, *Bacillus anthracis*, *Coxiella burnetii*, *Listeria monocytogenes*, *Clostridium chauvoei*, *Campylobacter* spp., *Chlamydia psittaci*, *Anaplasma marginale*, *Leptospira* spp. Causes leptospirosis, *Bordetella bronchiseptica*, *Clostridium perfringens*, *Staphylococcus intermedius*, *Bartonella henselae*, *Chlamydophila felis*, *Mycoplasma* spp, *Pasteurella multocida*, *Erwinia amylovora*, *Xanthomonas* spp., *Xanthomonas axonopodis*, *Ralstonia solanacearum*, *Pectobacterium* and *Dickeya* spp., *Agrobacterium tumefaciens*, *Pseudomonas syringae*, *Pseudomonas syringae* pv. *lachrymans*, *Xanthomonas campestris* pv. *campestris*, *Xanthomonas oryzae*, *Clavibacter michiganensis*, *Xylella fastidiosa*, *Pantoea* spp., *Burkholderia glumae*, *Xanthomonas citri*, *Xanthomonas translucens*, *Xanthomonas fragariae*, *Xanthomonas hortorum*, *Clavibacter michiganensis*, *Pseudomonas syringae* or a combination thereof.

[0079] In another example, target molecule to be detected and/or quantified using the device as disclosed herein is selected from the group consisting of a nucleotide, a polynucleotide, a nucleoside, a nucleic acid, a nucleic acid analogue, a peptide, a polypeptide, a protein, an antibody, a hormone, a salt, a metal, and a compound that produces color in a solvent.

[0080] In another example, the nucleic acid is a RNA or a DNA from a virus as disclosed herein, or a RNA or a DNA from a bacteria as disclosed herein.

[0081] In one example, the device as disclosed herein can detect 2-10, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, or more target molecules from a sample in a single tube, wherein the target molecule is selected from the group comprising a nucleic acid, an antigen, and an

antibody. In another example, the device as disclosed herein can detect 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more target molecules from a sample in a single tube, wherein the target molecule is selected from the group comprising a nucleic acid, an antigen, and an antibody.

- 5 [0082] In one example, the device is suitable or configured for one or more of amplification of nucleic acids including analysis of polymerase chain reactions including quantitative polymerase chain reactions, reverse transcription polymerase chain reaction, protein analysis, ligand analysis, fluorescence analysis of chemical reactions, or fluorescence analysis in the presence of particles, polymers, hydrogels, and
- 10 nanostructures, biomolecule interactions, detection of specific nucleic acid sequences, fluorescence protein analysis, bioluminescence enzymatic reactions, and chemiluminescence reactions. In another example, the reverse transcription polymerase chain reaction is for protein analysis, ligand analysis, biomolecule interactions, detection of specific nucleic acid sequences, fluorescence protein analysis, bioluminescence
- 15 enzymatic reactions, chemiluminescence reactions, fluorescence analysis of chemical reactions or fluorescence analysis in the presence of particles, polymers, hydrogels, and nanostructures. In another example, the device as disclosed herein is suitable or configured for clustered regularly interspaced short palindromic repeats and CRISPR associated protein (CRISPR-cas)- based nucleic acid, antigen, antibody, and aptamer or
- 20 chemical detection. In another example, the CRISPR based nucleic acid detection uses an effector complex comprising a Cas protein and at least one CRISPR RNA (crRNA) capable of specifically binding to a target nucleic acid molecule. In one example, the Cas protein is selected from the group consisting of Cas 9, Cas12a, Cas12b, Cas12c, Cas12d, Cas13a, Cas13b, Cas13c, Cas13d, Cas12e, and Cas14.
- 25 [0083] In another aspect, the present disclosure refers to a method of detecting and/or quantifying a target molecule in a sample, using the device as disclosed herein, the method comprising: providing the sample comprising the target molecule in the rotor; generating illumination and inducing the optical signal of the target molecule by the plurality of RGB LEDs; detecting the optical signal of the target molecule by the plurality of RGB sensors;
- 30 and transferring the data to the external device by the microcontroller.
- [0084] In one example, the sample to be detected and/or quantified using the disclosed detection method can be a biological, chemical or environmental sample as disclosed

herein. In another example, the target molecule can be a nucleotide, a polynucleotide, a nucleoside, a nucleic acid, a nucleic acid analogue, a peptide, a polypeptide, a protein, an antibody, a hormone, a salt, a metal, and a compound that produces color in a solvent, as disclosed herein.

5 [0085] In another example, the device has a capacity of 1, 2, 3, or 4 rotors and each rotor has a capacity to receive 1-200 samples, preferably 1-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-150, or 150-200 samples.

[0086] After illumination by the plurality of RGB LEDs, the target molecule was induced to generate an optical signal to be detected by the RGB sensor. The optical signal
10 is selected from the group consisting of a fluorescent signal, a colorimetric signal, an absorbance signal, a luminescence signal, a bioluminescence signal, and a chemiluminescence signal.

[0087] The microcontroller is selected from the group comprising a single-board Arduino Uno microcontroller (Arduino), ARM, AVR, 8051, PIC, AVR, XMEGA, SAM,
15 MSP430, AT MEGA, NXP, STM, LPC, GD, STM8 and STM32, and used to transfer the data related to the optical signal to the external device selected from the group comprising a smartphone, a tablet, a computer, a server or a cloud platform. The microcontroller comprises a Bluetooth module selected from the group comprising SPP-C, SPP2-0, CC2541, BT04-A, BK3231, BC417, CH-05, CH-08, CH-06, CH-10C, CH-07, CH-09,
20 NRF52832, NRF51822, JDY-09, CC2541, HM-19, and HM-17 for wireless communication with the external device, or a portal for cable connection with the external device.

[0088] The method of detecting and/or quantifying a target molecule in a sample as disclosed herein can be performed in one hour or less, for example about or less than 5
25 min, about or less than 10 min, about or less than 15 min, about or less than 20 min, about or less than 30 min, about or less than 40 min, about or less than 50 min, or about or less than 60 min.

[0089] In another aspect, the present disclosure refers to a method of monitoring viral or bacterial disease outbreaks and/or evolution, comprising performing a detection
30 method as disclosed herein. The detection method comprise the following steps: providing the sample comprising the target molecule derived from a virus or bacterium in the rotor; generating illumination and inducing the optical signal of the target molecule

by the plurality of RGB LEDs; detecting the optical signal of the target molecule by the plurality of RGB sensors; and transferring the data to the external device by the microcontroller.

- [0090] In some examples, the virus which causes disease outbreaks and/or evolution, to be detected by the method and the device as disclosed herein, can be selected from a group consisting of a double-stranded DNA virus, a single-stranded DNA virus, a double-stranded RNA virus, a positive sense RNA virus, a negative sense RNA virus, and a retrovirus, as disclosed herein. In some examples, the virus is selected from a group consisting of a coronavirus, an Ebola virus, measles, SARS, Chikungunya virus, Marburg, MERS, Dengue, Lassa, influenza, rhabdovirus, HIV, a hepatitis virus (including hepatitis A, B, C, D, or E), an influenza virus (including an influenza A or influenza B), a human respiratory syncytial virus, Sudan ebola virus, Bundibugyo virus, Tai Forest ebola virus, Reston ebola virus, Achimota virus, Aedes flavivirus, Aguacate virus, Akabane virus, Alethinophid reptarenavirus, Allpahuayo mammarenavirus, Amapari mmarenavirus, Andes virus, Apoi virus, Aravan virus, Aroa virus, Arumwot virus, Atlantic salmon paramyxovirus, Australian bat lyssavirus, Avian bomavirus, Avian metapneumovirus, Avian paramyxoviruses, penguin or Falkland Islands virus, BK polyomavirus, Bagaza virus, Banna virus, Bat herpesvirus, Bat sapovirus, Bear Canon mammarenavirus, Beilong virus, Bctacoronavirus, Bctapapillomavirus 1-6, Bhanja virus, Bokcloh bat lyssavirus, Boma disease virus, Bourbon virus, Bovine hepacivirus, Bovine parainfluenza virus 3, Bovine respiratory syncytial virus, Brazoran virus, Bunyamwera virus, Caliciviridae virus, California encephalitis virus, Candiru virus, Canine distemper virus, Canine pneumovirus, Cedar virus, Cell fusing agent virus, Cetacean morbillivirus, Chandipura virus, Chaoyang virus, Chapare mammarenavirus, Colobus monkey papillomavirus, Colorado tick fever virus, Cowpox virus, Crimean-Congo hemorrhagic fever virus, Culex flavivirus, Cupixi mammarenavirus, Dengue virus, Dobrava-Belgrade virus, Donggang virus, Dugbe virus, Duvenhage virus, Eastern equine encephalitis virus, Entebbe bat virus, Enterovirus A-D, European bat lyssavirus 1-2, Eyach virus, Feline morbillivirus, Fer-de- Lance paramyxovirus, Fitzroy River virus, Flaviviridae virus, Flexal mammarenavirus, GB virus C, Gairo virus, Gemycircularvirus, Goose paramyxovirus SF02, Great Island virus, Guanarito mammarenavirus, Hantaan virus, Hantavirus Z10, Heartland virus, Hendra virus, Hepatitis A/B/C/E, Hepatitis delta virus,

Human bocavirus, Human coronavirus, Human endogenous retrovirus K, Human enteric coronavirus, Human genital-associated circular DNA virus- 1, Human herpesvirus 1-8, Human mastadenovirus A-G, Human papillomavirus, Human parainfluenza virus 1-4, Human paraechovirus, Human picomavirus, Human smacovirus, Ikoma lyssavirus, Ilheus virus, Influenza A-C, Ippy mammarenavirus, Irkut virus, J-virus, JC polyomavirus, Japanese encephalitis virus, Junin mammarenavirus, KI polyomavirus, Kadipiro virus, Kamiti River virus, Kedougou virus, Khujand virus, Kokobera virus, Kyasanur forest disease virus, Lagos bat virus, Langat virus, Lassa mammarenavirus, Latino mammarenavirus, Leopards Hill virus, Liao ning virus, Ljungan virus, Lloviu virus, Louping ill virus, Lujo mammarenavirus, Luna mammarenavirus, Lunk virus, Lymphocytic choriomeningitis mammarenavirus, Lyssavirus Ozemoe, MSS12Y225 virus, Machupo mammarenavirus, Mamastrovirus 1, Manzanilla virus, Mapuera virus, Marburg virus, Mayaro virus, Measles virus, Menangle virus, Mercadeo virus, Merkel cell polyomavirus, Middle East respiratory syndrome coronavirus, Mobala mammarenavirus, Modoc virus, Moijang virus, Mokolo virus, Monkeypox virus, Montana myotis leukoencephalitis virus, Mopeia lassa virus reassortant 29, Mopeia mammarenavirus, Morogoro virus, Mossman virus, Mumps virus, Murine pneumonia virus, Murray Valley encephalitis virus, Nariva virus, Newcastle disease virus, Nipah virus, Norwalk virus, Norway rat hepacivirus, Ntaya virus, O'nyong-nyong virus, Oliveros mammarenavirus, Omsk hemorrhagic fever virus, Oropouche virus, Parainfluenza virus 5, Parana mammarenavirus, Parramatta River virus, Peste-des-petits-ruminants virus, Pichande mammarenavirus, Picomaviridae virus, Pirital mammarenavirus, Piscihepevirus A, Porcine parainfluenza virus 1, porcine rubulavirus, Powassan virus, Primate T-lymphotropic virus 1-2, Primate erythroparvovirus 1, Punta Toro virus, Puumala virus, Quang Binh virus, Rabies virus, Razdan virus, Reptile bomavirus 1, Rhinovirus A-B, Rift Valley fever virus, Rinderpest virus, Rio Bravo virus, Rodent Torque Teno virus, Rodent hepacivirus, Ross River virus, Rotavirus A-I, Royal Farm virus, Rubella virus, Sabia mammarenavirus, Salem virus, Sandfly fever Naples virus, Sandfly fever Sicilian virus, Sapporo virus, Sathuperi virus, Seal anellovirus, Semliki Forest virus, Sendai virus, Seoul virus, Sepik virus, Severe acute respiratory syndrome-related coronavirus, Severe fever with thrombocytopenia syndrome virus, Shamonda virus, Shimoni bat virus, Shuni virus, Simbu virus, Simian torque teno virus,

- Simian virus 40-41, Sin Nombre virus, Sindbis virus, Small anellovirus, Sosuga virus, Spanish goat encephalitis virus, Spondweni virus, St. Louis encephalitis virus, Sunshine virus, TTV-like mini virus, Tacaribe mammarenavirus, Taila virus, Tamana bat virus, Tamiami mammarenavirus, Tembusu virus, Thogoto virus, Thottapalayam virus, Tick-borne encephalitis virus, Tioman virus, Togaviridae virus, Torque teno canis virus, Torque teno douroucouli virus, Torque teno felis virus, Torque teno midi virus, Torque teno sus virus, Torque teno tamarin virus, Torque teno virus, Torque teno zalophus virus, Tuhoko virus, Tula virus, Tupaia paramyxovirus, Usutu virus, Uukuniemi virus, Vaccinia virus, Variola virus, Venezuelan Vesicular stomatitis Indiana virus, WU Polyomavirus, Wesselsbron virus, West Caucasian bat virus, West Nile virus, Western equine encephalitis virus, Whitewater Arroyo mammarenavirus, Yellow fever virus, Yokose virus, Yug Bogdanovac virus, Zaire ebolavirus, Zika virus, or Zygosaccharomyces bailii virus Z viral sequence, Canine Parvovirus, Canine Distemper Virus, Canine Coronavirus, Canine Influenza Virus, Feline Herpesvirus, Feline Calicivirus, Feline Leukemia Virus, Feline Immunodeficiency Virus, Canine Adenovirus - Type 1 and Type 2, Canine Herpesvirus, Tobacco Mosaic Virus, Potato Virus Y, Tomato Spotted Wilt Virus, Cucumber Mosaic Virus, Potato Virus X, Barley Yellow Dwarf Virus, Bean Common Mosaic Virus, Maize Dwarf Mosaic Virus, Soybean Mosaic Virus, Citrus Tristeza Virus, Rice Yellow Mottle Virus, Plum Pox Virus, Turnip Mosaic Virus, Aphid-Transmitted Yellow Dwarf Viruses, Cotton Leaf Curl Virus, Papaya Ringspot Virus, Cassava Mosaic Virus, Rice Tungro Virus, Apple Mosaic Virus, Sugarcane Mosaic Virus or a combination thereof. In another example, the virus is a coronavirus such as SARS-CoV-2.
- [0091] In some examples, the bacteria which causes disease outbreaks and/or evolution, to be detected by the method and the device as disclosed herein, can be a Gram-positive bacterium or a Gram-negative bacterium selected from the group consisting of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Bacillus anthracis*, *Bacillus cereus*, *Clostridium botulinum*, *Clostridium perfringens*, *Clostridium difficile* and *Clostridium tetani*, *Corynebacterium diphtheria*, *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Chlamydia trachomatis*, *Yersinia pestis*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Moraxella catarrhalis*,

Haemophilus influenzae, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Proteus mirabilis*, *Enterobacter cloacae*, *Serratia marcescens*, *Helicobacter pylori*, *Salmonella enteritidis*, *Salmonella typhi*, and *Vibrio cholera*, *Brucella abortus*, *Brucella melitensis*, *Brucella suis*, *Brucella canis*, *Brucella ovis*, *Brucella neotomae*, *Mycoplasma mycoides*,
5 *Clostridium perfringens*, *Mycobacterium avium* *Salmonella* spp., *Pasteurella multocida*, *Haemophilus parasuis*, *Actinobacillus pleuropneumoniae*, *Yersinia pestis*, *Francisella tularensis*, *Bacillus anthracis*, *Coxiella burnetii*, *Listeria monocytogenes*, *Clostridium chauvoei*, *Campylobacter* spp., *Chlamydia psittaci*, *Anaplasma marginale*, *Leptospira* spp. Causes leptospirosis, *Bordetella bronchiseptica*, *Clostridium perfringens*,
10 *Staphylococcus intermedius*, *Bartonella henselae*, *Chlamydomphila felis*, *Mycoplasma* spp, *Pasteurella multocida*, *Erwinia amylovora*, *Xanthomonas* spp., *Xanthomonas axonopodis*, *Ralstonia solanacearum*, *Pectobacterium* and *Dickeya* spp., *Agrobacterium tumefaciens*, *Pseudomonas syringae*, *Pseudomonas syringae* pv. *lachrymans*, *Xanthomonas campestris* pv. *campestris*, *Xanthomonas oryzae*, *Clavibacter*
15 *michiganensis*, *Xylella fastidiosa*, *Pantoea* spp., *Burkholderia glumae*, *Xanthomonas citri*, *Xanthomonas translucens*, *Xanthomonas fragariae*, *Xanthomonas hortorum*, *Clavibacter michiganensis*, *Pseudomonas syringae* or a combination thereof.

[0092] The target molecule can be a RNA or a DNA from a virus or a bacterium as disclosed herein.

20 [0093] Other microbe-caused disease can also be detected using the method and device as disclosed herein, such as one caused by an archaea, a protozoa, a fungi, an algae, a slime mode, a lichen, and a prion, highlighting the wide range of applications of the device.

[0094] **The present invention addresses the following problems of existing**
25 **technologies:**

[0095] **Complexity and Cost:** A common challenge with many POC devices for quantitative and high-throughput analysis of nucleic acids lies in their inherent complexity and high costs, restricting accessibility, particularly in resource-constrained settings. The intricate design and production of these devices typically entail expensive
30 components and specialized expertise. In contrast, the present device is strategically engineered to overcome these barriers, featuring a significantly lower initial price than other products with similar applications in the market. Moreover, it delivers substantial

cost savings throughout its lifespan, ensuring a cost-effective and accessible solution for a wide range of users.

[0096] **Versatility:** the present device is a versatile all-in-one instrument that combines the functionalities of Real-Time PCR, plate reader, and spectrophotometer.

5 This unique integration allows it to serve as a comprehensive solution for a wide range of analyses, effectively replacing the need for separate devices in many applications. Its multifunctional design not only enhances efficiency but also offers a cost-effective and streamlined approach to various analytical processes.

[0097] **Throughput:** While other POC devices often exhibit low throughput, the
10 present system stands out as it is easily designed for high-throughput quantitative analysis. This distinctive capability enables efficient processing of a large volume of samples, making the present device ideal for applications that demand rapid and extensive data analysis.

[0098] **Multiplexing:** Unlike other POC devices that face limitations in detecting
15 multiple targets simultaneously in one single eppendorf tube, the present device excels by effortlessly identifying a higher number of different signals in a single reaction during multiplexing. This advanced capability enhances the efficiency and accuracy of diagnostic testing, setting the present device apart in providing a more comprehensive and insightful analysis.

20 [0099] **Sensitivity and Specificity:** Achieving high sensitivity and specificity, especially in low-resource settings is challenging. The present device can accurately detect low concentrations of target molecules while minimizing false positives and false negatives is an ongoing challenge. The results of the present POC derive is comparable with (or better in some cases) with other relevant devices such as Real-Time PCR and
25 plate readers.

[00100] **User Training:** Current existing devices for quantitative measurements of target molecules require specific training, and errors in operation can lead to inaccurate results. Users with limited laboratory experience can easily use the present device effectively.

30 [00101] **Quality Control:** Maintaining quality control in POC settings can be challenging. Ensuring that devices remain calibrated and perform consistently over time

is critical to the reliability of results. The present device distinguishes itself by effortlessly and reliably performing quality control tasks.

[00102] **Power Requirements:** The operational demands of many POC devices often necessitate a stable power supply, posing significant challenges for conducting high-throughput quantitative analysis of biological molecules (such as DNA and RNA), particularly in remote areas. The present portable device has been meticulously engineered with energy efficiency in mind, demanding minimal power consumption to achieve optimal performance. Its innovative design allows it to seamlessly operate with a versatile range of batteries, providing users with unparalleled flexibility and convenience. This adaptability ensures that the device can efficiently function with various battery types, presenting a cost-effective and user-friendly solution suitable for a diverse array of applications and environments.

[00103] **Infrastructure:** Many POC devices (such as those for high-throughput quantitative analysis of nucleic acids) require an appropriate infrastructure for operation to endure harsh environment. The present device's infrastructure is designed to be highly adaptable, featuring a compact design, lightweight construction, or rugged casing to withstand varying environmental conditions and to ensure that the device remains operational and reliable during field use or while on the go.

[00104] **Data Management:** POC devices generate data that requires meticulous handling and storage. Establishing effective data transfer and storage mechanisms, particularly in resource-limited settings, can pose challenges. Unlike other POC devices that often lack this feature, the present device excels in meeting this essential need by securely transmitting information to a designated server or cloud platform. This ensures centralized storage, accessibility, and collaboration, enhancing the efficiency and convenience of data management, aggregating or summarizing data, ensuring reproducibility in research or analysis, enabling advanced analysis, facilitating error detection, supporting long-term research initiatives, aiding in data mining and pattern discovery, and fostering future analysis capabilities.

[00105] **Maintenance and Repair:** In remote or low-resource settings, maintaining and repairing POC devices can be difficult. Here a "fault detection system" has been designed which can automatically monitor and identify issues or faults in device or

processes and generate reports or alerts to inform operators about the problems detected.

The device is very simple to repair, and the components are easy to replace for users.

[00106] **Stability and Robustness:** Ensuring that the POC system can withstand environmental conditions, such as temperature fluctuations and humidity, and still
5 provide reliable results is a technical challenge. The present device demonstrates low- and high-temperature and humidity resistance, ensuring reliable performance even under challenging environmental conditions.

[00107] **The present invention also demonstrates the following advantages as compared to existing technologies:**

10 [00108] 1. No existing device seamlessly integrates RGB LEDs with RGB sensors to perform the real-time read-out of various rapid tests and quantify a broad spectrum of molecules (particularly nucleic acids) in both fluorometric and colorimetric assays. It not only enhances the efficiency of rapid tests but also provides precise quantification across a diverse range of molecules.

15 [00109] 2. By seamlessly integrating RGB LEDs and RGB sensors with an advanced thermal system, it provides simultaneous analysis across an extensive spectrum of molecules in a real-time based analysis.

[00110] 3. Realizing multiplex quantitative detection poses challenges, including limited signal reporting strategies, compromised sensitivity, potential cross-reactions, and
20 interference between recognition molecules and different analytes. Point-of-care (POC) multiplex biosensing faces additional complexities due to sample intricacies. Some conventional devices that offer practical multiplex detection clinically may be bulky and expensive, limiting their feasibility for widespread use, especially in resource-limited or POC settings. POC biosensing technology for multiplex quantitative detection has been
25 achieved in this invention. This versatile device can be utilized for CRISPR-Cas-based detection or real-time multiplex detection employing various fluorogenic probes.

[00111] The disadvantages of current mainly Real-Time based devices like GeneXpert Omni are, their price (over 15K\$), its high operating costs and its limited assay flexibility as well as its limited multiplexing capability (6 nucleic acid target). Considering the
30 combination of RGB LED and RGB sensors in the present device, it can be a significant expansion of Real-Time PCR multiplexing by detecting over 10 nucleic acid targets in a single sample. This is achieved in the PortaQuant by converting the peak emission spectra

for fluorogenic probes (such as those used in Real-Time PCR and CRISPR-Cas based detection) into RGB codes. Then, the experiment was performed by exposing the sample tubes to RGB LED light emitting at the peak emission spectra for given probes, and then recorded the fluorescence intensity for those probes under their respective excitation wavelengths. As a comparison, current existing multiplexed RT-PCR can detect 4-6 nucleic acid targets in a single sample.

[00112] The present device function has a broad overlap with those of Real-Time PCR. It can do detection and quantification of nucleic acid either in combination with CRISPR-Cas based or in a real-time based mode with fluorogenic dyes or probes.

[00113] Real-Time PCR uses a diverse range of optical systems that utilize a combination of light sources, filters, and detectors to quantify the fluorescence emitted during real-time PCR reactions.

[00114] As light source, current Real-Time PCR devices use individual or multiple LEDs (not RGB LED), which is positioned above each well so that each one is independently illuminated, or these can be arranged in a stationary array that excites multiple wells at a time. Halogen lamp and laser are also used as light source. Several filters are located between light source and target as well as from fluorescent light emitted from the wells to detector. In the present device, RGB LED was used, which is positioned in front of tubes with no filter.

[00115] In terms of Detector, Real-Time PCR machine use photodiode, CCD, or a photomultiplier tube. In the present device RGB sensors are used for detecting light from excited fluorophores.

[00116] 4. By using RGB LED and sensors, the present device address a few short coming of mobile-based POC devices. (1) mobile devices vary in their camera specifications and color reproduction, making accurate and consistent calibration for fluorescence detection across different mobiles challenging (2) The mobile device's camera sensitivity and specificity might not be optimized for detecting subtle fluorescence signals, potentially affecting the reliability of the results.

[00117] 5. The present device stands as a unique solution, offering an unparalleled extension in capacity from one sample to over 100, all accomplished with a remarkably small budget, setting it apart from any other available options. Particularly, in low-income countries, where resources are often constrained, this device opens avenues for

groundbreaking research and diagnostics, proving that POC technology for quantitative and high-throughput analysis can be both powerful and affordable.

[00118] 6. There is no device available on the market capable of performing the read-out of different types of rapid and quantitative test including both fluorometric and colorimetric test which can be used for a wide range of applications for home testing, POC testing, laboratory and hospital use, and education.

[00119] 7. Multiplex detection from a single sample is increasingly desirable, not only for disease diagnostics but across various sensing applications. It is shown that the versatility of device for CRISPR-Cas-based detection or real-time multiplex detection employing various fluorogenic probes.

[00120] 8. With a price of 1/50 to 1/100 of current RT-PCR machine, the present device can generate comparable results for high-throughput nucleic acid detection and quantification. It can also surpass the efficiency of some RT-PCR devices. The running cost of the present device can be as low as 0.5\$/sample.

[00121] 9. The real-time PCR machine is capable of quantitative detection using fluorophore dyes or fluorogenic probes. However, it lacks the ability to perform colorimetric analyses required for various assessments, such as colorimetric enzyme activity assays or food safety assays. On the other hand, sophisticated and expensive plate readers, among other similar devices, can carry out both fluorescence measurements and calorimetric analyses, but they are unable to perform DNA amplification. In contrast, the present device seamlessly integrates all these features in a cost-effective and user-friendly manner.

[00122] 10. The present device is designed to be compatible with both smartphones and computers, offering flexibility in its operation. This is a significant advantage, as many existing laboratory devices lack compatibility with mobile phones. Furthermore, the device comes equipped with a dedicated mobile app that performs all necessary analyses, eliminating the need for additional applications or software for data analysis.

[00123] 11. Laboratory devices are often susceptible to interference from dust, dirt, and humidity, posing challenges to their reliable operation. To address this issue, measures are implemented to isolate the present device from these environmental factors, by insulated materials, precise temperature controls, sealed enclosures, sponge filters, and

shock-absorbing components to mitigate environmental impacts and to ensure enhanced performance and longevity.

[00124] 12- The present device is equipped to measure concentration of molecules not only within the visible light range but also in the invisible light range. This capability
5 enables the measurement of various molecules within the invisible light spectra.

[00125] **Industrial Applicability**

[00126] It will be apparent that various other modifications and adaptations of the invention will be apparent to the person skilled in the art after reading the foregoing disclosure without departing from the spirit and scope of the invention and it is intended
10 that all such modifications and adaptations come within the scope of the appended claims.

[00127] The disclosed device is a portable and compact diagnostic instrument and a Point-of-Care Device which is used for the detection and quantification of biological (such as DNA, RNA, or protein) and chemical molecules in a liquid sample and is used in various scientific and research fields, including:

- 15 • **Molecular Biology:** It can be used to measure the amount of a specific DNA or RNA sequence in a biological sample. It is a tool for gene expression analysis, genotyping, detection of molecular markers, and gene copy number determination in high-throughput experiments.
- 20 • **Biochemistry:** It can be used to measure the absorbance, fluorescence, luminescence, or chemiluminescence of biological samples, for enzyme assays, protein quantification, nucleic acid quantification, protein-protein interaction, protein-DNA interaction, and ELISA (Enzyme-Linked Immunosorbent Assay) applications.
- 25 • **Medical Diagnostics:** It can be utilized in clinical diagnostics for the detection of infectious agents, genetic diseases, and monitoring the progression of diseases like cancer. It is employed for clinical chemistry tests such as Biochemical Tests, Microbiological Tests, Virological Tests, Immunological and Serological Tests, Parasitic Tests, Toxicological Tests, Genetic and Molecular Biological Tests, Urine Tests, Cardiac Enzyme Tests.
- 30 • **Microbiology:** It can be used for the detection of pathogens, such as bacteria, viruses, and fungi. It's also applied in environmental microbiology to monitor

microbial populations in various ecosystems. It can be also used for microbial growth and inhibition assays, as well as in antimicrobial susceptibility testing.

- **Genetic Research:** It can be employed in genetic research to study genetic variations, gene expression, mutations, and polymorphisms such as single nucleotide polymorphisms (SNPs). It can be used to investigate the genetic basis of various diseases and traits.
- **Immunology:** It can be utilized for immunological assays such as cytokine quantification, antibody-antigen interactions, and immune response studies.
- **Agricultural Biotechnology:** It can be used for genetically modified organism (GMO) testing, pathogen detection in crops, and crop quality assessment.
- **Forensics:** It can be employed in forensic science for DNA detection, paternity testing, and the analysis of biological evidence.
- **Chemistry:** It can be employed in the measurement of the absorption of UV or visible light by a sample, which can be used to determine the concentration of analytes and measurement the emission of light by a substance that has absorbed light to study the properties of organic and inorganic substances.

[00128] As used in this application, the singular form “a,” “an,” and “the” include plural references unless the context clearly dictates otherwise. For example, the term “a primer” includes a plurality of primers, including mixtures and combinations thereof.

[00129] As used herein, the term “comprising” means “including.” Variations of the word “comprising”, such as “comprise” and “comprises,” have correspondingly varied meanings. Thus, for example, a composition “comprising” X may consist exclusively of X or may include one or more additional unrecited components.

[00130] As used herein, the term “about” in the context of concentration of a substance, size of a substance, length of time, or other stated values means +/- 5% of the stated value, or +/- 4% of the stated value, or +/- 3% of the stated value, or +/- 2% of the stated value, or +/- 1% of the stated value, or +/- 0.5% of the stated value.

[00131] Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual

numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth
5 of the range.

[00132] The invention illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including", "containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions
10 employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by
15 preferred embodiments and optional features, modification and variation of the inventions embodied herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

[00133] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form
20 part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[00134] Unless otherwise indicated, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which the
25 invention belongs.

[00135] Other embodiments are within the following claims and non-limiting examples.

EXAMPLES

30 [00136] Non-limiting examples of the disclosure will be further described in greater detail by reference to specific Examples, which should not be construed as in any way limiting the scope of the disclosure.

[00137] Figures 1 presented the design of the device. Each illustration provides a detailed visual representation of the device's structure, components, and overall architecture. The flowchart (Figure 2) visually represents each step and process involved in the device's operation, providing a clear and structured overview of the entire workflow.

[00138] The capability of qPOCT to measure nucleic acids and enzyme activity precisely and quantitatively are demonstrated in a few examples:

[00139] **Example 1: CRISPR-Cas base quantitative detection of Covid**

[00140] The ability of qPOCT to rapidly detect nucleic acids with high sensitivity aid in disease diagnosis and monitoring, epidemiology, and general laboratory tasks.

[00141] There have been several mobile based devices to measure fluorescence signals from the cleavage of probes using CRISPR-enzyme activated by the presence of target. However, these devices have limitations such as sensitivity, multiplexing capability, sample complexity and background noise, throughput (the number of samples to run simultaneously), quantification accuracy, and user-friendliness. To address above mentioned challenge, a red, green, blue (RGB) sensor-based device was developed for accurate, sensitive, low cost, quantitative, robust, and user-friendly CRISPR-Cas based detection system. The Cas13-cd14 collateral cleavage activities in vitro were evaluated in order to use the device for the Cas13 SARS-CoV-2-based detection assays.

[00142] **Methods**

[00143] Fragment of DNA template (Broughton et al., 2020) of SARS-CoV-2 N gene (Target) for T7 in-vitro transcription (IVT) was synthesised and cloned in pUC57 by GenScript (Table 1). The amplification of N gene DNA oligo template were preformed using forward

(AATTCTAATACGACTCACTATAGGGCTGGTGTGTAACATGTTACCTTCTTCA TC) and reverse (CCTATTACTAGGTTCCATTGTTC) primers Phusion™ High-Fidelity PCR Master Mix (2X) (Thermo Fisher Scientific) with forward (TAATACGACTCACTATAG) and reverse (CTTTATGCTTCCGGCTCG) primers were used for amplification of template DNA. PCR assembly reaction in a 25-μL volume was set up according to manufacturer's instructions and using the cycling parameters according to manufacturer's recommendations, with an annealing temperature of 55 °C and 35 cycles. The integrity of amplified sequence was checked by Sanger sequencing.

PCR product was run on 2% agarose gel and 190bp template band was recovered from gel by GeneJET™ Gel Extraction Kit (Thermo Fisher Scientific). 3µl of gel recovered DNA was run on 2% agarose gel. The concentration of 190bp purified DNA was determined by Picodrop and Qubit Fluorometer.

- 5 [00144] TranscriptAid T7 High Yield Transcription Kit (Thermo Fisher Scientific) was used for ssRNA template synthesis using T7 RNA polymerase primer (TAATACGACTCACTATAG).

[00145] The ssRNA reaction components were combined at room temperature according to manufacturer's instructions using 1µg of template, mixed thoroughly, and

- 10 then centrifuged briefly to collect all drops and incubated at 37°C for 4 h in water bath. DNase I digestion directly after the IVT reaction was performed to prevent the template DNA from interfering with downstream applications of the RNA transcript. 1µL of DNase I (at 1 U/µL; included in the kit) was added into the reaction mix immediately after the IVT reaction and incubate at 37°C for 15 minutes. 0.5µL of the IVT product was
15 diluted in 10µL of DEPC-treated water and 10µL of the diluted sample was mixed with 10µL of 2X RNA Loading Dye Solution of the TranscriptAid T7 High Yield Transcription Kit and incubated at 70°C for 10 minutes and then was chilled on ice prior to loading. Samples were run on a 2% Agarose Gel against an RNA Ladder.

[00146] RNA purification from IVT reaction was performed using GeneJET RNA

- 20 Cleanup and Concentration kit (Thermo Fisher Scientific) as instructed by manufacturer. The purified RNA was stored at -80°C until use. 0.5 µL of the purified IVT product was diluted in 10 µL of DEPC-treated water and 10 µL of the diluted sample was mixed with 10 µL of 2X RNA Loading Dye Solution and was heated the sample at 70°C for 10 minutes and was chilled on ice prior to loading on a 2% Agarose Gel against an RNA
25 Ladder.

[00147] **Table 1.** Sense DNA template of SARS-CoV-2 N gene for production of RNA substrates (i.e. target sequences)

Name	Sequence
Fragment of SARS-CoV-2 N gene	5'CCAAATTGGCTACTACCGAAGAGCTACCAGACGAATTCGTGGTGGTGACG GTAAAATGAAAGATCTCAGTCCAAGATGGTATTTCTACTACCTAGGAACTGGG CCAGAAGCTGGACTTCCCTATGGTGCTAACAAAGACGGCATCATATGGGTTG CAACTGAGGGAGCCTTGAATACACCAAAAGATCACATTGGCACC CGCAATCC TGCTAACAAATGCTGCAATCGTGCTACAACCTTCCTCAAGGAACAACATTGCCAA AAGGCTTCTACGCAGAAGGGAGCAGAGGGCGGCAGTCAAGCCTCTTCTCGTT CCTCATCACGTAGTCGCAACAGTTCAAGAAATTCAACTCCAGGCAGCAGTAG GGGAACTTCTCCTGCTAGAATGGCTGGCAATGGCGGTGATGCTGCTCTTGCT TTGCTGCTGCTTGACAGATTGAACCAGCTTGAGAGCAAAATGTCTGGTAAAG GCCAACACAACAAGGCCAAACTGTCACTAAGAAATCTGCTGCTGAGGCTTC TAAGAAGCCTCGGCCAAAAACGTAAGTCCACTAAAGCATACAATGTAACACAA GCTTTCGGCAGACGTGGTCCAGAACAAACCAAGGAAATTTGGGGACCAG GAACTAATCAGACAAGGAACTGATTACAAACATTGGCCGCAAATTGCACAATT TGCCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGCATTGGCATGGAAGTC ACACCTTCGGGAACGTGGTTGACCTACACAGGTGCCATCAAATTGGATGACA AAGATCCAAATTTCAAAGATCAAGTCATTTTGCTGAATAAGCATATTGACGCA TACAAAACATTCCCACCAACAGAGCCTAAAAAGGACAAAAAGAAGAAGGCTG ATGAAACTCAAGCCTTACCGCAGAGACAGAAGAAACAGCAAACCTGTG-3'

[00148] Short 81 or 82-bp dsDNA made by annealing of complementary oligos were used to generate crRNAs (Fozouni et al. 2021) dsDNA coding sequence, First, concentrated complementary oligonucleotides (Table 2) were mixed together at a 1:1 molar ratio in a microcentrifuge tube and were diluted to a final concentration of 1 pmol/μl with a Tris or phosphate buffer (10 mM Tris, 1 mM EDTA, 50 mM NaCl (pH 8.0) or 100 mM sodium phosphate, 150 mM NaCl, 1 mM EDTA (pH 7.5)). To generate crRNA dsDNA, complementary oligonucleotides were annealed using thermocycler with a denaturation step of 95 °C for 5 min; then, the temperature gradually decreased by 1 degree per minute to 25 °C. The resultant 81 or 82-bp crRNA dsDNA was used to synthesize ssRNA templates (crRNA) (Table 3) using T7 RNA polymerase primer (TAATACGACTCACTATAG) as described in Example 3.

[00149] **Table 2.** Complementary oligonucleotides for synthesis of crRNA dsDNA for SARS-CoV-2. Complementary oligonucleotides were shown as a and b for crRNA.

Name	Sequence
Cd14-CoV crRNAa	TAATACGACTCACTATAGCAACTACAACCTGTCAAATTACAGGGTTCTGAAA CTTGTGCAATTTGCGGCCAATGTTTGTA
Cd14-CoV crRNAb	TTACAAACATTGGCCGCAAATTGCACAAGTTTCAGAACCTGTAATTTGACAG GGTGTAGTTGCTATAGTGAGTCGTATTA

[00150] **Table 3.** crRNA sequences (5' to 3') for SARS-CoV-2 detection.

Name	Sequence
Cd14-CoV crRNA	GCAACUACAACCCUGUCAAAUUACAGGGUUCUGAAACUUGUGCAA UUUGCGGCCAAUGUUUGUAA

[00151] To determine the ability of qPOCT for CRISPR-Cas based detection, a 50 μ L detection system was prepared that consisted of 200 nM Cas13-cd14, 100 nM gRNA, 250nM ssRNA reporter, and 0, 1.5625, 3.125, 6.25, 12.5, 25, 50 nM target ssRNA for enzyme in the reaction buffer (20 mM HEPES, 50 mM NaCl, 10 mM MgCl₂, 5% glycerol, 1 μ g/ml BSA, pH7.0). Fluorescence readouts were taken after 15 min. All experiments were performed using three replicates. The output light intensity (with samples (I)) to input light intensity (without sample (I₀)) ratio (I/I₀) were calculated for each red, green, and blue color. Subsequently, the resulting values were logarithmically transformed and multiplied by -1.

[00152] **Results**

[00153] Figure 3 to 5 displays the output light intensity to input light intensity ratio (I/I₀) calculated for each red, green, and blue color across varying concentrations of target. The values were logarithmically transformed and multiplied by -1. The mean values were utilized to create graphs, with the corresponding standard deviation from three replicates shown on each graph.

[00154] To create the RGB graph (Figure 6), the output (I) for different colors were multiplied. Simultaneously, the inputs (I₀) for various colors were also multiplied. Subsequently, the ratio (I/I₀) was calculated, and the negative logarithm was applied (Figure 6).

[00155] The limit of detection (LOD) was calculated using the 3 σ principle. Calculation is based on the RGB sense and standard deviation of the blank. For these reasons, the Eq (1) was used:

$$yD=yB+3\sigma_0 \quad \text{Eq (1)}$$

[00156] In this equation, yD is converted to concentration using the calibration function. yB represents the average value of the instrument blank response, and σ_0 denotes the standard deviation of the blank. In this experiment, the blank composed of all reaction components (with 50 nM target) except for the probe. Experiment was performed using 3 replicates and yD was calculated and the corresponding concentration were regarded as LOD. The linear curve (Figure 7) is considered within 0–12.5 nM/ μ L of

the target's concentration. Therefore, LOD was calculated based on the linear equation and was 0.097 nM.

[00157] Overall, over results demonstrated that qPOCT enables quantitative analysis of CRISPR-Cas-based detection without requiring a fluorescence plate reader or similar devices. This portable and cost-effective POC solution allows for the accurate detection of pathogens using CRISPR-Cas technology, offering laboratory-level precision comparable to expensive fluorescence plate readers or qRT-PCR instruments.

[00158] **Example 2: evaluation of qPOCT in combination with thermal cycling amplification**

10 [00159] In addition, CRISPR-Cas based detections mainly rely on preamplifications using isothermal enzymes such as recombinase polymerase amplification (RPA), and loop-mediated isothermal amplification (LAMP). However, these enzymes are relatively expensive. By integrating a thermal cycling mechanism into the device, the need for isothermal enzymes has been eliminated, opting for the amplification of the target using cost-effective Taq polymerase enzymes. To demonstrate this, thermal cycle amplification was combined with LbCas12a collateral cleavage activities for *Brucella* detection assay.

[00160] **Method**

[00161] In this study, sequence of *bp26* of *Brucella* (Genbank No. AF242532) was selected as a target sequence from the National Center for Biotechnology Information (NCBI) as previously described (Dang et al., 2023) and was synthesized and cloned in pUC19 (GenScript, USA).

[00162] Amplification was performed in qPOCT in a total volume of 25 μ l, with 2 μ l (50 ng/ μ l) of DNA, 12.5 μ l Q5® High-Fidelity 2X Master Mix (NEB), and 2.5 μ l of primer mix, and 8 μ l of molecular grade water. The forward primer (F) sequence was 5'-CAATGTTGGAAAAATTTGGATGAATCCGT-3', and the reverse primer (R) sequence was 5'-TTACTTGATTTCAAAAACGACATTGACCGATA-3'.

[00163] The PCR parameters are as follows: initial denaturation at 98°C for 30 sec, 30 cycles of denaturation at 98°C for 10 sec, annealing at 60°C for 30 sec, and extension at 72°C for 30 sec and a final extension at 72°C for 2 min.

30 [00164] Then, the following CRISPR reaction system was constructed to prepare 25 μ L reaction solution: 1.2 μ L EnGen® Lba Cas12a (NEB), 1 μ L probe, 0.3 μ L RNase inhibitor

(0.48 U), 1.5 μ L NEBuffer3.1, 5 μ L crRNA (400 nM), 4 μ L PCR product, and 12 μ L DDH₂O.

[00165] The probe was /56-FAM/CCCCCCCC/3IABkFQ/ and the crRNA sequence for collateral activity assay was 5'-

5 UAAUUUCUACUAAGUGUAGAUGAUGAAUCCGUCACGCUCGG-3' (Dang et al. 2023). The following concentration of probes were tested: 25, 50, 100, 200, and 400 nM. Fluorescence readouts were taken after 15 min.

[00166] **Result**

[00167] The results from the comparison of the probe concentrations was presented in Figure 8.

[00168] To create the RGB graph, the output (I) for different colors were multiplied. Simultaneously, the inputs (I_0) for various colors were also multiplied. Subsequently, the ratio (I/I_0) was calculated, and the negative logarithm was applied. The mean values were utilized to create graphs, with the corresponding standard deviation from three replicates shown on each bar.

[00169] The results demonstrated that CRISPR-mediated fluorescence signal intensities increased with increasing amounts of probes (from 25 nM to 400 nM).

[00170] **Example 3: Evaluation of RGB LED and sensor for multiplexing**

[00171] Multiplex detection from a single sample is increasingly desirable, not only for disease diagnostics but across various sensing applications. However, realizing multiplex detection poses challenges, including limited signal reporting strategies, compromised sensitivity, potential cross-reactions, and interference between recognition molecules and different analytes. Point-of-care (POC) multiplex biosensing faces additional complexities due to sample intricacies. Some devices that offer practical multiplex detection clinically may be bulky and expensive, limiting their feasibility for widespread use, especially in resource-limited or POC settings. Therefore, achieving multiplex detection is a well-studied yet challenging goal for current POC biosensing technologies. Realizing CRISPR/Cas multiplex biosensing holds promise for significantly advancing the capabilities of this technology in the biosensing field. Here a cost-effective and user-friendly device has been designed for multiplex detection in a single sample. This versatile device can be utilized for CRISPR-Cas-based detection or real-time multiplex detection employing various fluorogenic probes.

[00172] *Methods*

[00173] Two ssRNA probes, labeled with FAM or HEX at the 5' end and 3IABkFQ quencher at the 3' end (Table 4), were individually tested and combined in a single sample. The qPOCT was set up by converting the peak emission spectra for FAM (518 nm) and
5 HEX (535 nm) into RGB codes. Then, the experiment was performed by exposing the sample tubes to RGB LED light emitting at 518nm and 535 nm for FAM and HEX, respectively, and then recording the fluorescence intensity for both HEX and FAM under their respective excitation wavelengths. The experimental conditions were the same as described in Example 1 with three modifications: (1) the target concentration was 50 nM.
10 (2) the concentration of individual probes was 250 nM. For the mixed probe reaction, a concentration of 125 nM (250 nM total probe concentration) was utilized. (3) the distance from RGB LED and sensor to sample tubes has been reduced from 40 mm to 7.5 mm. Fluorescence measurements were taken after a 15-minute incubation period.

[00174] **Table 4.** Probes/reporters tested for multiplex analysis

Reporter RNAs	Sequence
FAM-----3IABkFQ	/56-FAM/rUuU rUuUu /3IABkFQ/
HEX-----3IABkFQ	/5HEX/rUuU rUuUu /3IABkFQ/

[00175] *Results*

[00176] The results for multiplex detection of two probes is presented in Figure 9 which displays the output light intensity to input light intensity ratio (I/I_0) calculated for each red, green, and blue color. The values were logarithmically transformed and multiplied
20 by -1. The mean values were utilized to create graphs, with the corresponding standard deviation from three replicates shown on each graph. The results demonstrated that the qPOCT can quantitatively distinguish two probes in a single sample. Additionally, the results suggested that the red color may be more suitable for the quantification of these two probes. Considering the nature of RGB LED and the sensor, the results suggested
25 that the device can be used for multiplex detection employing over 10 fluorogenic probes.

[00177] **Example 4: Evaluation of qPOCT for colorimetric determinations**

[00178] In this experiment, the device was used for quantitative analysis of color solution. Xylose and DNS (3,5-dinitrosalicylic acid) are commonly used in the context of enzyme activity assays, particularly for the determination of reducing sugars produced by
30 enzymatic reactions. xylose serves as a substrate for certain enzyme-catalyzed reactions, and DNS is applied for the quantitative measurement of reducing sugars produced during

these reactions. The combination of these components allows researchers to assess the activity of enzymes involved in the degradation of polysaccharides and other sugar-containing substrates.

[00179] **Methods**

- 5 [00180] Initially, various concentrations of xylose (0.2-3.5 mg/ml) were prepared. Then, 60 μ l of a sugar-containing samples were added to 120 μ l of DNS solution and boiled for 5 minutes. Finally, the resulting color intensity was measured. For each individual R, G, or B color, the output light intensity (I) to input light intensity (I_0) ratio (I/I_0) were calculated for each red, green, and blue color. Subsequently, the resulting
- 10 values were logarithmically transformed and multiplied by -1. The experiment was performed using three replicates.

[00181] **Results**

- [00182] Figure 10 presented output-to-input light intensity ratio (I/I_0) for each red, green, and blue color across varying concentrations of xylose. The data underwent
- 15 logarithmic transformation and multiplication by -1. The generated graph is based on mean values with the corresponding standard deviation derived from three replicates.

- [00183] The limit of detection (LOD) was calculated as described for Example 1. The blank was double distilled water. The linear curve (Figure 11) was established within the range of 0–2 mg/ml of the xylose's concentration. Based on the linear equation Eq1
- 20 ($yD=yB+3\sigma_0$), LOD was calculated based on the linear equation and was 0.114 mg/ml. Similar approach was used to measure LOD using plate reader Epoch (BioTek Instruments, Inc) (Linear range=0.2-1.4 (mg/ml))(Figure 12). The resulting LOD for plate reader Epoch (BioTek Instruments, Inc) was 0.299 (mg/ml), suggesting that qPOCT's LOD is approximately 61.87% better than the plate reader.

- 25 [00184] **Example 5. Demonstrating the versatility and precision of a novel RGB sensor and RGB LED for ELISA-based biomarker quantification: TSH as a benchmark example**

- [00185] Thyroid-stimulating hormone (TSH) plays a critical role in thyroid function regulation and is a key biomarker in diagnosing thyroid disorders. Accurate quantification
- 30 of TSH is essential in both clinical diagnostics and research settings, often performed using enzyme-linked immunosorbent assays (ELISA). Validation of new diagnostic

technologies is crucial to ensure precision, reliability, and consistency, particularly when compared to established conventional methods.

[00186] In this study, the performance of qPOCT for TSH quantification was evaluated against a plate reader Epoch (BioTek Instruments, Inc) using a widely utilized TSH ELISA MonoKIT. By generating standard curves and assessing internal control, the aim was to demonstrate the qPOCT's precision and reliability, thereby establishing its suitability for clinical and research applications. The use of TSH ELISA provided a robust benchmark, enabling direct comparisons and highlighting the qPOCT's capabilities.

[00187] **Methods**

10 [00188] The TSH MonoKIT was employed for all experiments. Standards with known TSH concentrations of 0, 0.5, 2.5, 5, 10, and 25 $\mu\text{IU/mL}$ were prepared and loaded into the assay plates. Each concentration was tested in triplicates. Two internal controls with predefined TSH concentrations within the standard range were included to assess reproducibility and accuracy. The experiment was conducted as follows: 50 μL of calibrators and controls were added to each well of the ELISA plate. Subsequently, 100 μL of enzyme-conjugated solution was introduced into each well. The plate was gently shaken for 30 seconds to ensure proper mixing, then covered and incubated at room temperature for 60 minutes. After incubation, the solutions were removed from the wells, and each well was washed with 300 μL of washing solution to eliminate unbound substances. Next, 100 μL of substrate solution, which generates a color reaction, was added to each well, and the plate was incubated for 15 minutes at room temperature in the dark. Finally, 50 μL of stop solution was added to each well, and the plate was gently shaken for 30 seconds to halt the enzymatic reaction. The absorbance was read at 450 nm using a microplate reader and qPOCT within 15 minutes of adding the stop solution.

25 [00189] Standard curves were generated by plotting TSH concentrations against their corresponding optical densities (OD). Linear regression analysis was performed to calculate the coefficient of determination (R^2), indicating the linearity and precision of each machine.

[00190] The internal control results were plotted against the standard curve for each machine to verify their placement within expected ranges.

[00191] **Results**

[00192] The results demonstrated high linearity in the standard curves generated by both machines, confirming their reliability in quantifying TSH. For plate reader the standard curve yielded an R^2 of 0.986, indicative of strong linear correlation and precision. For qPOCT, the standard curve yielded an R^2 of 0.981, similarly reflecting high
5 linearity and robust performance (Figure 13).

[00193] The internal control for both machines fell precisely within their expected ranges on the standard curves, further validating the accuracy of the measurements. This indicates that the qPOCT is capable of replicating the performance of microplate reader with a comparable level of precision and reliability.

10 [00194] The ability of qPOCT to maintain high linearity and accurately quantify TSH in this ELISA assay highlights its potential as a reliable alternative to traditional systems. Its performance in this study suggests that it could also handle a variety of other ELISA-based assays with similar precision.

[00195] Moreover, the results warrant further exploration of the qPOCT's potential to
15 enhance assay efficiency, particularly in high-throughput settings or complex workflows. By demonstrating compatibility with established TSH detection kits, this study lays the groundwork for broader applications of the novel diagnostic system in clinical and research environments.

20 [00196] **Example 6. Validation of a RGB sensor for high-precision multiplex detection of fluorophores**

[00197] The ability to simultaneously detect and quantify multiple fluorophores with high precision is essential for devices that can simultaneously detect and quantify multiple fluorophores with high precision in various fields, particularly in molecular diagnostics and laboratory research:

- 25 1) Real-time PCR machines are a prime example, as they rely on this capability for multiplex assays to identify multiple pathogens, genetic targets, or single nucleotide polymorphisms (SNPs) in a single reaction, enhancing efficiency and reducing costs.
- 30 2) Digital droplet PCR (ddPCR) systems utilize partitioning technology to achieve highly sensitive and precise detection of multiple targets, such as rare mutations, within a single sample.

3) Point-of-care diagnostic devices also capitalize on multiplex fluorescence assays for rapid and portable diagnostics in clinical settings.

4) Multiplex ELISA systems use technologies to measure multiple analytes like cytokines and hormones in a single sample, making them valuable for both clinical diagnostics and research applications.

[00198] This ability is critical across a range of applications:

1) In infectious disease diagnostics, it enables the simultaneous detection of multiple pathogens, such as SARS-CoV-2, Influenza A/B, and RSV, within a single assay, significantly improving diagnostic efficiency.

2) In cancer biomarker detection, this capability is used to quantify genetic mutations like EGFR, KRAS, and BRAF, or epigenetic markers such as methylation patterns, aiding in cancer diagnosis and personalized treatment.

3) Gene expression analysis benefits from this precision by allowing the measurement of reference genes, such as GAPDH and ACTB, alongside target genes, ensuring accurate normalization in studies of gene regulation or disease states.

4) In genetic testing, it facilitates the identification of single nucleotide polymorphisms (SNPs) or copy number variations, such as BRCA1/2 mutations associated with inherited diseases. Immunological assays leverage this technology for the detection of cytokines or immune markers like IL-6, TNF- α , and IFN- γ , which are essential for understanding inflammation and immune responses.

5) Environmental monitoring applications include identifying microbial species or toxins, such as Escherichia coli, Salmonella, or algal toxins, in water and soil samples. 6) In agriculture, this technology is employed to detect plant pathogens, such as Phytophthora infestans, or to analyze genetic traits in crops, improving disease management and breeding programs.

[00199] Several factors are critical when evaluating the ability of a device to simultaneously detect and quantify multiple fluorophores with high precision. First, the number of fluorophores that can be detected simultaneously is a key metric, as it determines the device's capacity for multiplexing and its utility in complex assays requiring the detection of multiple targets in a single reaction. High multiplexing capacity is particularly valuable in applications such as infectious disease diagnostics, cancer

biomarker detection, and environmental monitoring, where multiple analytes often need to be analyzed concurrently.

[00200] Multiplexing is a game-changing feature of instruments such as real-time PCR because it allows multiple targets to be detected and quantified at the same time in a single reaction. Rather than conducting separate reactions for each target, all can be analyzed at once, saving both time and resources. It's also more cost-effective since it cuts down on the need for extra reagents, consumables, and machine runtime which is a big advantage for handling large-scale studies or high-throughput experiments.

[00201] Multiplexing becomes even more important when working with limited samples, such as in clinical diagnostics or small-scale studies, it allows to make the most of the available material by avoiding the need to split samples across multiple reactions. Plus, it allows for a more comprehensive look at the data by enabling the simultaneous analysis of multiple genes, pathogens, or biomarkers. For instance, one could detect several pathogens in one test, track multiple genetic mutations in a cancer panel, or measure both target and reference genes in a single gene expression assay.

[00202] Another key benefit is that multiplexing reduces variability in experiments. By analyzing multiple targets under the same conditions, it ensures more consistent and reliable results compared to running separate reactions. This is especially valuable in diagnostics, where including internal controls in the same reaction improves the quality and confidence of the assay.

[00203] Multiplexing also plays a vital role in precision medicine. It supports the simultaneous analysis of genetic mutations, expression profiles, and disease biomarkers that are critical for tailoring treatments to individual patients. This feature has revolutionized how real-time PCR is used, making it more efficient, reliable, and versatile for applications ranging from molecular biology research to clinical diagnostics and personalized medicine.

[00204] Reproducibility is another essential factor, as consistent results across multiple runs ensure the reliability of the device for routine and high-stakes applications, such as clinical diagnostics or regulatory testing. Variability in detection can compromise data integrity and lead to inaccurate interpretations.

[00205] This study aimed to evaluate the performance of qPOCT by testing its fluorescence detection capabilities with 12 different fluorophores. The experiment

focused on determining the linearity of fluorescence detection across multiple concentrations, both individually and when mixed together. Probes containing fluorophores with and without quenchers were used to validate the qPOCT’s ability to handle both single-probe and multiplex conditions. The findings provide critical insights into qPOCT’s multiplexing level as well as its robustness and precision, demonstrating its potential for advanced applications in diagnostics and research.

[00206] **Methods**

[00207] In this study, the aim was to evaluate the performance of qPOCT for detecting up to 12 fluorophores and assess its ability to maintain linearity and reproducibility across different concentrations of fluorophores. The experiment involved testing a set of 12 different fluorophores, each paired with specific probes designed to target individual genes of bacteria, viruses, and mycoplasma associated with Bovine Respiratory Disease (BRD) (Goto et al., 2020). The fluorophores tested included CY3.5, ATTO 620, ATTO 655, 6-TET, AlexaFluor350, 6-VIC, TexRd-X, CY5.5, 6-FAM, 6-TAMRA, ATTO 647N, and Alexa 750 NHS (Table 5). All the probes were synthesized by GenScript, Singapore.

[00208] **Table 5.** List of probes and fluorophores used in this study. They are used to diagnose viruses and bacteria associated with Bovine Respiratory Disease (BRD).

Pathogen	Target gene	Fluorophore (5')	Probe sequence 5–3	Quencher (3')
BRSV	<i>N (Nucleocapsid protein)</i>	AlexaFluor350	ACCAAGACTTGTATGATGCTGCCAAAGCA	Dabcyl
BPIV3	<i>M (Membrane protein)</i>	6-VIC	ACAGCAATTGGATCAATAA	BHQ-1
BIDV	<i>PB1</i>	TexRd-X	AATGGACTTTCTCCTGGGACTGCT	BHQ-2
BHV1	<i>gE (glycoprotein E)</i>	CY5.5	TGCGGCCTCCGGGCTTTACGTCT	BHQ-3
BAdV7	<i>hexon</i>	CY3.5	TTCATCWCTGCCACWCAAAGCTTTTTT	3'BHQ-2
BToV	<i>N (Nucleocapsid protein)</i>	ATTO 620	CCAGCAGTCACTATCTTTGCCATTTGA	3'BHQ-2
BCoV	<i>N (Nucleocapsid protein)</i>	ATTO 655	ATTCCGACTAGGTTTCCGGCTGG	3'BHQ-3
BVDV	<i>5'-UTR</i>	6-TET	TGCTAYGTGGACGAGGGCATGC	3'BHQ-1
<i>Mycoplasma bovis</i>	<i>oppD</i>	6-FAM	TGCCAAACTTACCTATCGGTGACCT	BHQ-1
<i>Mannheimia haemolytica</i>	<i>LKTd</i>	6-TAMRA	ACAGATCGTCTTCGGGCACAATGA	BHQ-2
<i>Pasteurella multocida</i>	<i>pm1231</i>	ATTO 647N	TTGATGCCTTCTTTGCGGGTTTCG	BHQ-2
<i>Histophilus somni</i>	<i>bamE</i>	Alexa 750 NHS	TTGCTTACGTCCAAACCGTCGTGT	BHQ-3

[00209] For each fluorophore, probes with concentrations of 0, 12.5, 25, and 50 nM were used. The lyophilized probes were reconstituted with TE buffer. Reactions were performed in 40 μ L volumes in Eppendorf tubes. Probes were tested both with fluorophores and with the corresponding quencher, as well as probes that included only the fluorophore without the quencher (Figure 14). The fluorophore-only and fluorophore-quencher probes were tested separately, as well as mixed together in a single reaction tube to simulate multiplex conditions (Figure 15).

[00210] Each of the fluorophore sets was tested in triplicate to ensure reproducibility. The qPOCT was programmed to detect the fluorescence emitted by each fluorophore, and the signal was measured at each concentration. The data were analyzed to determine the correlation between fluorophore concentration and signal intensity, using the R^2 value as a measure of linearity.

[00211] The tests were designed to evaluate the qPOCT's performance both for single fluorophore detection and for multiplexed conditions, where multiple fluorophores are present in the same reaction. This setup allowed for a thorough assessment of the real-time PCR machine's ability to differentiate between fluorophores, accurately measure their signals, and maintain high precision in both single and mixed fluorophore reactions.

[00212] **Results**

[00213] Out of the 12 fluorophores tested, 10 (CY3.5, TET, Alexa Fluor 350, VIC, TexRd, CY5.5, FAM, TAMRA, ATTO 647N and Alexa 750 NHS) demonstrated excellent linearity ($R^2 > 0.9$) in both single-tube and mixed-probe formats, showcasing the RGB LED and sensor's high precision and compatibility with a wide range of fluorophores. This level of performance highlights the qPOCT's potential for advanced multiplexing applications (Figure 16).

[00214] Two fluorophores showed deviations in linearity under specific conditions. ATTO 620 showed R^2 of 0.74 and 0.56 in single and the mixed-probe format, respectively, indicating challenges in both single and multiplex detection. ATTO 655 demonstrated $R^2 = 0.96$ in the single-tube format, but significantly reduced linearity ($R^2 = 0.44$) in the mixed-probe format. These findings suggest potential interference or incompatibility issues with these fluorophores in complex conditions. Further optimization may be required for these specific cases.

[00215] The new qPOCT with built-in RGB LED and sensor exhibits exceptional performance in detecting and quantifying fluorescence signals from multiple fluorophores. With 10 of the 12 fluorophores achieving $R^2 > 0.9$ across single and mixed formats, this study highlights the RGB sensor reliability and precision in complex
5 multiplexing applications. While minor limitations were observed with ATTO 620 and ATTO 655 in mixed formats, the overall results validate the qPOCT with built-in RGB LED and sensors as a powerful tool for advanced multiplex diagnostic and research applications.

[00216] The results from this study provide strong evidence that the RGB LED and
10 sensors can not only can be utilized for detecting multiple fluorophores effectively but also has the potential to accommodate even higher levels of multiplexing in a single reaction. The experiment demonstrated that the qPOCT with integrated RGB LED and sensor was able to simultaneously detect and quantify signals from up to ten different fluorophores, each at varying concentrations, with high precision and minimal
15 interference between probes. This indicates that the qPOCT is highly capable of differentiating signals from a wide range of fluorophores, ensuring reliable data even when multiple targets are present in a single reaction.

[00217] Additionally, the successful performance of the qPOCT with ten different fluorophores, including both fluorophore-quencher and fluorophore-only probes,
20 demonstrates that the system can simultaneously manage a broad range of spectral signals without significant cross-talk or signal interference. This suggests that with further optimization, the qPOCT could efficiently handle even more probes and fluorophores in a single reaction without compromising signal clarity or accuracy.

[00218] Therefore, this study's results warrant the conclusion that the device and RGB
25 LED and sensor is not only suitable for high-level multiplexing with ten fluorophores but is also poised to handle more complex, higher-level multiplexing, extending its applications to areas requiring the simultaneous detection of even more targets in a single reaction. This feature opens up new possibilities for advanced and affordable multiplex diagnostic testing, gene expression profiling, and other applications that demand the
30 simultaneous analysis of numerous genetic markers or pathogens.

[00219] Linearity of detection across a range of fluorophore concentrations is equally important. A high degree of linearity, as reflected by R^2 values, demonstrates that the

device can accurately quantify targets over a wide dynamic range. This is critical for quantitative applications like gene expression analysis and very low copies of pathogens nucleic acids, where precise measurements of both low and high target concentrations are required.

- 5 [00220] Together, these factors—multiplexing capacity, reproducibility, and linearity—define the performance and versatility of a detection system and are essential for validating its applicability across diverse research and diagnostic scenarios.

[00221] **Example 7: Demonstrating the multiplexing of qPOCT for Real-Time PCR diagnostics**

- 10 [00222] The development of portable, user-friendly diagnostic tools is crucial to advancing molecular diagnostics, particularly in resource-limited or field settings. Real-time PCR (qPCR) has long been regarded as the gold standard for nucleic acid detection and quantification due to its sensitivity, specificity, and reliability. However, conventional qPCR systems are often hindered by their high cost, bulky designs, and
15 reliance on significant power supply and laboratory infrastructure. These limitations restrict their accessibility for on-site diagnostics by community veterinarians, physicians, and nurses.

- [00223] RGB LED and sensor with thermal cycling system have been combined in qPOCT to address these challenges by offering an inexpensive, portable, and efficient
20 solution for genetic testing. Its simple design supports high-volume, low-cost manufacturing, making it an ideal candidate for widespread adoption in decentralized diagnostic settings.

- [00224] One of the unique features of RGB sensor in qPOCT is its ability to detect a wide range of fluorophores with high precision. This capability enables the system to
25 perform advanced multiplexing, allowing the simultaneous detection and quantification of multiple targets in a single reaction. This feature not only increases the throughput of the system but also makes it highly suitable for complex diagnostic applications, such as the detection of multiple pathogens or genetic markers in a single assay.

- [00225] The present study aims to validate the performance of qPOCT in detecting and
30 quantifying multiple fluorophores simultaneously, highlighting its advanced multiplexing capability. By using a set of probes with various fluorophores in single and mixed-tube formats, we sought to demonstrate the qPOCT's precision, reproducibility, and

superiority compared to traditional qPCR systems. These results underscore qPOCT's potential as a transformative diagnostic tool, capable of addressing the needs of diverse fields including veterinary diagnostics, clinical testing, and environmental monitoring.

[00226] In this experiment, the ability of RGB sensor in combination of thermal system for DNA amplification with fluorescence detection was examined.

[00227] *Methods*

[00228] For validation purposes, a specialized Real-Time PCR kit (Senmurv HPV PCR Detection Kit) designed for laboratory diagnostics was utilized. This kit detects 14 high-risk human papillomavirus (HPV) types—67, 66, 59, 58, 56, 52, 51, 45, 39, 35, 33, 31, 18, and 16—along with two low-risk types, 6 and 11.

[00229] By employing this kit, the aim was to validate the ability of qPOCT to reliably detect and differentiate multiple targets in a single reaction.

[00230] The reagents were thawed at room temperature by placing the tubes on a rack. Once thawed, the contents of the tubes were gently mixed by pipetting or vortexing and briefly centrifuged to ensure all liquid collected at the bottom. For reaction preparation, 28 µL of HPV Master A1 (HMR1 Mix) (Table 6) was added to each PCR tube, followed by the addition of 2 µL of HPV Master B. Subsequently, 10 µL of positive control, 10 µL of negative DNA materials were used as negative controls, and 10 µL of nuclease-free water (for the contamination control) was added to separate PCR tubes.

[00231] The positive control was obtained from the manufacturer of the HPV detection kit. The negative controls were prepared using non-infected human genomic DNA, ensuring that these samples were free of HPV. During preparation, all components were maintained at a temperature of 2–8°C to ensure reagent stability.

[00232] **Table 6** illustrates the readings of each mix in the specified fluorescence channels, which indicate the HPV type present in the sample.

	FAM	HEX	Texas Red	Cy5
HMR1 MIX	HPV16	HPV6 & HPV11	Beta globin	HPV18
HMR2 MIX	HPV33	HPV31 & HPV35	HPV39	HPV45
HMR3 MIX	HPV51	HPV52	HPV56	HPV58
HMR4 MIX	HPV59	HPV66	HPV67	HPV68

[00233] The qPOCT was programmed to detect the fluorescence emitted by FAM, HEX, Texas-Red, and CY5 signals. The thermal cycling program included an initial pre-denaturation step at 95°C for 10 minutes, followed by 45 cycles of denaturation at 95°C

for 10 seconds, annealing with fluorescence acquisition at 55°C for 30 seconds, and extension at 72°C for 20 seconds.

[00234] Python was used to create a program for data processing. Initially, data from the sensor readings were retrieved and stored in an output file. The red, green, and blue (RGB) values for each cycle were multiplied together to produce a result referred to as (I). The RGB values measured in the absence of the reaction solution were similarly multiplied and designated as (I₀). The ratio of (I) to (I₀) was calculated, and then the -LOG₁₀ transformation was applied to this result. Since there were five readings per cycle, the results from each cycle were averaged to represent the level of fluorescent emission in each cycle. The baseline was determined by averaging the early cycles, and adjustments were made by subtracting the low fluorescence readings from cycles 3 to 12 (which usually correspond to background noise). After converting the raw data to corrected values, fluctuations were minimized using methods such as Savitzky-Golay smoothing, resulting in a more stable curve. To determine the threshold, the mean of the initial 10 cycles was calculated and then added to 3 times the standard deviation. The first cycle where the curve crossed this threshold was defined as the Ct value. Finally, the data was transformed into a sigmoid curve using the Dogbox method. In conclusion, the Python script was executed to display the plot, print relevant information, and save the processed data.

[00235] Samples were concurrently analysed using the Roche LightCycler® 96 as a reference system to validate the performance and accuracy of the qPOCT.

[00236] **Results**

[00237] The performance of the qPOCT was evaluated using HMR1 Mix, targeting fluorescence signals in FAM, HEX, Texas Red, and Cy5 channels. The generated amplification curves demonstrated characteristic sigmoidal profiles, indicating efficient amplification across all channels. The calculated cycle thresholds (Ct values) for the four fluorophores were 23, 22, 22, and 23 for FAM, HEX, TexasRed, and Cy5, respectively. These results closely align with those obtained using the Roche LightCycler® 96, which were 22, 22, 23, and 24 for FAM, HEX, Texas-Red, and Cy5, respectively.

[00238] Additionally, the uniformity of the sigmoidal curves across all channels indicates the robustness and precision of the novel device in handling multiplex reactions, affirming its potential for high-throughput diagnostic applications (Figure 17). Agarose

gel electrophoresis of the PCR products from both devices showed distinct amplified bands, further confirming the accuracy and reliability of the results obtained (Figure 18).

[00239] qPOCT has been designed to integrate the sensor technology into thermal cycling, enabling it to perform Real-Time PCR analyses with enhanced efficiency and portability. The qPOCT's streamlined design supports high-volume, low-cost manufacturing, ensuring that it is both affordable and widely accessible. Additionally, its low power consumption allows for battery operation, further enhancing its portability and making it suitable for field diagnostics in remote areas.

[00240] qPOCT provides real-time quantification of PCR products, eliminating the need for additional steps such as slab gel or capillary electrophoresis. This simplifies workflows and reduces processing time. Moreover, the integrated sensor technology supports the detection of multiple fluorophores simultaneously, allowing for high multiplexing capacity. This unique feature enables the simultaneous detection of multiple targets in a single assay, further enhancing the qPOCT's diagnostic capabilities.

[00241] Considering the cost-effective nature of the components and the advanced capabilities of the sensor, qPOCT stands out as a revolutionary solution that combines affordability, portability, and real-time PCR analysis. This makes it a compelling alternative to conventional Real-Time PCR machines, positioning it as a breakthrough tool in modern diagnostic applications.

[00242] **Example 8: Evaluation of analytical sensitivity of qPOCT**

[00243] Accurate determination of the limit of detection (LOD) is a crucial step in validating the performance of qPOCT. The LOD represents the lowest concentration or copy number of a target that can be reliably detected under the assay's specified conditions. For this study, the LOD of the newly developed qPOCT was evaluated. This validation is essential to establish the assay's sensitivity and ensure its utility for reliable detection, even at low target concentrations.

[00244] **Methods**

[00245] To determine the LOD, reactions were prepared with known target copy numbers. A *Brucella* gene (BCSP31) inserted into a vector was used as the target (Table 7). Reactions containing various target copy numbers (28×10^3 , 28×10^2 , 14×10^2 , 7×10^2 , 3.5×10^2 and 1.75×10^2 copy number/ml) were set up. The reactions were performed using the Real-Time PCR master mix (RealQ Plus 2x Master Mix Green Without ROX™,

Ampliqon) with 1 μ M of each forward and reverse primer (BCSP31-spF: TGGCTCGGTTGCCAATATCAA and BCSP31-spR: CGCGCTTGCCTTTCAGGTCTG) in a total reaction volume of 40 μ L. Amplification was carried out on qPOCT using the following thermal cycling program: initial
 5 denaturation at 95 °C for 5 minutes, followed by denaturation at 95 °C for 50 seconds, annealing at 60 °C for 45 seconds, and extension at 72 °C for 35 seconds, repeated for 35 cycles.

[00246] Ct values for each copy number were recorded, and a linear regression curve was plotted using Log₁₀(copy number) vs. Ct value (Figure 1). Finally, the LOD was
 10 calculated based on Equation (1).

$$\text{LOD} = 10^{\left(\frac{\text{Ct}-b}{m}\right)}$$

Where: m: Slope of the standard curve, b: Intercept of the standard curve Ct: maximum reliable Ct value. Therefore, LOD was calculated based on the linear equation and was
 1.42 $\times$ 10² copy number/ml of targets in the reaction. To ensure the accuracy of the
 15 reaction, 5 microliters of the polymerase chain reaction products were loaded onto a 1% agarose gel (Figure 2).

[00247] **Table 7.** DNA template of Brucella gene (BCSP31).

Name	Sequence
B.abortus BCSP31 gene encoding a 31- kDa cell surface protein, complete cds GenBank: M20404.1	5'GCGCCTATACGCGCGACCTGATCCAGTCTTTGCAATCGCGGTGCCATGTG CGTCTGGTGGGGGGCCGACGGGCTGGCGGCGATTGCCGAAGCGCATATTG CGGTGAAAGTTTCGATGAGGCGCTGGTCAATGGCGCAGATCGCGCCATGTTTT ATCGAAAAGGATGGCAAGCGCACCGATATCGTGGTGCTTGCCGTACGCATT ATCCGTTTCTCGTCAATGTATTGCGCCGCTCTGGCTCCATGGCCGGTGGATTG GCTGGACCCGGCGGAAGCCATTGCACGACGCATGAAATCGCTTTTGCCCTGC GCGAAGTGACGATGATGAATTTCAATCTCAAGATGACCTAGCATTCTTCACAT CCAGGAAACCCGACTATGCCATTGCGCGCTGATGCAGGGTTTTGGGCTGC GTTTTTAATCGTTTCAGTCGCGCTCTGGCGGCGCTTGTCGGCAAGCGATTGTA TTCTTTGGGAAATCCAGAATAATGGAATGCGGTGGTTGACAATCGGCCTCA AGCTTCCTATGTTTTCGGCATAATCTATGCGGGAAGAGGACTGGTATTATGA AATTCGGAAGCAAATCCGTGCGTTGGCTGTTGCGGCGGTGGCGGGCGCGA TTGCGTTGGGAGCGAGCTTTGCGGTTGCACAGGCCCGACATTTTTCCGTAT CGGCACTGGCGGCACAGCCGGAACCTATTATCCGATTGGTGGTCTGATCGC GAACGCGATTTCCGGCGCAGGCGAAAAGGGCGTSCCGGGTCTCGTGGC GACGGCCGTTTCTCGAATGGCTCGGTTGCCAATATCAATGCGATCAAGTCG GGCGCTCTGGAGTCGGGCTTTACGCAGTCAGACGTTGCCATTGGGCCCTATA ACGGCACCGGCTTTATGATGGCAAGGGCAAGGTGGAAGATTTGCGCCTTC TGGCGACGCTTTACCCGGAAACGATCCATATCGTTGCGCGTAAGGATGCAAA CATCAAATCGGTGCGAGACCTGAAAGGCAAGCGCGTTTCGCTGGATGAGCC GGGTTCTGGCACCATCGTCGATGCGCGTATCGTTCTGAAGCCTACGGCCTC ACGGAAGACGATATCAAGGCTGAACACCTGAAGCCGGGACCGGCGAGCGGA GAGGCTGAAAGATGGTGCGCTGCACGCCCTATTTCTTTGTTGGCGGCTATCC GACGGGCGCAATCTCGGAACTGGCCATCTCGAACGGTATTTGCTGCTTCC GATCTCGGGCCGGAAGCGGACAAGATTCTGGAGAAATATTCCTTCTTCTCG AAGGATGTGGTTCTGCGGAGGCTATAAGGACGTGGCGGAAACACCGACG CTTGCCGTTGCCGCACAGTGGGTGACGAGCGCCAAGCAGCCGACGACCT CATCTATAACATACCAAGGTTCTCTGGAACGAGGATACACGCAAGGCACTC GATGCGGGCCATGCGAAGGGCAACCTCATCAAGCTCGATAGTGGCAGGACG AGCCTCGGTATTCCGCTGCATCCCGGCGCAGAACGCTTTACAAGGAAGCG GGCGTGCTGAAATAATCCCTCAATGATCGGTTCTGATATCTTATCCGAATT GAAGGGTGACATTGCGGCAGCTCGTTATGCGCGCTGCTGCGCTCCGTTTT CCAGAGCGGTTCCGTTAGAACGGAATCGTTGGAACCGCTCTATCTCTTTGT TTTTACGCATTATCCGACGCAAAACCGTTTACGCTTTTGCTGGAAATGCTCT AGCCTATTGAAATGCACGACCGGCAAAAGTGGAGTTGGCCCGCATGACAGAA GAACAAAATGCAAAGCTT3'

[00248] **Results**

[00249] The limit of detection (LOD) of the qPOCT was experimentally determined to be 0.175 copies/ μ L, with the standard curve predicting a lower LOD of 0.142 copies/ μ L, demonstrating the device's high sensitivity and potential for detecting even lower concentrations of the target. A standard curve was generated by plotting the logarithm of the target copy numbers against the corresponding Ct values, demonstrating a strong linear correlation between Ct values and the log-transformed target copy numbers (Figure 19). This result confirms the quantitative accuracy of the qPOCT system over a broad dynamic range of target concentrations. In addition to the real-time amplification data, agarose gel electrophoresis was performed on the PCR products to verify the specificity and presence of the amplified target (Figure 20). The gel images showed distinct bands

corresponding to the expected amplicon size across all tested concentrations, including the lowest concentration of 3 copies, further confirming the device's sensitivity and reliability. Overall, the device demonstrated a highly competitive sensitivity, combining advanced diagnostic capability with portability, affordability, and ease of use, making it an ideal tool for point-of-care testing and resource-limited settings. Further optimization of assay components and reaction conditions is expected to enhance its sensitivity even further.

[00250] **Example 9. Evaluation of clinical sensitivity and specificity of a novel sensor**

10 [00251] This study aimed to evaluate the performance of a newly developed fluorescence detection sensor integrated into a real-time PCR platform. The emphasis was placed on validating the sensor's ability to detect multiple targets with high sensitivity and specificity. Using a commercially available HPV detection kit as the amplification platform, this experiment focused on the sensor's performance while acknowledging the
15 inherent sensitivity and specificity limitations of the kit itself.

[00252] **Methods**

[00253] Senmurv HPV PCR Detection Kit was used to evaluate the clinical sensitivity and specificity of the novel sensor. The experiment was conducted as described in Example 7. Reaction preparation involved adding 28 μL of HPV Master A1 (HMR1, HMR2, HMR3, and HMR4 mixes) and 2 μL of HPV Master B to each PCR tube. To
20 evaluate performance, 10 μL of positive control DNA, 10 μL of negative control DNA was included to rule out non-specific amplification, and 10 μL of nuclease-free water was added as a contamination control. 100 positive controls and 100 negative controls were used for this study. The positive control was obtained from the manufacturer of the HPV
25 detection kit. The 100 negative controls were prepared using non-infected human genomic DNA, ensuring that these samples were free of HPV.

[00254] The qPOCT was programmed to detect the fluorescence emitted by FAM, HEX, Texas Red, and CY5 signals. The thermal cycling program included an initial pre-denaturation step at 95°C for 10 minutes, followed by 45 cycles of denaturation at 95°C
30 for 10 seconds, annealing with fluorescence acquisition at 55°C for 30 seconds, and extension at 72°C for 20 seconds.

[00255] Sensitivity and specificity were calculated using the following formulas:

$$\text{Sensitivity} = (\text{True Positives}) / (\text{True Positives} + \text{False Negatives}) \times 100$$
$$\text{Specificity} = (\text{True Negatives}) / (\text{True Negatives} + \text{False Positives}) \times 100$$

[00256] **Results**

[00257] The sensor demonstrated a sensitivity range of 89–98% and a specificity range of 93–100% across 100 internal positive samples and 100 negative control samples. These results are consistent with the performance metrics of the HPV detection kit itself, which reported sensitivity and specificity values ranging from 90% to 98% and 92% to 100%, respectively, depending on the HPV genotype (Table 8).

[00258] The calculated sensitivity and specificity closely align with the reported performance of the commercial HPV detection kit, emphasizing the reliability of the sensor in detecting HPV genotypes. Importantly, the results suggest that discrepancies from perfect sensitivity and specificity values may, to some extent, reflect the performance limitations of the kit itself rather than the sensor.

[00259] By using the positive controls provided by the HPV kit, the reliability of the device's results was further validated, showcasing its compatibility with existing diagnostic kits.

[00260] By focusing on the fluorescence detection capabilities of the sensor, this study provides strong evidence for its utility in real-time diagnostic applications. The sensor's high accuracy and consistency make it a promising candidate for integration into diagnostic workflows. Moreover, the affordability of the components used to construct the device, coupled with its ability to process small sample volumes, enhances accessibility. Considering the versatility of the sensor, the device can be adapted for a wide range of applications, including colorimetric analysis, environmental monitoring, food safety testing, and home-based health diagnostics. This adaptability positions the dual-sample device as a valuable tool for both clinical and non-clinical applications.

[00261] Future work may focus on optimizing the sensor's performance with different kits or platforms, further enhancing its utility in clinical and point-of-care settings.

[00262] **Table 8.** Evaluation of clinical sensitivity and specificity for HPV using qPOCT

Target	Sensitivity (%)	Specificity (%)
HPV16	95	98
HPV6 or HPV11	98	100
HPV18	95	100
HPV31 or HPV35	92	97
HPV33	91	97
HPV39	96	92
HPV45	88	95
HPV51	91	95
HPV52	93	97
HPV56	90	95
HPV58	91	98
HPV59	91	94
HPV66	91	93
HPV67	91	97
HPV68	89	94

[00263] **Example 10. Design and development of mqPOCT: a portable two-sample diagnostic device**

[00264] A compact and highly portable diagnostic device that integrates the RGB LED and sensor technology with thermal cycling capabilities has been developed. This device is designed to process up to two samples simultaneously, making it particularly suitable for low-throughput applications, including home use, field diagnostics, and resource-limited settings. Its portability and capability for multiplex analysis combined with the flexibility to adapt for various applications, highlights its potential as a versatile diagnostic tool.

[00265] **The structure of mqPOCT**

[00266] **RGB LEDs (14):** two RGB LEDs are utilized to produce light with precise wavelengths. These LEDs can emit light in various spectra (e.g., red, green, blue) and adjust intensity based on RGB codes to suit the fluorescence requirements of the experiment.

[00267] **RGB Color Sensors (TCS230) (13):** two TCS230 color sensors are employed to analyze the fluorescence of samples. These sensors offer high precision in detecting

fluorescence intensity and differentiating wavelengths. The use of two sensors allows the device to process two samples simultaneously, significantly increasing the speed of experiments.

[00268] **Power supply Input (2):** The standard power input port provides energy for the device. It supplies sufficient power to operate high-consumption components like heating elements, fans, sensors, and the microcontroller. The design ensures stability and protects the device from power fluctuations.

[00269] **Power Switch (3):** A high-current-capable power switch allows the device to be turned on or off. It safely manages the main electrical flow to the circuit, providing reliable operation.

[00270] **Cooling Fan (5):** The device includes a high-performance cooling fan for thermal management. It ensures the heating block and internal components remain at optimal temperatures, especially during sensitive PCR cycles, preventing overheating and maintaining stable operation.

[00271] **Fan Driver (6):** Precise fan control is achieved through an advanced fan driver, which adjusts fan speed using Pulse Width Modulation (PWM). This functionality allows automatic speed adjustment based on the device's temperature conditions, reducing energy consumption and noise levels.

[00272] **Heating Element Driver (7):** An accurate heating element driver is used to regulate the thermal block temperature. This driver works in conjunction with the PID algorithm to ensure precise temperature control during different experiment stages.

[00273] **Noise Filter (9):** A high-quality noise filter is integrated to reduce electromagnetic interference and power supply fluctuations. This ensures accurate sensor readings and consistent heating element performance, free from unwanted disruptions.

[00274] **Main board (MEGA 2560) (11):** The core of the device is an Arduino MEGA 2560 microcontroller, which manages all components, including the fan, heating element, sensors, and Bluetooth module. It processes data using precise algorithms and sends commands to maintain optimal conditions.

[00275] **Heat Sink (12):** A specially designed aluminum heat sink is used to dissipate heat from sensitive components like sensors and the microcontroller. It prevents damage from high temperatures and extends the device's lifespan.

[00276] **Temperature Sensor (15):** The device includes a high-precision digital temperature sensor that continuously monitors the thermal block's temperature. The microcontroller uses this data with the PID algorithm to precisely control thermal cycles.

[00277] **Element (16):** A 12V, 40W heating element is integrated to rapidly heat the thermal block. It can quickly reach and maintain the required temperatures for various PCR cycles.

[00278] **Heating Pad (22):** A specialized heat pad is placed above the thermal block to prevent sample evaporation during the PCR process. This ensures that the samples remain intact, improving experiment accuracy.

[00279] **Heat transfer copper block (18):** A critical component of the device is the copper block, which ensures efficient heat transfer and reduces optical noise. This block:

- Houses the temperature and color sensors, RGB LEDs, and heating elements.
- Is made from high-purity copper, offering excellent thermal conductivity.
- Contains two sample chambers designed to hold the test samples securely.
- Acts as a protective shield, preventing ambient light from affecting fluorescence readings.
- Not only transfers heat effectively but also minimizes optical noise, ensuring accurate fluorescence detection.

[00280] **External housing (4):** which houses all the equipment, electronic components, and heating equipment, as well as its small dimensions, makes it easy to carry.

[00281] **Rear Access Door (8):** which provides access to the inside of the main body.

[00282] **Test chamber lid (23):** This door with a hinged mechanism allows quick access to the test sample. This door is where the heat pad is located to prevent liquid vapor from escaping inside the chamber.

[00283] **Test chamber door retaining pin (21):** This pin is installed to hold the door in place.

[00284] **Bluetooth Module (10):** The device features a powerful Bluetooth module for wireless communication with mobile or desktop applications. This module enables:

- Real-time monitoring of experiment data
- Remote configuration of experiment settings
- Storage and sharing of experiment results for further analysis

[00285] **Touch and LCD (1):** This device features a 3.5-inch TFT LCD touchscreen with high resolution, serving as the main user interface. The screen displays critical information, such as:

- 5 • Temperature, number of cycles, experiment type, remaining time, thermal profiles, and fluorescence graphs.
- Through the touchscreen, users can easily configure settings, such as:
 - The number of cycles
 - Adjusting different temperatures for each PCR stage
 - Selecting the experiment type (SYBR Green, probe-based assays, etc.)
 - 10 ○ Displaying error or warning messages
 - The user interface is designed to be user-friendly, offering a seamless and intuitive experience for users.

[00286] Figures 21 presented the design of the device. Each illustration provides a detailed visual representation of the device's structure, components, and overall
15 architecture. The flowchart (Figure 22) visually represents each step and process involved in the device's operation, providing a clear and structured overview of the entire workflow.

[00287] **Example 11. Validation of mqPOCT**

[00288] The device was assessed for analytical and clinical sensitivity and specificity.
20 Analytical sensitivity was determined as described in Example 8 by measuring the limit of detection (LOD) using serial dilutions of target DNA. The LOD was defined as the lowest concentration of target DNA consistently detected in multiple replicates.

[00289] The clinical sensitivity and specificity of mqPOCT was assed using 50 positive and 50 negative samples using a commercially available HPV detection kit as described
25 in Examples 7 and 9.

[00290] **Methods**

[00291] The LOD of mqPOCT was determined as described in Example 8 using a *Brucella* gene (BCSP31) and reactions containing various target copy numbers (28×10^3 , 28×10^2 , 14×10^2 , 7×10^2 , 3.5×10^2 and 1.75×10^2 copy number/ml).

30 [00292] For clinical sensitivity and specificity, a total of 100 samples were used, comprising 50 positive and 50 negative samples. Positive samples were obtained from the HPV detection kit provided by the manufacturer, while negative controls consisted of

non-infected human genomic DNA. Each reaction was prepared by combining 14 μL of HPV Master Mix A1 (containing HMR1, 2, 3, and 4 mixes) with 1 μL of HPV Master Mix B and 5 μL of either sample DNA, positive control, or negative control. all reactions were performed under optimized cycling conditions using the integrated thermal cycler.

5 [00293] **Results**

[00294] The experimentally determined limit of detection (LOD) of the qPOCT device was 0.175 copies/ μL , while the standard curve estimated an even lower LOD of 0.123 copies/ μL , underscoring the device’s high sensitivity and its capability to detect minimal target concentrations. The standard curve, created by plotting the logarithm of target copy
10 numbers against the corresponding Ct values, exhibited a strong linear relationship, highlighting the device’s precision in quantifying target DNA (Figure 23).

[00295] The mqPOCT’s performance was assessed using the HMR1 Mix, which targeted fluorescence signals in the FAM, HEX, Texas Red, and Cy5 channels. The resulting amplification curves displayed characteristic sigmoidal profiles, reflecting
15 efficient amplification across all channels. The calculated cycle threshold (Ct) values for FAM, HEX, Texas Red, and Cy5 were 24, 21, 22, and 23, respectively. The consistency of the sigmoidal curves across all channels highlights the device’s robustness and precision in managing multiplex reactions, demonstrating its suitability for high-throughput diagnostic applications (Figure 24).

20 [00296] Then, the clinical sensitivity and specificity were evaluated using 100 samples. The device achieved clinical sensitivity and specificity values comparable to the commercially available HPV detection kit used as a reference. Sensitivity values ranged between 90% and 98%, and specificity values were between 94% and 100% across different HPV types (Table 9). These results validate the clinical diagnostic capability of
25 the device in scenarios requiring low-throughput diagnostics.

[00297] **Table 9.** Evaluation of clinical sensitivity and specificity for HPV using mqPOCT

Target	Sensitivity (%)	Specificity (%)
HPV16	96	98
HPV6 or HPV11	96	100
HPV18	94	100
HPV31 or HPV35	94	100
HPV33	92	96
HPV39	94	98
HPV45	90	96
HPV51	92	96
HPV52	94	98
HPV56	88	96
HPV58	92	98
HPV59	94	96
HPV66	92	94
HPV67	92	96
HPV68	90	96

[00298] This two-sample mqPOCT device provides an innovative solution for applications requiring high portability and simplicity. Its smaller size and low-cost design make it accessible for diverse settings, including at-home diagnostics, remote fieldwork, and resource-constrained environments. Furthermore, the advanced sensor technology integrated into the device is capable of detecting both fluorescence and colorimetric changes, enabling its use in a wide range of diagnostic and analytical applications.

[00299] Potential applications extend beyond Real-Time PCR and include immunodiagnosics, spectrophotometry, isothermal amplification methods, environmental monitoring, pathogen detection, food safety testing, and point-of-care biomarker analysis. The mqPOCT’s multiplexing capacity, versatility and affordability, coupled with its precise analytical capabilities, underscore its potential as a transformative tool in modern diagnostics.

[00300] *Cost Analysis and economic feasibility of qPOCT and mqPOCT*

[00301] The development of qPOCT and mqPOCT offers an affordable and versatile solution for advanced multiplex diagnostic applications, designed for large-scale production to ensure accessibility and cost-effectiveness. These devices provide significant advantages in affordability and functionality compared to conventional machines while maintaining robust capabilities for multiplex Real-Time PCR, ELISA-style assays, and nanobead-based analyses.

[00302] qPOCT is engineered for high-throughput analysis, handling 24 to 100 samples depending on the rotor size, making it ideal for applications requiring larger sample processing capacity. The total estimated cost of qPOCT for large-scale production is approximately \$129.7. This includes key components such as RGB LED (WS2812) (\$0.5), RGB sensor (tcs230)(\$5), main board (Arduino 2560 R3)(\$15), touch and LCD screen (\$12.5), temperature sensor (DS18B20) (\$2), Bluetooth module (\$4.5), stepper motor (\$4.5), motor driver (\$5), motor coupling (\$1), fan (\$15), heater(40w-12v)(\$6), heater drive (\$4), heat sink (\$0.5), power key (\$0.2), noise filter (\$3), and power supply (\$1). Other structural and functional elements, including external housing, internal compartment, base part, shaft, main rotor, and lid are projected to cost approximately \$50.00 in large-scale manufacturing using injection molding or other mass production techniques.

[00303] mqPOCT, a compact and portable version of qPOCT, is optimized for two-sample analyses and designed for applications where portability and ease of use are essential. Its total estimated cost for large-scale production, is approximately \$119.6. This includes components such as two RGB LED (WS2812) (\$1), two RGB sensor (tcs230)(\$10), two main board (Arduino 2560 R3)(\$30), touch and LCD screen (\$12.5), temperature sensor (DS18B20) (\$2), Bluetooth module (\$4.5), fan (\$9), heat transfer copper block (\$11), two heat sink (\$1), element (\$2.6), noise filter (\$3), and heating pad (\$3). The structural components of mqPOCT, such as external housing, test chamber lid, test chamber lid retaining pin, and rear access door are now estimated to cost around \$30 for large-scale production.

[00304] In comparison to conventional multiplexing Real-Time PCR machines or plate readers capable of 4-plex or higher analyses, which are priced between \$15,000 and \$50,000, qPOCT and mqPOCT remain a groundbreaking advancement in affordability. These traditional devices, while functional, are significantly more expensive and often less portable. In contrast, qPOCT and mqPOCT achieve multiplexing and real-time analysis capabilities for less than 1% of the cost of conventional systems, making them exceptionally suited for community health centers, remote diagnostics, and widespread deployment in resource-limited settings.

[00305] Additionally, when compared to plate readers used for multiplex ELISA assays, which can cost between \$25,000 and \$70,000, both qPOCT and mqPOCT offer a

more affordable alternative while still maintaining high-performance capabilities. These traditional ELISA readers, while highly specialized, often come with higher costs and limitations in portability, making qPOCT and mqPOCT ideal solutions for a broader range of diagnostic applications, particularly in settings with budget constraints.

- 5 [00306] The affordability of these devices, coupled with their ability to detect fluorescence and colorimetric signals, highlights their versatility. They can support a broad range of applications, including pathogen detection, immunodiagnosics, chemical assays, and nanobead-based multiplex analyses. The integration of advanced sensor technology, capable of reading multiple fluorophores or bead signals simultaneously,
- 10 further enhances their functionality. These cost-effective devices demonstrate the potential to revolutionize diagnostic testing by providing high-quality, accessible solutions for diverse healthcare and research needs.

[00307] The device disclosed herein has wide applications:

- 15 • Biotechnology and Pharmaceutical Industry: It can be used for quality control, monitoring bioprocesses, and verifying the identity of microorganisms in bioproduction. it can be used for quality control in the pharmaceutical industry to measure drug components.
- 20 • Food and Beverage Safety and Quality Control: It can be used to detect foodborne pathogens, allergens, and contaminants. It can be also used to determine factors such as food color, nutrient content, and the presence of contaminants or adulterants.
- 25 • Chemistry: It can be employed in chemical analysis for the quantitative determination of the concentration of chemical compounds, often through colorimetry or fluorometry assays.
- Material Science: It can be used to characterize and study the optical properties of materials such as polymers, and coatings.
- 30 • Environmental Science: It can be utilized for the detection and quantification of microorganisms in environmental samples, water quality testing, and monitoring of microbial communities in natural environments. It can be also used to analyze environmental samples, such as water quality testing, measuring the concentration of pollutants, and monitoring air quality.

- Education and Research: It can be a tool in educational institutions and research laboratories for teaching and conducting experiments in biology, biochemistry, genetics, chemistry, agriculture, and physics.

References:

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- Fozouni et al. Amplification-free detection of SARS-CoV-2 with CRISPR-
5 Cas13a and mobile phone microscopy. *Cell.* 2021 21;184(2):323-333.e9;
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Claims

1. A portable device for detecting and/or quantifying a target molecule in a sample, the device comprising:
 - 5 a sample chamber comprising an outer shell and a rotor for receiving the sample comprising the target molecule;
 - a plurality of red, green, blue (RGB) LEDs for generating illumination and inducing an optical signal of the target molecule;
 - a plurality of RGB sensors for detecting the optical signal of the target molecule;
 - and
 - 10 a microcontroller communicatively coupled to the RGB sensors, for connecting to an external device and transferring data;
 - wherein integrated LED of the RGB sensor has been removed.
2. The device of claim 1, wherein the RGB sensor is selected from the group comprising TCS230, AS73211, TCS3200, TCS3400, TCS34715, TCS34727, colorPAL, SEN-11195, EV3, GY-33, VCNL4010, VCNL4040, MAX44009, ISL29125, BH1745, APDS-9960, AS7262, and AS7263.
- 15 3. The device of claim 1 or 2, wherein the device comprises 2-10 RGB sensors, or more than 10 RGB sensors through the incorporation of a transistor switch.
4. The device of any one of claims 1-3, wherein the RGB sensor detects an optical signal selected from the group consisting of a fluorescent signal, a colorimetric signal, an absorbance signal, a luminescence signal, a bioluminescence signal, and a chemiluminescence signal.
- 20 5. The device of claim 4, wherein the optical signal is within the visible light range or the invisible light range.
- 25 6. The device of claim 1, wherein the RGB LED is selected from the group comprising gallium nitride (GaN), indium gallium nitride (InGaN), gallium phosphide (GaP), and gallium arsenic (GaAs), wherein the RGB LED generates illumination with a wavelength of 300–1100 nm.
7. The device of claim 6, wherein the RGB LED is positioned without a filter between the RGB LED and the sample.
- 30 8. The device of claim 6, wherein the RGB LED is positioned with a filter between the RGB LED and the sample.
9. The device of claim 6, wherein the RGB LED is positioned at different locations and distances to the sample.
- 35 10. The device of any one of claims 1-9, wherein number of the RGB sensor is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more.

11. The device of any one of claims 1-10, wherein the ratio of the number of the RGB sensor and the RGB LED is 1:1 or 1:2.
12. The device of claim 1, wherein the microcontroller is selected from the group comprising a single-board Arduino Uno microcontroller (Arduino), ARM, AVR, 8051, PIC, AVR, XMEGA, SAM, MSP430, AT MEGA, NXP, STM, LPC, GD, STM8 and STM32.
13. The device of claim 12, wherein the microcontroller comprises a Bluetooth module selected from the group comprising SPP-C, SPP2-0, CC2541, BT04-A, BK3231, BC417, CH-05, CH-08, CH-06, CH-10C, CH-07, CH-09, NRF52832, NRF51822, JDY-09, CC2541, HM-19, and HM-17 for wireless communication with the external device, or a portal for cable connection with the external device.
14. The device of claim 13, wherein the external device is selected from the group comprising a smartphone, a tablet, a computer, a server or a cloud platform.
15. The device of claim 1, further comprising a thermal cycler thermally coupled to the sample chamber, wherein the thermal cycler comprises a heating element selected from the group comprising ceramic, carbon, iron, steel, glass, strip, silicon rubber, and belt, and a fan selected from the group comprising axial, centrifugal, tangential, and composite fan.
16. The device of claim 15, wherein the microcontroller comprises a temperature sensor and a microcontroller program, being associated with the thermal cycler and configured to control a temperature profile of the sample chamber to perform a reaction.
17. The device of claim 16, wherein the temperature profile is 1-100 °C, preferably 10-90 °C.
18. The device of any one of claims 15-17, wherein the thermal cycler is suitable for use with a Taq polymerase.
19. The device of any one of claims 15-17, wherein the thermal cycler is suitable for use with an isothermal enzymes suitable for a reaction selected from the group comprising recombinase polymerase amplification (RPA), loop-mediated isothermal amplification (LAMP), Rolling Circle Amplification (RCA), Multiple Displacement Amplification (MDA), Transcription-mediated Amplification (TMA), Nucleic Acid Sequence-Based Amplification (NASBA), Ligase Chain Reaction (LCR), and Helicase-dependent Amplification (HDA).
20. The device of claim 1, wherein the rotor has a capacity to receive 1-200 samples, preferably 1-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-150, or 150-200 samples.
21. The device of claim 1, wherein the device has a capacity of 1, 2, 3, or 4 rotors.
22. The device of claim 1, further comprising a motor and a speed control for rotating the rotor.

23. The device of claim 1, wherein the outer shell has a body made of a material selected from the group comprising aluminum, plastic, polycarbonate, steel, ABS, PLA, PET, PETT, Nylon, PVA, HIPS, PA, HDPE, POM, PP, TPU, TPE, PMMA, PVC, and PEEK.
- 5 24. The device of claim 1, wherein the rotor has a body made of a material selected from the group comprising aluminum, plastic, polycarbonate, and steel, ABS, PLA, PET, PETT, Nylon, PVA, and HIPS.
25. The device of claim 1, wherein the device is operatable with a battery selected from the group comprising cadmium battery, Lithium polymer battery, Lithium ion battery, 10 Nickel-metal hydride battery, Nickel-cadmium battery, Mercury battery, Zinc-carbon battery, and Silver oxide battery.
26. The device of claim 1, wherein microcontroller is programmed for fault detection to automatically monitor and identify faults.
- 15 27. The device of claim 26, further comprising a LCD screen for displaying results and errors, the LCD screen being selected from the group comprising character, graphic, TFT, OLED LCD, PLS, LTPS, IGZO, AMOLED, and RETINA.
28. The device of claim 1, wherein the sample is a biological, chemical or environmental sample.
- 20 29. The device of claim 28, wherein the biological sample is obtained from a tissue sample, saliva, blood, plasma, sera, stool, urine, sputum, mucous, lymph, synovial fluid, cerebrospinal fluid, ascites, pleural effusion, seroma, pus, or swab of skin or a mucosal membrane surface or a combination thereof.
- 25 30. The device of claim 28, wherein the chemical sample is obtained from a pharmaceutical substance, a chemical reagent, a cosmetic product, an industrial effluent, a pollutant, a hazardous material, a polymer, an adhesive, a solvent, an oil, a gas, a metal alloy, a nanomaterial, a fuel, a cleaning agent, a dye, or a combination thereof.
- 30 31. The device of claim 28, wherein the environmental sample is obtained from a food, a beverage, a paper surface, a fabric surface, a metal surface, a wood surface, a plastic surface, a soil sample, a freshwater sample, a waste water sample, a saline water sample, exposure to atmospheric air or other gas sample, or a combination thereof.
32. The device of claim 28, wherein the biological, chemical or environmental sample is a crude sample and/or wherein the target molecule is not purified or amplified from the sample.
- 35 33. The device of any one of claims 28-31, wherein the sample contains a microbe selected from a bacteria, a virus, an archaea, a protozoa, a fungi, an algae, a slime mode, a lichen, and a prion.

34. The device of claim 33, wherein the virus is selected from a group consisting of a double-stranded DNA virus, a single-stranded DNA virus, a double-stranded RNA virus, a positive sense RNA virus, a negative sense RNA virus, and a retrovirus.
35. The device of claim 34, wherein the virus is selected from a group consisting of a coronavirus, an Ebola virus, measles, SARS, Chikungunya virus, Marburg, MERS, Dengue, Lassa, influenza, rhabdovirus, HIV, a hepatitis virus (including hepatitis A, B, C, D, or E), an influenza virus (including an influenza A or influenza B), a human respiratory syncytial virus, Sudan ebola virus, Bundibugyo virus, Tai Forest ebola virus, Reston ebola virus, Achimota virus, Aedes flavivirus, Aguacate virus, Akabane virus, Alethinophid reptarenavirus, Allpahuayo mammarenavirus, Amapari mammarenavirus, Andes virus, Apoi virus, Aravan virus, Aroa virus, Arumwot virus, Atlantic salmon paramyxovirus, Australian bat lyssavirus, Avian bomavirus, Avian metapneumovirus, Avian paramyxoviruses, penguin or Falkland Islands virus, BK polyomavirus, Bagaza virus, Banna virus, Bat herpesvirus, Bat sapovirus, Bear Canon mammarenavirus, Beilong virus, Betacoronavirus, Betapapillomavirus 1-6, Bhanja virus, Bokeloh bat lyssavirus, Boma disease virus, Bourbon virus, Bovine hepatitis virus, Bovine parainfluenza virus 3, Bovine respiratory syncytial virus, Brazoran virus, Bunyamwera virus, Caliciviridae virus, California encephalitis virus, Candiru virus, Canine distemper virus, Canine pneumovirus, Cedar virus, Cell fusing agent virus, Cetacean morbillivirus, Chandipura virus, Chaoyang virus, Chapare mammarenavirus, Colobus monkey papillomavirus, Colorado tick fever virus, Cowpox virus, Crimean-Congo hemorrhagic fever virus, Culex flavivirus, Cupixi mammarenavirus, Dengue virus, Dobrava-Belgrade virus, Donggang virus, Dugbe virus, Duvenhage virus, Eastern equine encephalitis virus, Entebbe bat virus, Enterovirus A-D, European bat lyssavirus 1-2, Eyach virus, Feline morbillivirus, Ferde- Lance paramyxovirus, Fitzroy River virus, Flaviviridae virus, Flexal mammarenavirus, GB virus C, Gairo virus, Gemycircularvirus, Goose paramyxovirus SF02, Great Island virus, Guanarito mammarenavirus, Hantaan virus, Hantavirus Z10, Heartland virus, Hendra virus, Hepatitis A/B/C/E, Hepatitis delta virus, Human bocavirus, Human coronavirus, Human endogenous retrovirus K, Human enteric coronavirus, Human genital-associated circular DNA virus- 1, Human herpesvirus 1-8, Human mastadenovirus A-G, Human papillomavirus, Human parainfluenza virus 1-4, Human paraechovirus, Human picornavirus, Human smacovirus, Ikoma lyssavirus, Ilheus virus, Influenza A-C, Ippy mammarenavirus, Irkut virus, J-virus, JC polyomavirus, Japanese encephalitis virus, Junin mammarenavirus, KI polyomavirus, Kadipiro virus, Kamiti River virus, Kedougou virus, Khujand virus, Kokobera virus, Kyasanur forest disease virus, Lagos bat virus, Langat virus, Lassa mammarenavirus, Latino mammarenavirus, Leopards Hill virus, Liao ning virus, Ljungan virus, Lloviu virus, Louping ill virus, Lujo mammarenavirus, Luna mammarenavirus, Lunk virus, Lymphocytic choriomeningitis mammarenavirus, Lyssavirus Ozemoe, MSS12Y225 virus, Machupo mammarenavirus, Mamastrovirus 1, Manzanilla virus, Mapuera virus, Marburg virus, Mayaro virus, Measles virus, Menangle virus, Mercadeo virus, Merkel cell polyomavirus, Middle East respiratory syndrome coronavirus, Mobala mammarenavirus, Modoc virus, Mojiang virus, Mokolo virus, Monkeypox virus, Montana myotis leukoencephalitis virus, Mopeia lassa virus reassortant 29, Mopeia mammarenavirus, Morogoro virus, Mossman virus, Mumps virus, Murine pneumonia virus, Murray Valley encephalitis virus, Nariva

- virus, Newcastle disease virus, Nipah virus, Norwalk virus, Norway rat hepatitis virus, Ntaya virus, O'nyong-nyong virus, Oliveros mammarenavirus, Omsk hemorrhagic fever virus, Oropouche virus, Parainfluenza virus 5, Parana mammarenavirus, Parramatta River virus, Peste-des-petits-ruminants virus, Pichande mammarenavirus, Picomaviridae virus, Pirital mammarenavirus, Piscihpevirus A, Porcine parainfluenza virus 1, porcine rubulavirus, Powassan virus, Primate T-lymphotropic virus 1-2, Primate erythroparvovirus 1, Punta Toro virus, Puumala virus, Quang Binh virus, Rabies virus, Razdan virus, Reptile bornavirus 1, Rhinovirus A-B, Rift Valley fever virus, Rinderpest virus, Rio Bravo virus, Rodent Torque Teno virus, Rodent hepatitis virus, Ross River virus, Rotavirus A-I, Royal Farm virus, Rubella virus, Sabia mammarenavirus, Salem virus, Sandfly fever Naples virus, Sandfly fever Sicilian virus, Sapporo virus, Sathuperi virus, Seal anellovirus, Semliki Forest virus, Sendai virus, Seoul virus, Sepik virus, Severe acute respiratory syndrome-related coronavirus, Severe fever with thrombocytopenia syndrome virus, Shamonda virus, Shimon bat virus, Shuni virus, Simbu virus, Simian torque teno virus, Simian virus 40-41, Sin Nombre virus, Sindbis virus, Small anellovirus, Sosuga virus, Spanish goat encephalitis virus, Spondweni virus, St. Louis encephalitis virus, Sunshine virus, TTV-like mini virus, Tacaribe mammarenavirus, Taila virus, Tamana bat virus, Tamiami mammarenavirus, Tembusu virus, Thogoto virus, Thottapalayam virus, Tick-borne encephalitis virus, Tioman virus, Togaviridae virus, Torque teno canis virus, Torque teno douroucouli virus, Torque teno felis virus, Torque teno midi virus, Torque teno sus virus, Torque teno tamarin virus, Torque teno virus, Torque teno zalophus virus, Tuhoko virus, Tula virus, Tupaia paramyxovirus, Usutu virus, Uukuniemi virus, Vaccinia virus, Variola virus, Venezuelan Vesicular stomatitis Indiana virus, WU Polyomavirus, Wesselsbron virus, West Caucasian bat virus, West Nile virus, Western equine encephalitis virus, Whitewater Arroyo mammarenavirus, Yellow fever virus, Yokose virus, Yug Bogdanovac virus, Zaire ebolavirus, Zika virus, or Zygosaccharomyces bailii virus Z viral sequence, Canine Parvovirus, Canine Distemper Virus, Canine Coronavirus, Canine Influenza Virus, Feline Herpesvirus, Feline Calicivirus, Feline Leukemia Virus, Feline Immunodeficiency Virus, Canine Adenovirus - Type 1 and Type 2, Canine Herpesvirus, Tobacco Mosaic Virus, Potato Virus Y, Tomato Spotted Wilt Virus, Cucumber Mosaic Virus, Potato Virus X, Barley Yellow Dwarf Virus, Bean Common Mosaic Virus, Maize Dwarf Mosaic Virus, Soybean Mosaic Virus, Citrus Tristeza Virus, Rice Yellow Mottle Virus, Plum Pox Virus, Turnip Mosaic Virus, Aphid-Transmitted Yellow Dwarf Viruses, Cotton Leaf Curl Virus, Papaya Ringspot Virus, Cassava Mosaic Virus, Rice Tungro Virus, Apple Mosaic Virus, Sugarcane Mosaic Virus or a combination thereof.
36. The device of claim 35, wherein the virus is a coronavirus.
37. The device of claim 36, wherein the coronavirus is SARS-CoV-2.
38. The device of claim 33, wherein the bacteria is a Gram-positive bacterium or a Gram-negative bacterium selected from the group consisting of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Bacillus anthracis*, *Bacillus cereus*, *Clostridium botulinum*, *Clostridium perfringens*, *Clostridium difficile* and *Clostridium tetani*, *Corynebacterium diphtheriae*, *Listeria monocytogenes*, *Escherichia*

- coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Chlamydia trachomatis*, *Yersinia pestis*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Moraxella catarrhalis*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Proteus mirabilis*, *Enterobacter cloacae*, *Serratia marcescens*,
5 *Helicobacter pylori*, *Salmonella enteritidis*, *Salmonella typhi*, and *Vibrio cholera*, *Brucella abortus*, *Brucella melitensis*, *Brucella suis*, *Brucella canis*, *Brucella ovis*, *Brucella neotomae*, *Mycoplasma mycoides*, *Clostridium perfringens*, *Mycobacterium avium* *Salmonella* spp., *Pasteurella multocida*, *Haemophilus parasuis*, *Actinobacillus pleuropneumoniae*, *Yersinia pestis*, *Francisella tularensis*, *Bacillus anthracis*,
10 *Coxiella burnetii*, *Listeria monocytogenes*, *Clostridium chauvoei*, *Campylobacter* spp., *Chlamydia psittaci*, *Anaplasma marginale*, *Leptospira* spp. Causes leptospirosis, *Bordetella bronchiseptica*, *Clostridium perfringens*, *Staphylococcus intermedius*, *Bartonella henselae*, *Chlamydophila felis*, *Mycoplasma* spp., *Pasteurella multocida*, *Erwinia amylovora*, *Xanthomonas* spp., *Xanthomonas axonopodis*,
15 *Ralstonia solanacearum*, *Pectobacterium* and *Dickeya* spp., *Agrobacterium tumefaciens*, *Pseudomonas syringae*, *Pseudomonas syringae* pv. *lachrymans*, *Xanthomonas campestris* pv. *campestris*, *Xanthomonas oryzae*, *Clavibacter michiganensis*, *Xylella fastidiosa*, *Pantoea* spp., *Burkholderia glumae*, *Xanthomonas citri*, *Xanthomonas translucens*, *Xanthomonas fragariae*, *Xanthomonas hortorum*,
20 *Clavibacter michiganensis*, *Pseudomonas syringae* or a combination thereof.
39. The device of claim 1, wherein the target molecule is selected from the group consisting of a nucleotide, a polynucleotide, a nucleoside, a nucleic acid, a nucleic acid analogue, a peptide, a polypeptide, a protein, an antibody, a hormone, a salt, a metal, and a compound that produces color in a solvent.
- 25 40. The device of claim 39, wherein the nucleic acid is a RNA or a DNA from a virus of any of claims 34-37, or a RNA or a DNA from a bacteria of claim 38.
41. The device of claim 39 or 40, wherein the device is for detecting 2-10 target molecules from a sample in a single tube, wherein the target molecule is selected from the group comprising a nucleic acid, an antigen, and an antibody.
- 30 42. The device of claim 39 or 40, wherein the device is for detecting 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more target molecules from a sample in a single tube, wherein the target molecule is selected from the group comprising a nucleic acid, an antigen, and an antibody.
- 35 43. The device of claim 1, wherein the device is suitable or configured for one or more of amplification of nucleic acids including analysis of polymerase chain reactions including quantitative polymerase chain reactions, reverse transcription polymerase chain reaction, protein analysis, ligand analysis, fluorescence analysis of chemical reactions, or fluorescence analysis in the presence of particles, polymers, hydrogels, and nanostructures, biomolecule interactions, detection of specific nucleic acid
40 sequences, fluorescence protein analysis, bioluminescence enzymatic reactions, and chemiluminescence reactions.
44. The device of claim 43, wherein the reverse transcription polymerase chain reaction is for protein analysis, ligand analysis, biomolecule interactions, detection of specific

nucleic acid sequences, fluorescence protein analysis, bioluminescence enzymatic reactions, chemiluminescence reactions, fluorescence analysis of chemical reactions or fluorescence analysis in the presence of particles, polymers, hydrogels, and nanostructures.

- 5 45. The device of claim 1, wherein the device is suitable or configured for clustered regularly interspaced short palindromic repeats and CRISPR associated protein (CRISPR-cas)- based nucleic acid, antigen, antibody, and aptamer or chemical detection.
- 10 46. The device of claim 45, wherein the CRISPR based nucleic acid detection uses an effector complex comprising a Cas protein and at least one CRISPR RNA (crRNA) capable of specifically binding to a target nucleic acid molecule.
47. The device of claim 46, wherein the Cas protein is selected from the group consisting of Cas 9, Cas12a, Cas12b, Cas12c, Cas12d, Cas13a, Cas13b, Cas13c, Cas13d, Cas12e, and Cas14.
- 15 48. A method of detecting and/or quantifying a target molecule in a sample, using the device of any one of claims 1-47, the method comprising:
- providing the sample comprising the target molecule in the rotor;
- generating illumination and inducing the optical signal of the target molecule by the plurality of RGB LEDs;
- 20 detecting the optical signal of the target molecule by the plurality of RGB sensors; and
- transferring the data to the external device by the microcontroller.
49. The method of claim 48, wherein the method is performed in one hour or less.
- 25 50. A method of monitoring viral or bacterial disease outbreaks and/or evolution, comprising performing a method as in any one of claims 48-49.