Supplementary Material

Real-time colorimetric LAMP methodology for quantitative nucleic acids detection at the point-of-care

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In-house developed Android application

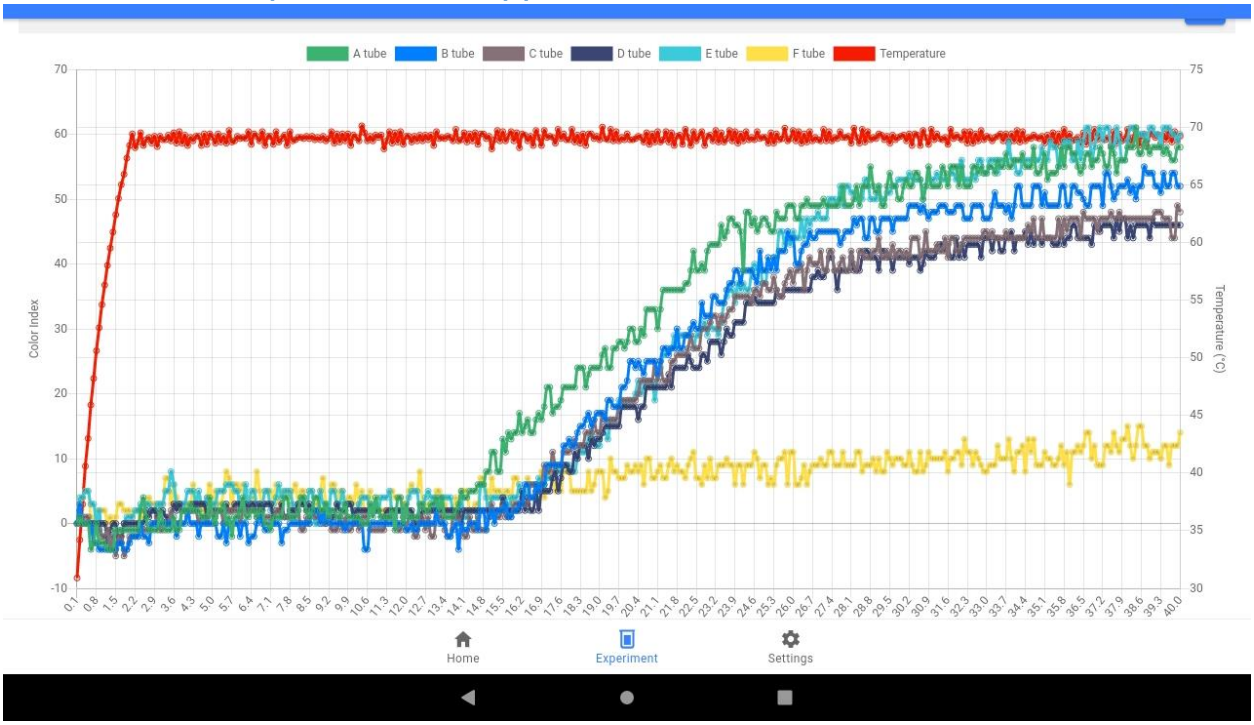


Fig. S1: Screenshot of the in-house developed Android application. The settings to be adjusted include the temperature, run time, type of dye, time interval for capturing images and option for USB storage.

Digital image analysis

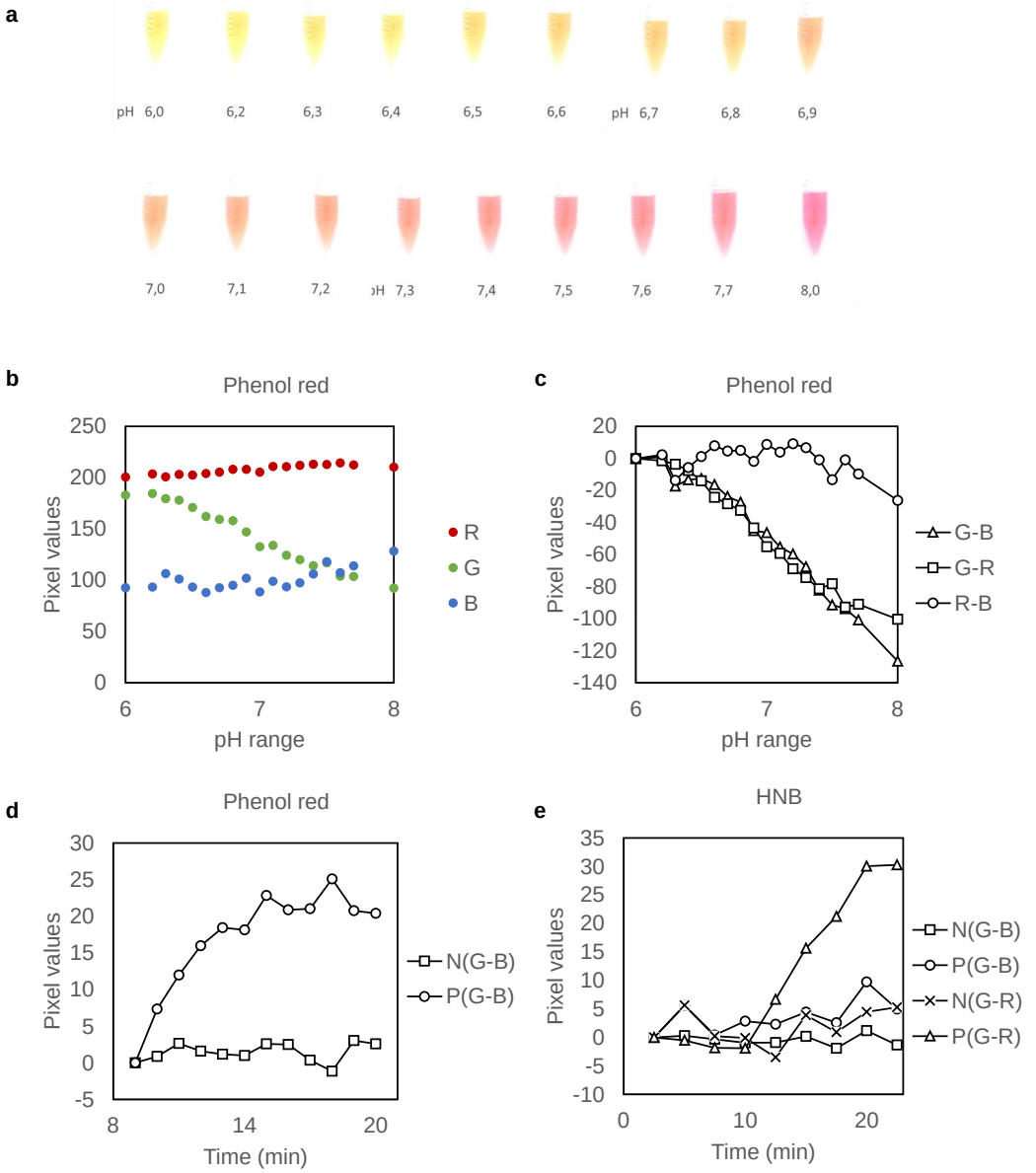


Fig. S2: (a) Series of images corresponding to different pH values between pH 6 and 8 in respect to the phenol red indicator. Image source: https://www.testallcolour.com/blog/post/what-is-phenol-red-in-swimming-pools/. (b) Raw pixel values extracted from several images (see a) correlating different pH values to color change using the phenol red pH indicator. (c) phenol red: change in pixels as function of pH using data from figure S2b and following three formulas; Green-Blue (G-B), Green-Red (G-R) and Red-Blue (R-B). (d) Change in pixels of phenol red based LAMP reactions spiked with 0 (N) and 10^5 (P) lysed bacteria by applying the Green-Blue formula. The first 8 minutes were omitted. Reactions took place in a pre-warmed oven at 63oC with a glass door that allowed video capturing with a camera placed outside the door. (e) Change in pixels of HNB based LAMP reactions spiked with 0 (N) and 10^5 (P) bacteria by applying the Green-Blue and Green-Red formulas. With the HNB indicator, the Green-Red formula resulted in better discrimination (Fig. S2e); this could be explained by the fact that the purple to sky blue transition involved more prominent changes in the green and red than in the blue channel.

Performance evaluation

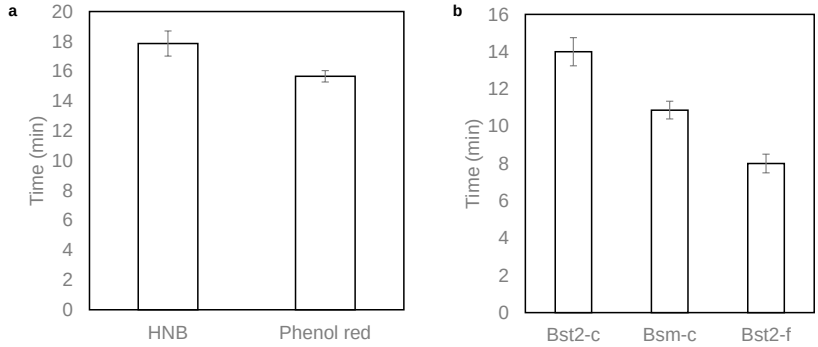


Fig. S3: (a) Variation in the time point (min) in which LAMP preparations (containing the same amount of starting template but different color indicator) show a change in the slope of the real-time colorimetric curve when changing the position of the tube inside the tubes holder (see Fig. 1b). Each bar is the average of 3 replicates at 2 different slots in the holder (total of 6 measurements). (b) Comparison of the speed of detection of a LAMP reaction containing 10 bacteria as starting template using different combinations of 2 enzymes (Bst2, Bsm), 2 colorimetric indicators (HNB, phenol red) and inside 2 real time systems (qcLAMP device, BIORAD). Bst2-c: Bst2 warm start polymerase mixed with either phenol red or HNB, tested with qcLAMP; Bsm-c: Bsm polymerase (20 Units) with HNB, tested with qcLAMP; Bst2-f: Bst2 warm start with LAMP fluorescent dye tested in a real-time PCR machine.

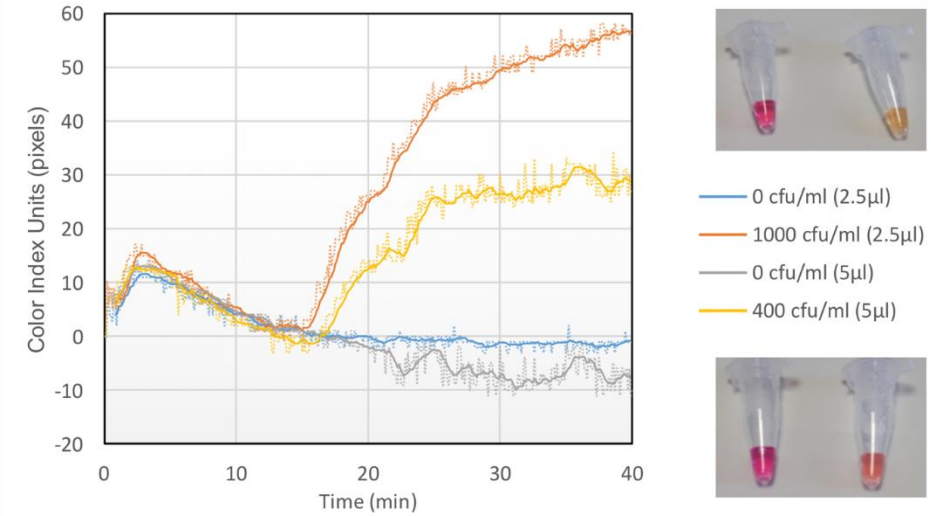


Fig. S4: Left: Real time colorimetric curves monitored during qcLAMP amplification performed with 2 different bacteria concentrations (400 and 1000 CFU/ml) spiked in saliva samples. The two concentrations were chosen based on previously reported detection limits of bacteria using end-point colorimetric LAMP or biosensors[1, 2]. Both were successfully detected in less than 17 min while zero background signal was monitored for the negative controls. Right, top: Picture of end-point reactions with 0 or 1000 CFU/ml after 40 min of incubation. Right bottom: Picture of end-point reactions with 0 or 400 CFU/ml after 40 min of incubation.

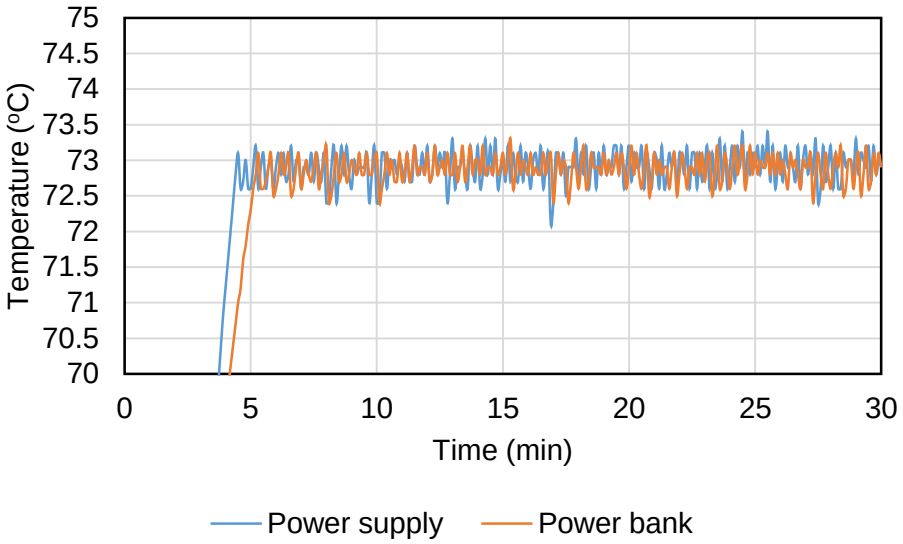
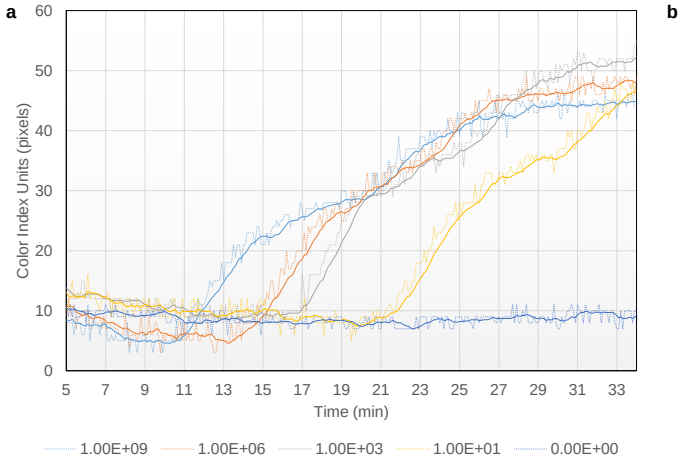


Fig. S5: Temperature stability during operation of the qcLAMP device with a power bank and in comparison to a standard power supply.

qcLAMP curves for Influenza/SARS-CoV-2



b

NUMBER OF SAMPLE	TIME-TO-POSITIVE (MIN)
1	9.7
2	10.2
3	10.9
4	10.1
5	11.7
6	10.2
7	9.4
8	10.5
9	10.4
10	10.1
11	9.2
12	10.6
13	10.6
14	10.2
15	9.9
16	10.2
17	9.6
18	9.9
19	9.7
20	10.5
21	10.7
AVARAGE TIME (MIN)	10.2
STANDARD DEVIATION (min)	0.6

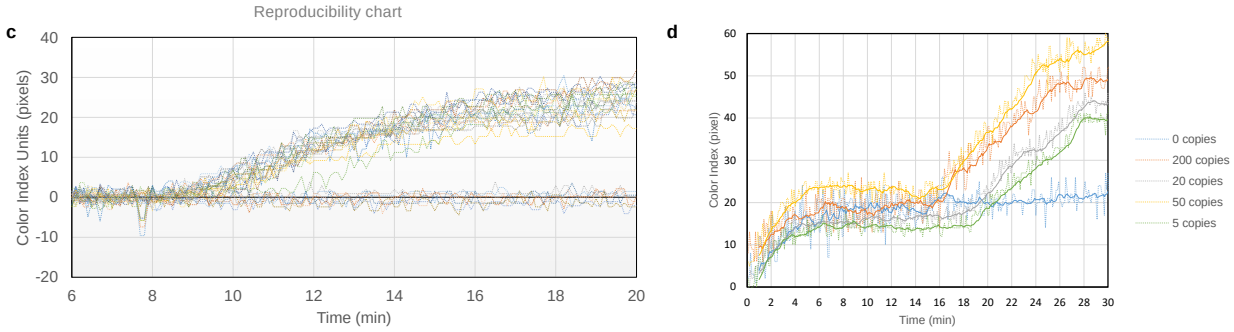


Fig S6: (a) Typical real time colorimetric LAMP curves for Influenza A. (b) Average time-to-positive for 21 positive samples with the same initial target concentration (10^9 copies/reaction). (c) Real-time curves of 28 samples (21 positive, 7 negative). (d) Real time curves for 0 to 200 copies of SARS-CoV-2 synthetic RNA.

Electronics design and smartphone app development

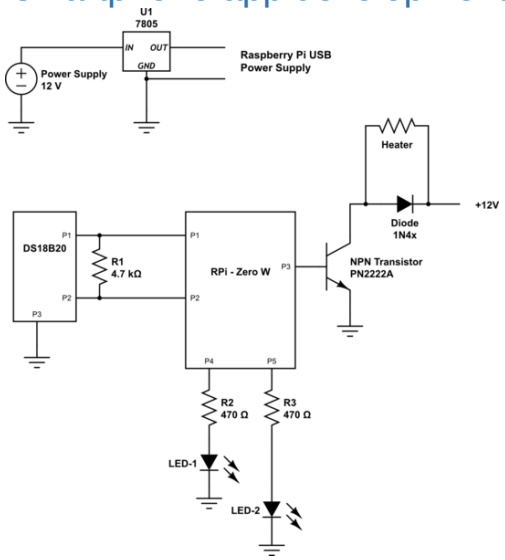


Fig. S7: Schematic representation of the custom PCB RPi Zero W Hat for controlling temperature sensor (DS18B20), Heating element, and LEDs.

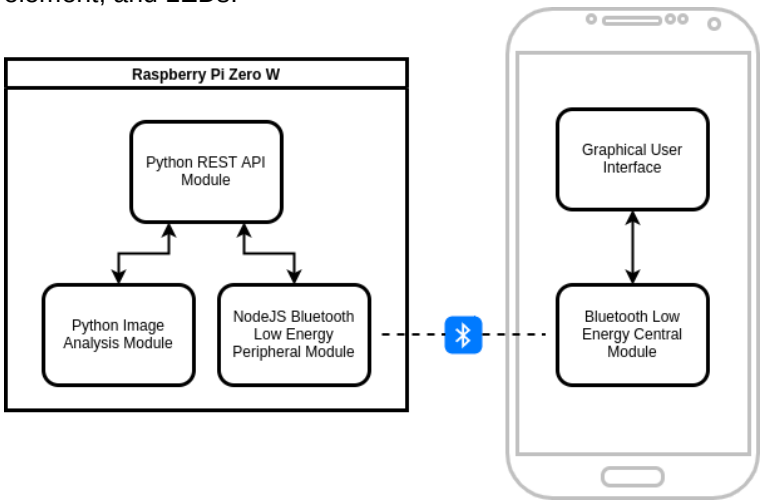


Fig. S8: Systems' architecture at software layer.

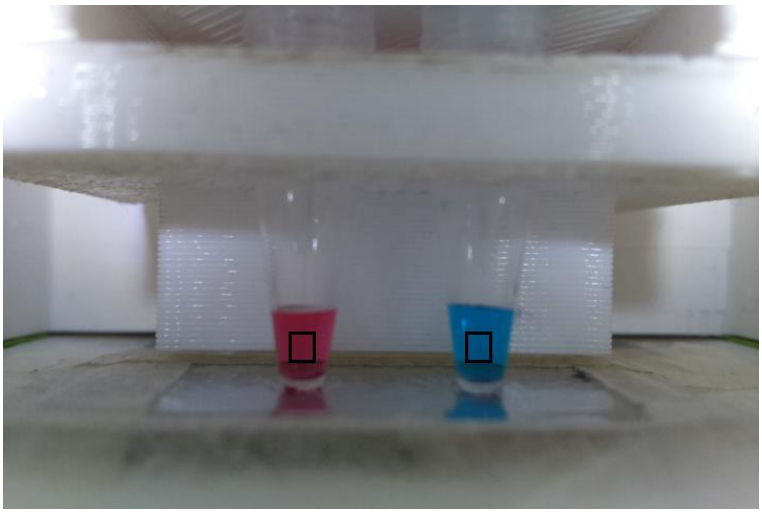


Fig. S9: Snapshot of the reaction tubes in the qcLAMP device. The black rectangles correspond to the analysis area.

References

1. Papadakis, G. et al. 3D-printed Point-of-Care Platform for Genetic Testing of Infectious Diseases Directly in Human Samples Using Acoustic Sensors and a Smartphone. *ACS Sens* **4**, 1329-1336 (2019).
2. Hsieh, K.W., Patterson, A.S., Ferguson, B.S., Plaxco, K.W. & Soh, H.T. Rapid, Sensitive, and Quantitative Detection of Pathogenic DNA at the Point of Care through Microfluidic Electrochemical Quantitative Loop-Mediated Isothermal Amplification. *Angew Chem Int Edit* **51**, 4896-4900 (2012).