

How to Cite:

Iqbal, F., Asif, M. S., Qureshi, A. G., Shah, J. A., Abdikaxarovich, S. A., Adil, M. N., & Hussain, A. (2023). RPA-Based colorimetric detection of SARS-Cov-2 (Covid-19) and its physiological effects. *International Journal of Health Sciences*, 6(S7), 6804–6818.
<https://doi.org/10.53730/ijhs.v6nS7.13862>

RPA-Based colorimetric detection of SARS-Cov-2 (Covid-19) and its physiological effects

Faisal Iqbal

Department of biological Sciences, International Islamic University, Islamabad, Pakistan; ORCID: 0000-0001-7596-9180.

Mohammad Shahzad Asif

Bachelor of Medicine and Bachelor of Surgery, University College of Medicine and Dentistry North Canton, Ohio, USA.

Aniqa Gulfam Qureshi

Bachelor of Medicine and Bachelor of Surgery, Jiggangshan University, China.

***Junaid Ali Shah**

College of Life Sciences, Jilin University, Changchun 130012, China; Fergana medical institute of public health Uzbekistan, Fergana 150110, Uzbekistan.

*Corresponding author email: junaid1316@mails.jlu.edu.cn

Sidikov Akmal Abdikaxarovich

Rector, Fergana medical institute of public health Uzbekistan, Fergana 150110, Uzbekistan.

Muhammad Naveed Adil

Department of Animal Genomics & Biotechnology, PIASA, Quaid-e-Azam International University Islamabad Pakistan.

Abrar Hussain

Department of Biological Sciences, International Islamic University Islamabad, Pakistan.

Abstract--The SARS-CoV-2 coronavirus outbreak is extremely concerning and poses a threat to the public health system. SARS-CoV-2 is a pathogen that affects people and caused fever, dry cough, dizziness and severe respiratory disease. By media time, greater than 662 million people were infected and 6.6 million people were died globally. The pandemic coronavirus was hurt the third world countries due to weak health infrastructure. A timely response is crucial for commercially and easily accessible resources since an actual pandemic emergency does not provide the necessary timescale for the test of innovative ways. Coronavirus SARS-CoV-2 was infected

individuals without any indication and could still transfer the virus to others. The success of the quarantine effort during the SARS-CoV-2 eruption depended heavily on the identification of the infectious agent. It is thus urgent to develop a rapid and accurate detection method for coronavirus SARS-CoV-2 and control the disease spreading. Here demonstrated an isothermal based SARS-CoV-2 amplification with quick colorimetric detection. The inorganic phosphate (Pi) was detected via colorimetric technique from SARS-CoV-2 after amplification by using the basic recombinase polymerase amplification (RPA) technique. The accuracy and simplicity of this amplification and colorimetric detection strategy provide cost efficient and reliable alternative, particularly in resource limited labs. SARS-CoV-2 infected patient faced different physiological problems, here reported blood pressure and heart.

Keywords---Recombinase Polymerase Amplification, COVID-19, real time quantitative polymerase chain reaction, physiological problems,

Introduction

Millions of people around the world have been infected by the SARS-CoV-2 coronavirus. The developing countries were suffering with SARS-CoV-2 more as compared to developed countries and these countries have horrible condition because they have lack of funds and hurt the economy and pitiable setups for the pandemic SARS-CoV-2 virus detection. With quick blowout of SARS-CoV-2, there is a huge burden on clinic centers for identification of SARS-CoV-2 and some infected persons have no signs but virus still transfer to others. The physiological effects of SARS-CoV-2 virus damage the human health and given serious cause for concerns globally. Therefore, a critical need to develop a detection system that should be simple and no need of trained personnel, sensitive, quick output response and low cost.¹⁻⁵

The SARS-CoV-2 virus is caused serious respiratory infection. The molecular biology based instrument like real time quantitative polymerase chain reaction (RT-qPCR) is used to detect the SARS-CoV-2.⁶⁻⁸ This technique has been used by center for disease control (CDC) and other related department globally.⁹⁻¹⁵ The RT-qPCR gold standard was suggested by the center for disease control (CDC) and world health organization (WHO) for the detection of positive cases. Though, faith on real time quantitative polymerase chain reaction (RT-qPCR) frequently lead to dependence on centralized laboratory facilities for testing of SARS-CoV-2.¹⁶⁻²⁰ Now a days RT-qPCR based methodologies are developed and performed in the labs. SARS-CoV-2 physiologically effect the different part of the body and sometime it was cause for death. However, RT-qPCR has numerous limitation such as high need purity sample and reach to tremendously expensive laboratory instrumentation with long reaction time as well. In addition, RT-qPCR need skilled recruits and sophisticating amenities for sampling preparation, while this issue make practical application limited in many cases and thus can delay the required fast prescription.⁷⁻⁹

Here a molecular detection based approach was described, the recombinase polymerase amplification (RPA) was performed for SARS-CoV-2 and simple colorimetric detection of amplified product. A well-established, quick and trustable technique recombinase polymerase amplification (RPA) was used to amplify minor quantity of target sequence at a single temperature reaction, removing thermal cycling equipment. The isothermal amplification technique has permitted color examination with using the dyes like malachite green in the absence of costly instrument. This recombinase polymerase amplification (RPA) technique provide amplification in short time as compared to thermal cycler. The amplified sample of SARS-CoV-2 was used to trace out the inorganic phosphate (Pi). After RPA, SARS-CoV-2 amplified sample with pre addition of inorganic pyrophosphatase (PPase) as a catalyst to hydrolyze the pyrophosphate (PPi) into inorganic phosphate (Pi). The Pi and acidic molybdate was converted into phosphomolybdate complex and change the color in blue form. The benefits of this amplification and colorimetric detection system is simple, fast, low cost, and less used of bulky equipment to help the low resource lab settings.

Different physiological problems were reported during SARS-CoV-2 such as high blood pressure and heart rate. A weak immune system is less able to control the high blood pressure and fight against the coronavirus.

Materials and methods

RNA extraction

A SARS-CoV-2 positive samples were obtained according to the principles specified in the Helsinki declaration who were clinically diagnosed with SARS-CoV-2, total five sample were obtained. Venipuncture was used to obtain blood samples, which were then placed in heparin-containing VACUETTE® blood collection tubes. For RNA extraction, the serum from the blood samples was separated. Human SARS-CoV-2 viral RNA was extracted using the QIAamp viral mini kit.

Primer design for RPA amplification

Primers were designed ranging (product length 103 bp) from 487–589 bp of SARS-CoV-2 genome (GenBank consent no. >MT240479.1 Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/PAK/Gilgit1/2020) by using the NCBI software. The length of primer was 20 bp. The primers position were 5'-3', Forward primer, TGAGCTGGTAGCAGAACTCG (IDT, USA), ranging 487-506, Reverse primer, CTTGCGGTAAGCCACTGGTA (IDT, USA), ranging 589-570.

RT (Reverse transcription) Reaction

The 10 µl containing 5 µg of total RNA was heated at 65 °C for 5 to 10 minutes prior to performing the RT reaction, after which the mixture was cooled on ice. The following component was set up in a 1.5 ml eppendorf tube then 10.0 µl denatured RNA was warmth, 3.0 µl (10x) PCR buffer (Invitrogen), 2.5 µl (10mM) dNTPs (Biocompare), 6.0 µl (25mM) MgCl₂ (Sigma-Aldrich), 1.0 µl (1.5µg) random primers (Invitrogen), 0.5 µl (200U/ µl) SuperScript II reverse transcriptase

(Invitrogen) and 17.0 μl water (Sigma-Aldrich) was used for reverse transcription reaction. Leave the samples at 25°C for 10 minutes then for 1 hour it was incubated at 42°C. The cDNA was denatured at 95°C then it was placed on ice.

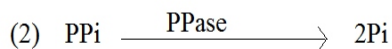
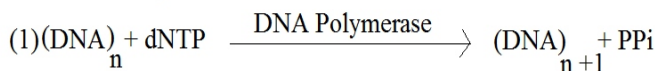
RPA assay

RPA is an isothermal amplification technique to execute the reaction at 37°C-42°C and it can be an alternative of PCR technique. A very less amount of chemical and instrument was used for RPA. A 5 μl cDNA product was used for RPA reaction. TwistAmp®Basic Kit (TwistDx™) was used for RPA reaction. The concentration of reverse primer was 2.4 μl (10 μM , IDT, USA) and forward primer was 2.4 μl (10 μM , IDT, USA), but the rehydration buffer was 29.5 μl , nuclease free water 12.2 μl (Sigma-Aldrich) and magnesium acetate was 2.5 μl (280 mM). Positive control was executed to test the activity of kit components. The positive control primer mix was 6 μl , rehydration buffer 29.5 μl , positive control template 10 μl and magnesium acetate was 2.5 μl (280 mM). The positive control template strand was 143 bp. Negative control was performed by using the nuclease free water replacement of cDNA with all other reagents. The RPA experimental reaction was performed on water bath (JOANLAB, China) at 42°C. This is one of the main benefit of RPA to perform it without using any expensive apparatus.

Inorganic Phosphate (Pi) based detection of SARS-CoV-2

SARS-CoV-2 amplified samples were used for inorganic phosphate (Pi) detection. The SARS-CoV-2 each sample 20 μl , positive control 20 μl and inorganic pyrophosphatase (NEB) was 0.2 μl (2 units) was used while colorimetric reagents such as potassium antimonyle tartrate 1.4 μl , ammonium molybdate 0.2 μl , sulfuric acid 0.8 μl and ascorbic acid 0.3 μl . The colorimetric reagents and amplified samples were mixed to make the phosphomolybdate complex in blue color. The sample solution alteration into blue color form means the presence of inorganic phosphate (Pi) in sample solution.

(A) Reaction equation



Phosphomolybdate complex

Figure 1: (A) Amplification reaction, it is presenting in chemical structural form, the working principle of tracing inorganic phosphate (Pi) for the amplification of

nucleic acid. The SARS-CoV-2 genomic DNA was released the pyrophosphate (PPi) as a byproduct during nucleotide base incorporation. (1) Reaction equation, DNA is presented the target template DNA. The hydrolysis and incorporation of nucleotide base result in the extension of DNA and release of PPi (2) Conversion of pyrophosphate (PPi) to inorganic phosphate (Pi) by using the catalyst inorganic pyrophosphatase (PPase). (3) Molybdate and inorganic phosphate (Pi) reaction for phosphomolybdate complex to change the sample solution color in blue form.

SARS-CoV-2 based physiological effects

SARS-CoV-2 effects different parts of the body and caused such as high blood pressure and heart rate was reported. The blood pressure and pulse pressure was measured with digital device (OMRON, BP7200).

Results

Gel electrophoresis

Gel electrophoresis was performed for the analysis and separation of DNA fragment size. The electric current was applied for the separation of DNA molecules. The movement of shorter molecules are fast while large molecules are slow in the gel electrophoresis.

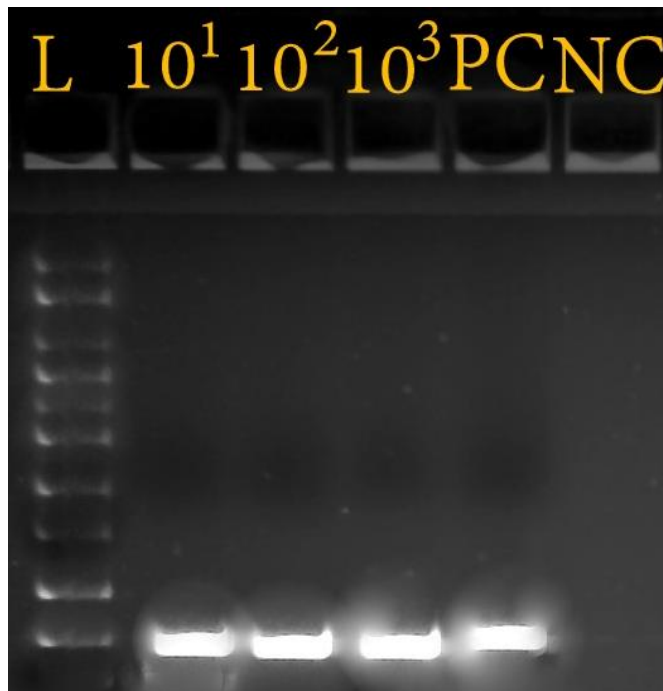


Figure 2: SARS-CoV-2 gel electrophoresis result. Recombinase polymerase amplification (RPA) based SARS-CoV-2 (>MT240479.1 Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/PAK/Gilgit1/2020) samples were executed on the gel electrophoresis for the analytical analysis purpose. L is a

DNA ladder (Protein Ark) and that was used in agarose gel electrophoresis for achieving different length form. It was used as a guide to calculate the size of SARS-CoV-2 DNA molecules, which were separated based on how they moved across a gel under an electrical field. The size of DNA ladder was 100 bp, while the SARS-CoV-2 amplified DNA samples were used as a serial dilution (10^1 , 10^2 and 10^3) form. The serial dilution (10^1 , 10^2 and 10^3) of SARS-CoV-2 amplified DNA samples were used 5 μ l each in gel electrophoresis. The positive control (PC) of TwistAmp[®]Basic Kit (TwistDx[™]) with 143 bp fragment size. The last one is negative control (NC) with no band was generated. The 1.5% gel was used for agarose gel electrophoresis.

Inorganic phosphate (Pi) colorimetric detection result

The isothermal amplification based SARS-CoV-2 genomic DNA was generated the pyrophosphate (PPi) as a byproduct during nucleotide base incorporation phenomena. The inorganic pyrophosphatase (PPase) was used as a catalyst to hydrolyze the pyrophosphate (PPi) into inorganic phosphate (Pi) by using the colorimetric method.

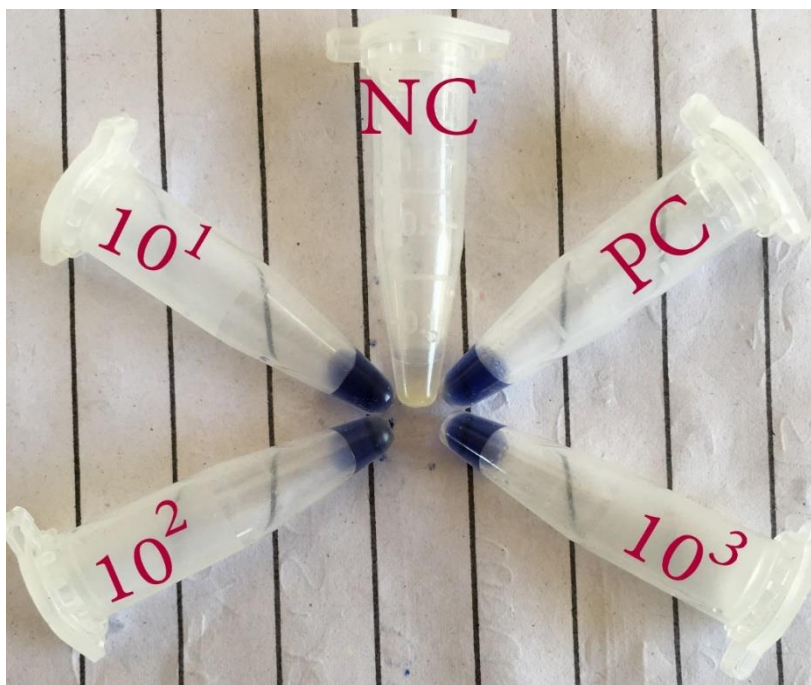


Figure 3: Inorganic phosphate (Pi) detection of SARS-CoV-2 genomic DNA. After amplification of SARS-CoV-2 genomic DNA, here described a simple approach for visual detection of inorganic phosphate (Pi). Throughout DNA amplification, complementary nucleotides were incrementally added to the developing strand.

The pyrophosphate (PPi) was released, catalyst inorganic pyrophosphatase (PPase) was used to convert the pyrophosphate (PPi) into inorganic phosphate (Pi) then Pi was reacted with ammonium molybdate in the presence of ascorbic acid,

antimonyle potassium tartrate and sulfuric acid to form a phosphomolybdate complex. A visual readout was achieved with naked eyes. The SARS-CoV-2 genomic DNA was amplified in serial dilution ($10^1, 10^2, 10^3$) form and PC is positive control. The presence of inorganic phosphate (Pi) in the sample solution was caused to change the color in blue form, while NC is negative control and showing no color it means absence of inorganic phosphate (Pi) in the solution.

SARS-CoV-2 effects on blood pressure and heart rate

Blood pressure and pulse pressure reading list

For 14 days, regular measures of the blood pressure and pulse rate were taken. The readings were measured two time (morning and night). The patient weight was decreased from 64 kg to 63.7 kg. Heart log app was installed to the iPhone 6s (Apple store) free of cost. Digital device (OMRON, BP7200) was used to measure the blood pressure and pulse rate. All the 14 days data (OMRON, BP7200) was used for heart log app for graphics.

Blood pressure List

day	Morning	Blood pressure	Night	Blood pressure	Check	Weight	Comment
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12	8:00	135/86/96	20:00	133/85/93		64.0	Vegetables, bread, butter and butter milk, chicken and rice. Stress
13	8:00	134/86/90	20:00	132/84/89		64.0	Vegetables, bread, butter and butter milk, chicken and rice. Feeling Stress
14	8:00	138/88/98	20:00	135/86/96		64.0	Vegetables and bread. Stomachache
15	8:00	138/88/95	20:00	135/88/95		63.9	Vegetables and bread. Slight cold
16	8:00	140/90/99	20:00	138/89/97		63.9	Vegetables and bread. Slight cold, dizziness, fever
17	8:00	140/90/99	20:00	138/88/98		63.8	Vegetables and bread. Slight cold, dizziness, body pain, headache, fever
18	8:00	133/86/92	20:00	131/86/90		63.7	Vegetables and bread. Slight cold, dizziness, body pain, headache
19	8:00	130/85/88	20:00	128/84/87		63.7	Mangos, vegetables and bread. Slight cold
20	8:00	127/82/80	20:00	125/80/80		63.7	Mangos, vegetables and bread.
21	8:00	123/81/74	20:00	121/80/73		63.7	Mangos, vegetables and bread.
22	8:00	121/81/69	20:00	120/80/68		63.7	Mangos, vegetables and bread. Feel so good
23	8:40	120/80/65	20:00	118/80/65		63.7	Mangos, vegetables and bread. Feel so good
24	8:00	118/79/60	20:00	118/79/60		63.7	Mangos, vegetables and bread. Feel so good
25	8:00	118/79/59	20:00	117/79/59		63.7	Mangos, vegetables and bread. Feel so good
26							
27							
28							
29							
30							
31							

Figure 4: Blood pressure and pulse rate reading list. Systolic pressure refers to the highest force the heart can generate when beating, it is first reading in the blood pressure. The second-to-last number represents the diastolic pressure, or the pressure in arteries between heartbeats, while the last one is pulse rate or heart rate, it is the quantity of heartbeats a person experiences in a minute. The blood pressure readings is measured in millimeters of mercury (mmHg).

Chart of blood pressure and heart rate

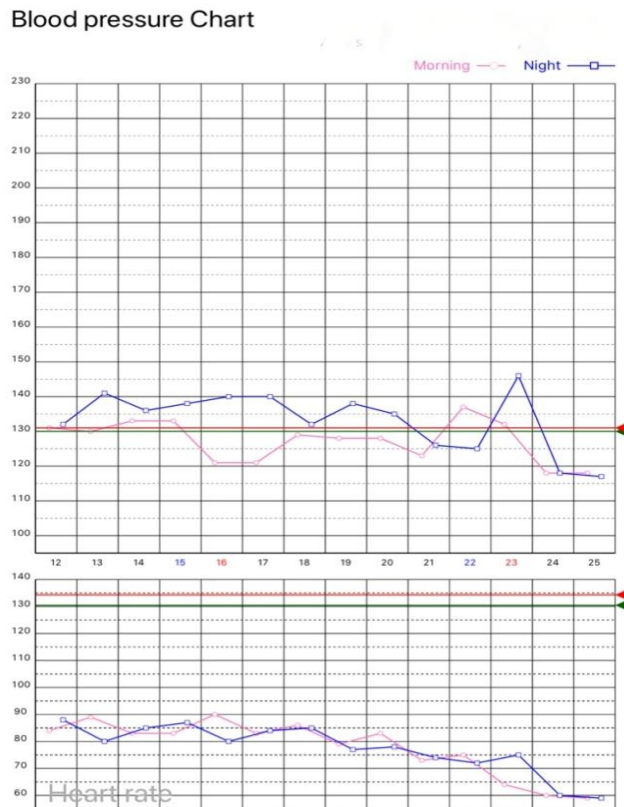


Figure 5: Blood pressure and heart rate chart. The two weeks blood pressure and heart rate chart during SARS-CoV-2 are described, the pink line are presenting morning and blue line are presenting night time data. The fluctuation of blood pressure and heart rate chart data is a sign that how coronavirus was effect the infected person.

Histogram chart

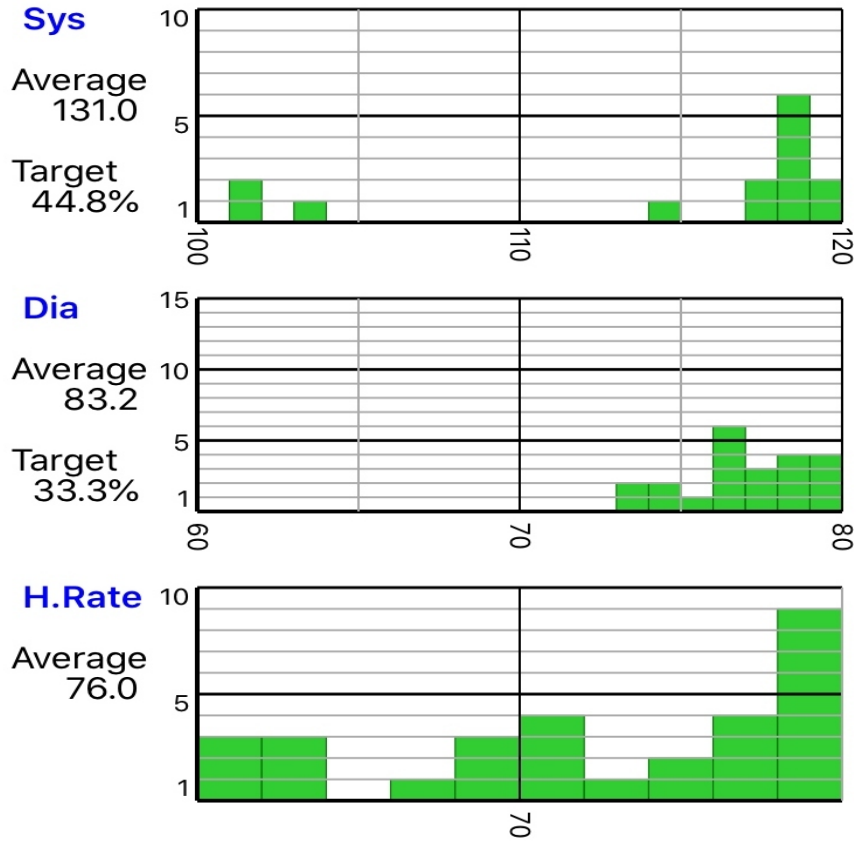


Figure 6: Histogram chart for blood pressure and heart rate. The histogram is a basically frequency distribution of a set of continues data. The two weeks data of systolic, diastolic and heart rate in histogram presented separately during SARS-CoV-2.

Pulse pressure

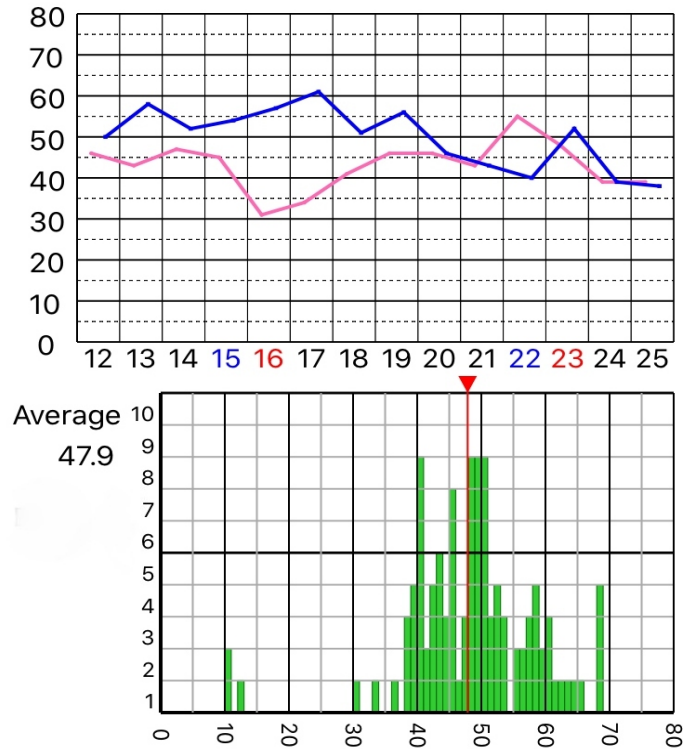


Figure 7: Pulse pressure. The chart is showing pulse pressure (PP), two weeks pulse pressure data presenting in two lines, the pink line is presenting morning and blue line is presenting the night based data of pulse pressure during SARS-CoV-2. While histogram is presenting the two weeks pulse pressure (PP) average data.

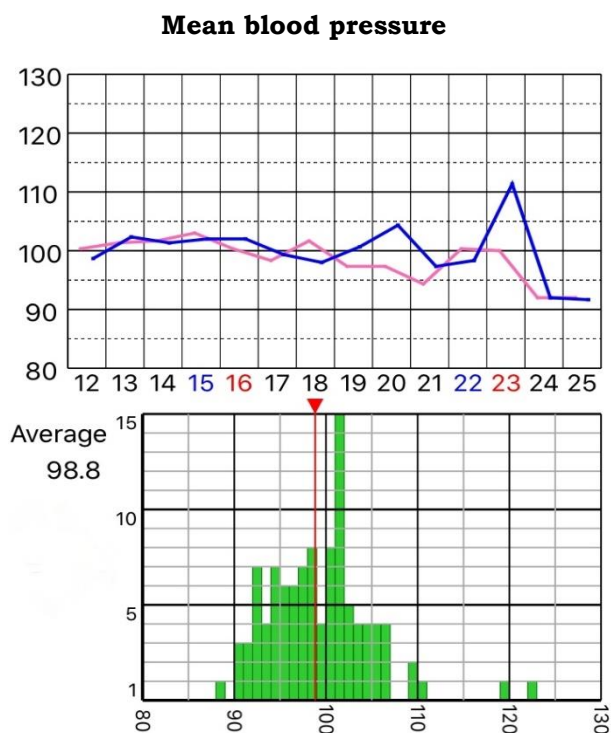


Figure 8: Mean blood pressure (MBP). The two weeks morning (pink line) and night (blue line) mean blood pressure (MBP) was calculated during SARS-CoV-2. While histogram presenting the average mean blood pressure (MBP).

Discussion

The difficulty of viral identification is of utmost relevance, particularly in developing countries and during emergencies. The availability of testing infrastructure based on RT-qPCR was acknowledged as a significant challenge globally in the context of the COVID-19 pandemic. Scientist are working around the globe day and night on COVID-19. Real-time quantitative polymerase chain reaction (RT-qPCR) techniques were recommended as the gold standard for the official detection of positive cases by the centers for disease control (CDC) and the world health organization (WHO). However, the reliance on centralized lab facilities for COVID-19 testing due to the use of real-time quantitative polymerase chain reaction (RT-qPCR) is common. The existing resources available for widespread COVID-19 testing by RT-qPCR would definitely not be enough in developing economies like Latin America, India, Pakistan, and African nations, so these nations are woefully lacking in the finance and bad health infrastructure of this pandemic infection. Clearly, these nations may not be able to set up enough centralized facilities to do quick, extensive COVID-19 testing. The COVID-19 RT-qPCR test currently takes 1 to 3 days to get diagnostic results, despite the terrible conditions in wealthy countries. The available PCR labs are overworked with samples, understaffed to run the tests, contending with instrumentation

backlogs, and dealing with challenging logistics to move fragile and infectious samples while maintaining the cold chain (Gonzalez et al., 2020).

Here demonstrated a very simple embodiment of RPA reaction, executed on the water bath for isothermal amplification and then further assisted to perform the colorimetric reaction by using the pyrophosphatase (PPase) for pyrophosphate (PPi), so it was used to enable the quick and highly accurate identification of SARS-CoV-2 amplified sample. The significance of these SARS-CoV-2 results in the context of pandemic COVID-19 does not lie in its novelty but rather in its applicability. Some cost considerations are made here, as the market value of conventional RT-qPCR apparatus is in the range of \$10,000 to \$40,000 USD, while on the other side a simple water bath price range is less than 100 USD, used for RPA. This difference is so much significant during pandemic or endemic crisis when rational investment of resources are very critical. While the RT-qPCR platform's quantitative capabilities are beyond dispute. Many countries have capability to fast, effective and massive established diagnostic center base on RT-qPCR is questionable. The pandemic experience in China, United States, England, Italy, Spain, Brazil, and Pakistan has blatantly shown that centralized labs were not the best option in times of emergency. RPA have 20 minute amplification time, flexible and fast colorimetric detection response that RT-qPCR platforms cannot deliver.

Here, a fundamentally novel method for visual detection of amplified SARS-CoV-2 genomic DNA after isothermal amplification is reported. The inorganic phosphate (Pi) was used to detect. Following the incorporation of complementary nucleotides into the developing strand during DNA polymerization, the two phosphates were released as pyrophosphate (PPi) as a byproduct with the addition of inorganic pyrophosphatase (PPase). Pi was subsequently reacted with ammonium molybdate in the presence of antimony potassium tartrate, sulfuric acid, and ascorbic acid to form a blue phospho-molybdenum. With just the naked eye, a qualitative visual readout was achieved. In this study, demonstrated the use of simple robust RPA amplified samples for the colorimetric detection of inorganic phosphate (Pi). In the case of COVID-19 pandemics, this straightforward approach would be ideal for the rapid deployment of diagnostic efforts. So the early detection is important to control the blowout of disease.

Here described a patient without history of cardiovascular disease while infected with COVID-19. Our main finding was blood pressure and heart rate observations. The blood pressure was increase during COVID-19, when blood pressure increase the pulse rate was increase and the heart rate definitely increase, it is a serious condition. Health risk linked to high blood pressure include heart disease. It is important to note that people with higher blood pressure seem to be more at risk of complication from COVID-19 than those whose high blood pressure is managed with medication. The patient got fever, dizziness, headache, flu and stomach problem during COVID-19. Healthy food are very important and especially have beta carotene and fiber, both are active to low the blood pressure, while polyphenols are helpful to control the heartbeat.

Conclusion

The challenge of SARS-CoV-2 detection is important particularly underdeveloped countries and especially in emergency situation. Infrastructure accessibility for testing based on RT-qPCR in the COVID-19 pandemic condition is recognized as a significant challenge globally. In less developed countries like Pakistan, India, Bangladesh and some African countries have current available resource for huge COVID-19 testing by RT-qPCR are insufficient. PCR labs that are easily accessible are overrun with samples and understaffed with qualified workers to carry out the test.

The COVID-19 virus is posing serious new public health issues and expanding the usefulness of diagnostic tests, so the early detection is important to control the virus. Here, demonstrated a simple RPA reaction on the water bath for amplification reaction, the inorganic phosphate (Pi) was used for colorimetric detection of SARS-CoV-2. The number of freshly integrated dNTPs is quantitatively inversely correlated with the pyrophosphate (PPi). To assess the usefulness of this method as an alternative to RT-qPCR, it is obviously essential to provide evidence of these results using genuine human samples for positive SARS-CoV-2. In situations where RT-qPCR is either impossible or not available, this straightforward approach, nevertheless, may considerably helpful for COVID-19 testing. This research study also shown that SARS-CoV-2 association with blood pressure and heart rate fluctuation may be concern.

Declaration of interest: Author declare no competing interest.

References

1. L. Yu, S. Wu, X. Hao, X. Li, X. Liu, S. Ye, H. Han, X. Dong, X. Li, J. Li, N. Liu, J. Liu, W. Zhang, V. Pelechano, H. W. Chen and X. Yin. Rapid Detection of COVID-19 Coronavirus Using a Reverse Transcriptional Loop-Mediated Isothermal Amplification (RT-LAMP) Diagnostic Platform. *Clinical Chemistry*, 2020, 66, 975-977.
2. A. M. Lim, R. Pranata, I. Huang, E. Yonas, Y. A. Soeroto and Supriyadi. Multiorgan Failure With Emphasis on Acute Kidney Injury and Severity of COVID-19: Systematic Review and Meta-Analysis. *Canadian Journal of Kidney Health and Disease*, 2020, 7, 1-12.
3. H. Shi, X. Han, N. Jiang, Y. Cao, O. Alwalid, J. Gu, Y. Fan and C. Zheng. Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study. *Lancet Infect. Dis*, 2020, 20, 425-434.
4. T. Notomi, H. Okayama, H. Masubuchi, T. Yonekawa, K. Watanabe, N. Amino and T. Hase. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res*, 2000, 28, e63.
5. W. Xu, X. Xue, T. Li, H. Zeng and X. Liu, Ultrasensitive and Selective Colorimetric DNA Detection by Nicking Endonuclease Assisted Nanoparticle Amplification. *Angew Chem Int Edit*, 2009, 48, 6849-6852.
6. N. Tomita, Y. Mori, H. Kanda and T. Notomi. Loop-mediated isothermal amplification (LAMP) of gene sequences and simple visual detection of products. *Nat Proc*, 2008, 3, 877-882.

7. F. Zhang, J. Wu, R. Wang, L. Wang and Y. Ying. Tracing phosphate ions generated during DNA amplification and its simple use for visual detection of isothermal amplified products. *Chem Commun*, 2014, 50, 8416-8419.
8. P. Craw and W. Balachandran. Isothermal nucleic acid amplification technologies for point-of-care diagnostics: a critical review. *Lab on a Chip*, 2012, 12, 2469-2486.
9. S. Motomizu and Z. H. Li. Trace and ultratrace analysis methods for the determination of phosphorus by flow-injection techniques. *Talanta*, 2005, 66, 332-340.
10. B. Hu, H. Guo, P. Zhou and Z. Shi. Characteristics of SARS-CoV-2 and COVID-19. *Nature Reviews Microbiology*, 2021, 19, 141-154.
11. F. Zhang, J. Wu, R. Wang, L. Wang and Y. Ying. Portable pH-inspired electrochemical detection of DNA amplification. *Chemical Communications*, 2014, 50, 8416-8419.
12. Z. Luo, C. Yea, H. Xiao, J. Yin, Y. Liang, Z. Ruan, D. Luo, D. Gao, Q. Tan, Y. Li, Q. Zhang, W. Liu and J. Wu. Optimization of loop-mediated isothermal amplification (LAMP) assay for robust visualization in SARS-CoV-2 and emerging variants diagnosis. *Chemical Engineering Science*, 2022, 251, 117430.
13. B.W. Raddatz, E.Y.S. Kim and L.M. Imamura. Development of an optimized colorimetric RT-LAMP for SARS-CoV-2 assay with enhanced procedure controls for remote diagnostics. *Sci Rep*, 2022, 12, 21424.
14. G. D. Suarez, D. A. Suarez, Y. Y. K. Tang, J. X. Zhang, J. Li, S. Nagl and P. P. H. Cheung. Uncovering mechanisms of RT-LAMP colorimetric SARS-CoV-2 detection to improve assay reliability. *Anal. Methods*, 2022, 14, 378-382.
15. J. Song, B. Cha, J. Moon, H. Jang, S. Kim, J. Jang, D. Yong, H.J. Kwon, I. Lee, E. K. Lim, J. Jung, H. G. Park and T. Kang. Smartphone-Based SARS-CoV-2 and Variants Detection System using Colorimetric DNAzyme Reaction Triggered by Loop-Mediated Isothermal Amplification (LAMP) with Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). *ACS Nano*, 2022, 16, 11300-11314.
16. M. H. Choi, G. S. R. Kumara, J. Lee and Y. J. Seo. Point-of-care COVID-19 testing: colorimetric diagnosis using rapid and ultra-sensitive ramified rolling circle amplification. *Anal Bioanal Chem*, 2022, 414, 5907-5915.
17. C. Amaral, W. Antunes, E. Moe, A. G. Duarte, L. M. P. Lima, C. Santos, I. L. Gomes, G. S. Afonso, R. Vieira, H. S. S. Teles, M. S. Reis, M. A. R. DaSilva, A. M. Henriques, M. Fevereiro, M. R. Ventura, M. Serrano and C. Pimentel. A molecular test based on RT-LAMP for rapid, sensitive and inexpensive colorimetric detection of SARS-CoV-2 in clinical samples. *Sci Rep*, 2021, 11, 16430.
18. S. Wu, X. Liu, S. Ye, J. Liu, W. Zheng, X. Dong and X. Yin. Colorimetric isothermal nucleic acid detection of SARS-CoV-2 with dye combination. *Heliyon*, 2021, 7, e06886.
19. W. S. Zhang, J. Pan, F. Li, M. Zhu, M. Xu, H. Zhu, Y. Yu and G. Su. Reverse Transcription Recombinase Polymerase Amplification Coupled with CRISPR-Cas12a for Facile and Highly Sensitive Colorimetric SARS-CoV-2 Detection. *Anal. Chem*, 2021, 93, 4126-4133.
20. Y. He, L. Wang, X. An and Y. Tong. All-in-one in situ colorimetric RT-LAMP assay for point-of-care testing of SARS-CoV-2. *Analyst*, 2021, 146, 6026-6034.