PharmacologyOnLine
Archives • 2021 • vol.2 • 1093-1101

REVIEW ARTICLE

A LAB-ON A CHIP DEVICE FOR DIAGNOSIS OF SARS COV- 2 BY SOLID PHASE PCR IN RESOURCE LIMITED SETTINGS: A PROTOCOL

Usha Adiga

Professor, Nitte (Deemed to be University), KS Hegde Medical Academy(KSHEMA),Department of Biochemistry,Mangalore, Karnataka

*ushachidu@yahoo.com

Abstract

Objective of the study is to develop a lab on a chip device which works on the principle of solid phase PCR, for the diagnosis of COVID19 and to compare its sensitivity, specificity and cost effectiveness with that by real time reverse transcriptase PCR(RT-PCR).

In this cross-sectional study, three hundred symptomatic patients, suspected of COVID-19 will be screened by the proposed new lab on a chip technique as well as by RT-PCR. The number of extra positive cases detected by **lab on a chip device** gives us the yield. Cost effectiveness analysis will be done by calculating incremental cost effectiveness ratio(ICER) and average cost effectiveness ratio(ACER) for the tests. The major constraint for implementing RT-PCR as a routine screening technique in India appears to be its high cost per test as well as its false negative results. Hence we propose a new technique which could be sensitive, specific as well as cost-effective as compared to RT-PCR in Indian settings.

Data will be analyzed using SPSS (Statistical package for Social Sciences) version 21. Sensitivity, specificity, Positive predictive values will be computed. Comparison of sensitivity and specificity of RT-PCR and lab on a chip device will be carried out using McNemar's test. Receiver operating curves will be generated separately to assess the utility of RT-PCR, our proposed new technique and area under the curve (AUC) will be determined. The cut off for the sensitivity without significant decrease in specificity will be chosen for each of the test.The significance level will be set as $p< 0.05$.

The study outcome may be a sensitive ,specific and cost effective technique as well as a device which may be helpful in early diagnosis, isolation and treatment of COID-19 in resource limited settings.

Keywords: *COVID-19, RT-PCR, lab on a chip,solid phase PCR*

Introduction

The coronavirus belongs to a family of viruses that may cause various symptoms such as pneumonia, fever, breathing difficulty, and lung infection [1]. The World Health Organization (WHO) used the term 2019 novel coronavirus to refer to a coronavirus that affected the lower respiratory tract of patients with pneumonia in Wuhan, China on 29 December 2019. The WHO announced that the official name of the 2019 novel coronavirus is coronavirus disease (COVID-19) [2]. Current reference name for the virus is severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Susceptibility to this infection seems to be associated with age, gender and other associated co-morbid conditions [3]. COVID-19 has been declared as a Public Health Emergency of International Concern by the WHO [4]. Recently, study on the early transmission dynamics of COVID-19 has reported human-to-human spread of the infection[5]. Therefore, it is very essential to diagnose COVID-19 infection precisely so that isolation and treatment can be done effectively.

Rationale of the study

RT-PCR has been the gold standard for the diagnosis of COVID 19 infection.However the limitations of the method are false negative results, inability of the method to detect the infection in the early stage, variable results with different samples, variable results when done in different intervals, expensive instrumentation, need for well trained personnel and so on. So, it is the need for the hour to develop a device/method that is sensitive,can detect the cases at the early stage, cost effective, portable so that it will be very useful in resource limited settings.

Diagnostic approaches for the detection of COVID-19 infection

Currently, the real-time reverse transcriptase polymerase chain reaction (RT-PCR) amplification of the viral RNA is considered as the "gold standard". However, initial results of RT-PCR, in the early phase of infection is not always positive in COVID-19 infection [6,7]. In such situations, chest computed tomographic (CT) images could play an important role to detect the lesions in the lung parenchyma in suspected patients. However lung pathology may not be reflected in CT images as well irrespective of whether RT-PCR is positive or negative [6-9].

Hao et al described clinical features of atypical 2019 novel coronavirus pneumonia with an initially negative RT-PCR assay[10]. Along with false negative results, other major constraint for implementing RT-PCR as a routine screening technique in India appears to be its high cost per test and time duration required.

Li et al reported data of 610 hospitalized patients from Wuhan, clinically diagnosed with COVID-19 during the 2019 outbreak. They found that the RT-PCR results performed at different points of time, were variable. They also found a potentially high false negative rate of RT-PCR testing for SARS-CoV-2 in hospitalized patients, clinically diagnosed with COVID-19[11]. Fluctuating results of RT-PCR may be due to insufficient viral load in the specimen, laboratory error during sampling, or improper sample transportation methods [12].

It must be appreciated that no matter how accurate and fast testing methods are used in the laboratory, the diagnosis of viral pneumonias caused by SARS-CoV-2 involves collecting the correct specimen from the patient at the right time. SARS-COV-2 have been detected from a variety of upper and lower respiratory sources including throat, nasal nasopharyngeal, sputum, and bronchial fluid [13-16]. Wang et al have just reported that the SARS-CoV-2 RNA was detected only in 32% of OP swabs, which was significantly lower than that in NP swabs (63%)[17].

The main IVD assays used for COVID-19 employ real-time reverse transcriptase polymerase chain reaction (RT-PCR) that takes a few hours. But the assay duration has been shortened to 45 min by Cepheid. Abbott has developed a point of care molecular assay that decreased the assay duration to just 5 min. Most molecular tests have been approved by the United States Food and Drug Administration (FDA) under emergency use authorization (EUA) and are Conformité Européenne (CE) marked.

Several serological immunoassays have been developed by IVD companies for the detection of SARS-CoV-2 viral proteins and antibodies in the serum or plasma. The most widely used biomarkers for the detection of SARS-CoV-2 infection in commercial immunoassays (rapid lateral flow immunoassay (LFIA) tests, automated chemiluminescence immunoassay (CLIA), manual

ELISA, and other formats) are IgM and IgG antibodies produced in suspects from the 2nd week of viral infection. IgM can be detected in the patient samples from 10 to 30 days after SARS-CoV-2 infection, while IgG can be detected from 20 days onwards [18]. The IgM response occurs earlier than that of IgG, but it then decreases and disappears. On the other hand, IgG can persist after infection for a long time and may have a protective role.

Apart from the molecular diagnostics, numerous LFIA based rapid POC tests have been developed by several companies, which enable the detection of IgM and IgG antibodies produced in suspects in response to SARS-COV-2 infection. One of the most prominent rapid tests is the COVID-19 test developed by BioMedomics, USA, which detects IgM and IgG antibodies in suspects in just 10 min [19]. It requires minimal sample volume, i.e., 20 µL of finger-pricked blood or 10 µL of serum/plasma. It does not require any instrument or trained staff and, thus, it can be employed at any place and time, especially in developing nations with limited healthcare resources and remote settings. The assay is ideal for primary healthcare workers for the rapid testing of COVID-19 suspects. Another prospective test is the SARS-CoV-2 rapid by Pharmacyt AG, Germany [20], which employs only two drops of finger-pricked blood sample from the suspects and can provide results in 20 min. The results obtained by the rapid test correlated well with those achieved by RT-PCR. The most exciting advance is the DPP COVID-19 IgM/IgG test launched recently by Chembio Diagnostics, USA, which has already received FDA EUA. It is a POC rapid LFIA test that provides results in just 15 min using finger-pricked blood sample.

The accurate diagnosis of people infected with the SARS-CoV-2 is essential to curb the global spread of COVID-19. However, the current RT-PCR based diagnostic assays are not robust, as they are still missing several infected cases [21-23]. Moreover, they can only be performed in well-equipped central laboratories by highly skilled analysts. Therefore, they are of limited utility and cannot be deployed widely, such as in developing nations, remote locations, and regions with decentralized laboratories. The delay in diagnosing people until after they have passed the disease onto many others is contributing to the continued global spread of COVID-19. The rapid LFIA and automated CLIA tests for IgM and IgG could complement the existing COVID-19 testing by RT-PCR. However, there is a need to stringently evaluate the clinical performance of commercial tests before they are used for the diagnosis of COVID-19.

Hence we propose to develop a lab on a chip device ,working on solid phase PCR, which could be sensitive, specific as well as cost effective compared to other diagnostic method.

We would like to develop a lab on a chip device, that enables multiplexed, sensitive assays that may be superior to PCR based laboratory assays and may provide high-quality, fast precision diagnostics for COVID-19.

Lab on a chip , paper microfluidics represents a promising technology as it is user friendly, low cost technology, using paper as the solid matrix for managing the fluids in complex networks[24-27. Until recent years, this technology has been applied to immunoassays. Nonetheless, with the development of isothermal amplification, it has recently served the identification of nucleic acid targets with techniques such as RT-RPA [28-31] which is particularly suitable for paper-based applications as its working temperature (between 37–42 °C) requires neither large thermal energy nor cycle control. Considering the chemical reactivity of paper and the biochemical complexity of the amplification reagents[32,33].

Aim of the study would be to develop solid phase PCR based **Lab on a chip device** for the diagnosis of COVID-19 and compare its diagnostic sensitivity , specificity and cost-effectiveness with those of RT-PCR in resource limited settings. We would like to demonstrate that such technique can be implemented in underserved communities at the point of need cost effectively.

Cost effectiveness analysis

Cost-effectiveness analysis is an important tool to assist clinicians, scientists and policymakers in determining the efficiency of healthcare interventions, guiding societal decision-making on the financing of healthcare services and establishing research priorities. Diverse approaches to

synthesize evidence have been considered in biomedical research , including economic evaluations of healthcare interventions[34-36]. At the same time, decision-making in health care requires an understanding of the state of economic evaluation at a national level, where the completeness of the reporting is generally less well understood but where specific priorities are often set. Cost effectiveness analysis (CEA) compares two diagnostic tests, where the costs are identified in monetary terms and the outcomes in non-monetary terms.

Measurement of cost effectiveness could be made in two different ways:

1. ACER – Average Cost Effectiveness Ratio
2. ICER – Incremental Cost Effectiveness Ratio

It helps a decision maker to compare one diagnostic test to other thereby quantifying the opportunity cost of decisions.

Sudden emergence of SARS-CoV-2 and its potential to cause a pandemic posed an unsurmountable challenge to the public health system of India. However, concerted efforts of various arms of the Government of India resulted in a well-coordinated action at each level. India has successfully demonstrated its ability to establish quick diagnosis of SARS-CoV-2 at NIV, Pune, and the testing laboratories.

Novelty/Innovation

The study may demonstrate that solid phase PCR-based **Lab on a chip** device can deliver precision diagnostics for COVID19 in low-resource, underserved settings with a sensitivity that is higher than that of the current COVID-19 diagnostic tests used in the field and with performance that is similar to that of a laboratory-based real-time PCR test. These diagnostic devices could have a meaningful, positive impact on the provision of mass screening and treatment in campaigns to eliminate infectious disease. These campaigns have had limited success to date in combating COVID19 transmission, which has been linked to the inability of current field-based diagnostic tools to detect low level infections. Thus, the availability of easy-to-use, highly sensitive nucleic acid amplification tests, such as those provided by this device, could potentially detect these missed cases and reduce the opportunity for transmission. This would have a significant impact on public health in areas where COVID-19 is highly prevalent.

Study Objectives

- To develop a solid phase PCR based **Lab on a chip device** for the diagnosis of COVID 19
- To compare sensitivity , specificity and cost effectiveness of lab on a chip device versus real time RT-PCR in the detection of COVID -19 .

Methods

i. Study design: Cross-sectional

Study Center: The study will be carried out in the Molecular Genetics division of Central research Laboratory, KS Hegde Medical Academy, Mangalore, Karnataka, India.

ii. Sample size calculation

A sample size of 300 suspected COVID-19 cases will be included in the study. Sample size is also based on estimating output indicators , where the upper bound of the 95% CI does not overlap with 5% for estimates in which the observed prevalence of false-negative RT-PCR results is below 5% and where the lower bound of the 95% CI does not overlap with 5% for estimates in which the observed prevalence is above 5% .

iii. Project implementation plan

Study participants

Inclusion criteria: Patients presenting to the fever clinic with the complaints of fever and respiratory symptoms like cough, sneezing, sore throat or breathlessness or pneumonia in all the age groups , of either gender, presenting to the Justice KS Hegde Charitable Hospital.

Exclusion criteria: Lung disorders of other etiologies, fever due other established causes

A pre-tested semi-structured questionnaire containing socio-demographic details of patients like age, gender, place of residence, will be collected after formal written consent maintaining confidentiality of identity. The participants will be analyzed for laboratory tests and cost effective analysis.

Sample Collection

Oropharyngeal/nasopharyngeal swabs/throat swabs will be collected using sterile Dacron/nylon flocked swabs,tongue depressor , with appropriate method. Collected samples will be transported to the laboratory in viral transport medium.

A.Laboratory Investigations:

Symptomatic individuals will be screened for COVID-19 infection by using both RT-PCR and solid phase PCR based Lab on a chip device.

Procedure for Real time RT -PCR

RNA will be extracted from specimens using the NucleoSpin Dx Virus kit. RNA extracted from 100 µl of original sample, will be eluted in 100 µl of elution buffer. RT PCR kit,MY Lab,(Batch no –PP00005-C-032001) which is validated and approved by Indian Council of Medical Research will be used for COVID-19 detection.

Experimental design of Solid phase PCR based Lab on a chip device

Huber *et al.* proposed a method to allow the reaction to proceed in the liquid phase and on the surface of the solid phase simultaneously, which dramatically increased the product yield on the solid support[37].The liquid-phase amplification produced DNA templates to initiate amplification on the solid phase and the accumulation of targets in a solution also served to accelerate the solid-phase amplification. The same extended strategy of this method will be used to develop an approach where RT-PCR occurs in the liquid solution and nested amplification occurs on the microarray elements with specific oligonucleotide probes. For the liquid-phase PCR, three pairs of primers will be designed to amplify conserved region of SARS COV2 on the matrix . For the solid-phase amplification, three nested probes will be spotted on the glass surface to target the templates generated in the liquid. Though a multitude of unbound primers will be added to the liquid phase, the potential for primer interference is counteracted by the superior specificity of nested amplification on the solid surface.

The working model for on-chip solid-phase PCR

- The viral RNA and RT-PCR mixture will be pumped into the chamber
- The RNA will be reverse transcribed to cDNA in the liquid
- cDNA will be amplified with freely moving PCR primers.
- The newly amplified PCR amplicons in the liquid phase interact with the nested probes immobilized on the solid support
- The matched probes will be extended by the polymerase
- In the next cycle, the forward primers in the liquid phase will be annealed to the extended probes
- Complementary strands will be generated and serve as new templates for the solid-phase amplification. After the reaction, PCR products remain attached to the glass slide through covalent binding and could be directly visualized as the forward primers will be labeled with Cy5 dyes.
- Detection of SARS COV 2 will be achieved by examining the specific patterns of the microarray.

Preparation of DNA microarrays on glass substrate

Microarray will be produced on an unmodified glass substrate by simple UV cross-linking according to a method developed [38].Briefly, a glass chip of 10 mm × 10 mm will be diced from a 0.5 mm glass wafer using a Dicing Saw (Disco, Japan) and will used without any pre-treatment or modification. The three oligonucleotide probes with poly(T)10–poly(C)10 tails will be diluted in 150 mM sodium phosphate buffer (pH 8.5) to a final concentration from 5 to 50 µM and will be spotted using a non-contact array nano-plotter 2.1 (GeSim, Dresden,

Germany). The array will be spotted at the centre of the glass chip and each probe will be repeated four times for easy identification of the reaction products. The spots will be allowed to dry and then exposed to UV irradiation at 254 nm with an energy of 0.3 J cm^{-2} for 10 min (Stratalinker 2400, Stratagene, CA, USA). Subsequently, the glass chip will be washed under agitation in 0.1× standard saline citrate (SSC) with 0.1% (w/v) sodium dodecyl sulphate (SDS) (Promega, WI, USA) solution for 10 min, then wiil be rinsed in deionized water and dried under nitrogen.

Microfabrication

The PCR microchamber will be fabricated in polydimethylsiloxane (PDMS) by rapid prototyping. The mask layout will be designed using a CAD program. The master for molding will be fabricated in SU-8 50 (MicroChem, MA, USA) by the standard photolithography.

A 10 : 1 mixture of PDMS pre-polymer and curing agent (Sylgard 184, Dow Cornig, MI, USA) will be stirred thoroughly and then will be poured onto the master and cured for 1 h at 65 °C. After curing, the 1 mm thick PDMS replica will be peeled from the master. Inlet and outlet holes will be punched by a needle. The PDMS substrate and the glass chip with DNA microarray will be sealed by plasma bonding. Both PDMS and glass substrates will be exposed to oxygen plasma for 30 s at 100 W with an oxygen flow rate of 240 ml min^{-1} (Plasma Processor 300, PVA TePla, Germany). Right after the removal from the plasma chamber, the substrates will be brought into conformal contact where an irreversible seal formed spontaneously.

On-chip solid-phase PCR

25 µl PCR reaction mix will be prepared, consisting of 10 µl of 5 x RT-PCR buffer. 1 µl of 10 mM DNTP mix, 1 µl of 2 µl /reaction enzyme mix, 2.5 µl of 2.5 µg $µl^{-1}$ BSA (Onestep RT-PCR kit, Qiagen, Germany), three pairs of primers with a final concentration of 1 µM for forward primers and 0.5 µM for reverse primers, and 5 µl RNA sample. 2 µl of the mixture will be loaded into the microchamber. The whole chip will be placed in a homemade chip holder and the inlet and outlet will be then sealed by pressing rubber plugs down on the holes. The chip holder will be put on a flatbed thermocycler (MJ Research Inc., MA, USA). PCR will be carried out according to the following program: 15 min at 50 °C for reverse transcription, followed by 40 cycles of 1 s at 95 °C, 5 s at 54 °C and 3 s at 72 °C, and finally 3 min at 72 °C for extension. After the cycling, the microchamber will be washed with 0.1× SSC per 0.1% SDS and deionized water.

The microarray in the microchip will be scanned by an array scanner (LaVision BioTec, Germany). Fluoresence imaging and processing software (LaVision BioTec, Germany) will be used to quantify the spots by calculating the average pixel intensity inside the defined spots.

B. Cost effective analysis of Diagnostic techniques

Incremental cost effectiveness ratio (ICER) and average cost ratio(ACER) will be calculated as follows:

Incremental cost effectiveness ratio : It compares the incremental cost divided by the incremental effect. This can be described in an incremental cost effectiveness ratio (ICER), and can be expressed in an equation:

ICER = C1 – C2/E1 – E2 where C1 and E1 are the cost and effect due to RT-PCR/ paper microfluidic device , and C2 and E2 are the cost and effects of RDT, effect(E1 & E2) being the number of cases detected. A high ICER indicates more expenditure for better health outcome while compared to a lower ICER .Hence an intervention with a lower ICER would be preferred.

Average Cost-effectiveness Analysis: Cost-effectiveness ratio of each diagnostic test will be calculated and the two ratios are compared . Specifies the cost of an intervention required to achieve each unit of effect. Average cost-effectiveness ratio (ACER) =

$$\frac{\text{Cost of diagnostic test}}{\text{No. of positive cases detected}} = \text{Cost per unit of effect}$$

iv. Ethical considerations : University ethics committee approval will be sought before starting the study .Informed consent will be obtained from the study subjects.

v.Statistical Analysis

Quantitative data will be expressed in mean and standard deviation. Data thus collected will be coded, validated and entered into Microsoft Excel version 2010 and analyzed using SPSS (Statistical package for Social Sciences) version 21. Sensitivity, specificity, Positive predictive values will be computed. Comparison of sensitivity and specificity of RT-PCR and solid phase PCR based Lab on a chip device will be carried out using McNemar's test. Receiver operating curves will be generated separately to assess the utility of RT-PCR, our proposed new technique for RNA diagnostics and Area under the curve (AUC) will be determined. The cut off for the sensitivity without significant decrease in specificity will be chosen for each of the test.The significance level will be set as $p < 0.05$.

Expected outcome

Primary output measures

Comparison of sensitivity and specificity of RT-PCR versus solid phase PCR based lab on a chip device in diagnostics of COVID-19.

Secondary output measure

Cost effectiveness analysis of diagnostic tests ,NAT by RT-PCR and solid phase PCR based lab on a chip device in detecting COVID-19

Future plans and Applicability of the study

The study may demonstrate that solid phase PCR based Lab on a chip devices can deliver precision diagnostics for COVID 19 in low-resource, underserved settings with a sensitivity that is higher than that of the current diagnostic tests used in the field and with performance that is similar to that of a laboratory-based real-time PCR test. These diagnostic devices may have a meaningful, positive impact on the provision of mass screening and treatment in campaigns to eliminate infectious disease . Thus, the availability of easy-to-use, highly sensitive nucleic acid amplification tests, such as those provided by this device, could potentially detect these missed cases and reduce the opportunity for transmission. This would have a significant impact on public health in areas affected with COVID -19.It could also inform current thinking within governments and nongovernmental organizations concerning improvements in the effectiveness and cost-effectiveness of prophylactic approaches to control diseases (where new precise diagnostic tools are required to rapidly and accurately target where treatment is needed).

Economic assessments of diagnostic tests are inherently difficult than assessments of therapeutic interventions because of uncertainty about the relation between diagnosis and end result or outcomes of care. Towards the end, this study would evaluate the economic feasibility of introduction of **solid phase PCR based lab on a chip device** as a diagnostic tool for COVID-19. This study would help in planning out further strategies for the effective management and treatment of individuals detected by the test. It would also dive into newer research areas to establish the subsequent decrease the morbidity and mortality associated with COVID-19 given appropriate facilities for early treatment after detection would be mandated at policy level.

Conclusion

The Implications of this study from the patient's perspective would mean early diagnosis which forms the tenet of control of the disease by increasing the yield. Early diagnosis at community level would translate into application of efficient prevention mechanisms of spread of the infection . The cost effectiveness analysis would provide scientific basis for adoption of the best test for the diagnosis, given the economic feasibility of the study. Early diagnosis will aid the clinician in providing timely treatment by reducing the morbidity and mortality due to SARS COV 2 infection.

Conflicts of interest: None

References

1. WMHC. Wuhan Municipal Health and Health Commission's Briefing on the Current Pneumonia Epidemic Situation in Our City. 2020. http://wjw.wuhan.gov.cn/front/web/showDetail/2019123108989. Accessed 1 Feb 2020.
2. WHO. Novel Coronavirus–China. 2020. https://www.who.int/csr/don/12- january-

2020-novel-coronavirus-china/en/. Accessed 1 Feb 2020.
3. Fehr AR, Channappanavar R, Perlman S. Middle East respiratory syndrome: emergence of a pathogenic human coronavirus. Annu Rev Med 2017;68: 387–99.
4. WHO. Statement on the second meeting of the International Health Regulations (2005) Emergency Committee regarding the outbreak of novel coronavirus (2019-nCoV). 2020
5. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, et al. Early transmission dynamics in Wuhan, china, of novel coronavirus-infected pneumonia. N Engl J Med 2020. doi:10.1056/NEJMoa2001316.
6. Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, et al. Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases. Radiology 2020:200642. doi:10.1148/radiol.2020200642.
7. Fang Y, Zhang H, Xie J, Lin M, Ying L, Pang P, et al. Sensitivity of chest CT for COVID-19: comparison to RT-PCR. Radiology 2020:200432. doi:10.1148/radiol. 2020200432.
8. Shi H, Han X, Jiang N, Cao Y, Alwalid O, Gu J, et al. Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, china: a descriptive study. Lancet Infect Dis 2020. doi:10.1016/S1473-3099(20)30086-4.
9. Pan F, Ye T, Sun P, Gui S, Liang B, Li L, et al. Time course of lung changes on chest CT during recovery from 2019 novel coronavirus (COVID-19) pneumonia. Radiology 2020:200370.
10. Hao W. Clinical features of atypical 2019 novel coronavirus pneumonia with an initially negative RT-PCR assay. J Infect 2020. doi:10.1016/j.jinf.2020.02.008
11. Yafang L ,Yao L , Li J , Chen L , Song Y , Cai Z5 , Yang C. Stability Issues of RT-PCR Testing of SARS-CoV-2 for Hospitalized Patients Clinically Diagnosed with COVID-19. doi: 10.1002/jmv.25786
12. Qian M, Yi Q, Qihua F, Ming G. Understanding the influencing factors of nucleic acid detection of 2019 novel coronavirus. Chinese Journal of Laboratory Medicine. February 8, 2020. DOI: 10.3760/cma.j.issn.1009-8158.2020.0002.
13. Su S, Wong G, Shi W, et al. Epidemiology, Genetic Recombination, and Pathogenesis of Coronaviruses. Trends Microbiol. 2016;24(6):490-502. doi: 410.1016/j.tim.2016.1003.1003. Epub 2016 Mar 1021.
14. Ksiazek TG, Erdman D, Goldsmith CS, et al. A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med. 2003;348(20):1953-1966. doi: 1910.1056/NEJMoa030781. Epub 032003 Apr 030710
15. Charlton CL, Babady E, Ginocchio CC, et al. Practical Guidance for Clinical Microbiology Laboratories: Viruses Causing Acute Respiratory Tract Infections. Clin Microbiol Rev. 2019;32(1).(pii):32/31/e00042-00018. doi: 00010.01128/CMR.00042-00018. Print 02019 Jan.
16. Falsey AR, Formica MA, Walsh EE. Simple method for combining sputum and nasal samples for virus detection by reverse transcriptase PCR. J Clin Microbiol. 2012;50(8):2835. doi: 2810.1128/JCM.01473-01412. Epub 02012 Jun 01412.
17. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. Jama. 2020;11(2762997)
18. Tan W, Lu Y, Zhang J, Wang J, Dan Y, Tan Z, He X, Qian C, Sun Q, Hu Q et al. Viral kinetics and antibody responses in patients with COVID-19. medRxiv 2020.
19. COVID-19 IgM/IgG Rapid Test. Available online https://www.biomedomics.com/products/infectiousdisease/covid-19-rt/ (accessed on 2 April 2020).
20. SARS-CoV-2 Rapid Test. Available online: https://www.pharmact.eu/ (accessed on 2 April 2020).
21. Rothe C, Schunk M, Sothmann P, Bretzel G, Froeschl G, Wallrauch C, Seilmaier M. Transmission of 2019-nCoV Infection from an asymptomatic contact in Germany. N Engl J Med 2020; 382: 970–971.
22. Huang P, Liu T, Huang L, Liu H, Lei M, Xu W, Hu X, Chen J, Liu B. Use of chest CT in

combination with negative RT-PCR assay for the 2019 novel coronavirus but high clinical suspicion. Radiology 2020; 295: 22–23.
23. Xie X, Zhong Z, Zhao W, Zheng C, Wang F, Liu J. Chest CT for typical 2019-nCoV pneumonia: Relationship to negative RT-PCR testing. Radiology 2020. [CrossRef] [PubMed](in press)
24. Martinez AW et al. Patterned paper as a platform for inexpensive low volume portable bioassays. Angew Chem Int Ed 2007; 46: 1318–1320.
25. Parolo C, Merkoçi A. Paper-based nanobiosensors for diagnostics. Chem Soc Rev 2012; 42: 450–457.
26. Cate D M et al. Recent developments in paper-based microfluidic devices. Anal Chem 2015; 87: 19–41.
27. Glavan A C et al. Rapid fabrication of pressure-driven open-channel microfluidic devices in omniphobic RF paper. Lab Chip 2013;13:2922–2930.
28. Rohrman BA, Richards-Kortum R R. A paper and plastic device for performing recombinase polymerase amplification of HIV DNA. Lab Chip 2012; 12: 3082–88.
29. Linnes JC et al. Paper based molecular diagnostic for Chlamydia trachomatis. RSC Adv 2014; 4:42245–42251.
30. Connelly T, Rolland P, Whiteside M.Paper Machine for molecular diagnostics.Anal Chem 2015; 87:7595–601.
31. Rodriguez N M et al. A fully integrated paperfluidic molecular diagnostic chip for the extraction, amplification, and detection of nucleic acids from clinical samples. Lab Chip 2016;16: 753–763.
32. Pelton R. Bioactive paper provides a low cost platform for diagnostics. Trends Anal Chem2009; 28: 925–942.
33. Credou J, Berthelot T. Cellulose from biocompatible to bioactive material. J Mater Chem2014; 2: 4767–4788 .
34. Elixhauser A, Luce BR, Taylor WR, Reblando J. Health care CBA/CEA: an update on the growth and composition of the literature. Med Care. 1993;31:JS1–JS11. JS18-149.
35. Elixhauser A, Halpern M, Schmier J, Luce BR. Health care CBA and CEA from 1991 to 1996: an updated bibliography. Med Care. 1998;36:MS1–9. MS18-147.
36. Neumann PJ, Stone PW, Chapman RH, Sandberg EA, Bell CM. The quality of reporting in published cost-utility analyses, 1976–1997. Ann Intern Med. 2000;132:964–72.
37. Huber M, Losert D, Hiller R, Harwanegg C, Mueller MW and Schmidt WM, *Anal. Biochem* 2001; **299**: 24–30 .
38. Gudnason H, Dufva M, Bang DD ,Wolff A. *BioTechniques* 2008; **45**: 261–271 .