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Rapid and simple detection of SARS-CoV-2 with point-of-care COVID-19 testing

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COVID-19 as a public health concern of the world has spread worldwide and a combination of various methods including tomography imaging, reverse transcription-polymerase chain reaction (RT-PCR), enzyme-linked immunosorbent assay (ELISA) and cell culturing were developed to detect and identify SARS-CoV-2. Due to the absence of specific antiviral agents or vaccines for COVID-19 treatment, early detection and identification are vital. An alternative, sensitive, fast point-of-care (POC) detection tool that can be routinely used by health care providers utilizing biological fluids as a specimen before starting an emergency process is desired. Efforts are underway to develop more effective diagnostic and surveillance technologies with loop-mediated isothermal amplification (LAMP) tests, antibody testing and microfluidic RT-PCR devices (Lab-on-a-chip). Point-of-care diagnostics are promising candidates in SARS-Cov-2 detection and encourage scientists to improve their technologies beyond conception. The reverse transcription LAMP (RT-LAMP) method developed for SARS-CoV-2 could detect the virus even in saliva samples in less than an hour (Harapan et al., 2020). Lab-on-a-chip devices contain a small size chip, microchannel, microelectrodes and microheater. Cell lysis, DNA extraction and PCR amplification stages could be integrated on these microchips (Sharma et al., 2020). Because of the rapid detection, small volume of the specimen and integration with PCR in a portable tiny system, these devices are promising for SARS-CoV-2 detection (Huang et al., 2018). The validity and sensitivity of all the above-mentioned methods need to be improved for salivary specimen usage; in case of improvement, they might provide an opportunity for salivary detection of the virus without a waiting period and complex analytical infrastructure.

Keywords: *COVID-19; RT-PCR; polymer chip; thermocycler; emergency situations; biosensors*

INTRODUCTION

The family of coronaviride are responsible for respiratory, hepatic, neurological and enteric manifestations of the new epidemy of COVID-19. *Coronaviride* family has four general classes including, alpha, beta, gamma and delta coronaviruses (Harapan et al., 2020). The crown-like shape of the virus under electron microscope has been attributed to the term coronom (Sharma et al., 2020). A broad range of mammels such as humans, animals and rodents could be infected via coronaviruses. High recombination and mutation rate leads to the rapid adjusting of the virus in the new host (Sharma et al., 2020; Huang et al., 2018).The common symptoms of this pandemic health concern include fever, cough, myalgia, plummeted leukocytes and opacities (Chan et al., 2020; Hoffmanet al., 2020). Headaches, abdominal pain, hem-

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optysis and diarrhea are considered as other symptoms (Harapan et al., 2020).However, many cases are asymptomatic and the validity of testing assays is low, and thus the real number of affected patients is certainly underestimated. This in turn increases the spreading of the disease (To et al., 2020). In addition, the symptoms are commonly similar to normal cold and flu. So, accurate and early diagnosis of the disease is crucial in order to prohibit the fatality. In particular, early detection allows the clinicians to avoid serious complications in COVID-19 patients. Also, identification of negative individuals helps the removal of unnecessary quarantine time. In the current paper, novel developments in the diagnosis of COVID-19 and innovations especially in the context of biosensors and point of care settings will be discussed.

MATERIAL AND METHODS

1. Rapid and portable detection devices

The nonspecific manifestations of COVID-19 require an urgent need for rapid diagnosis of suspected individuals and an exact screening (Yang et al.,2020). Point of care settings which are rapid, efficient, cost-effective and do not need experts are demanded (Nguyen et al., 2018). These devices could be utilized in emergency situations (Baroneet al., 2020).This would inhibit rapid spreading of the diseases (Nguyen et al., 2020). However, the current standard methodology is based on the real-time reverse transcription-polymerase chain reaction (rRT-PCR) with five steps (Berry et al., 2016) that include sample collection, storing the specimens, transmission to the laboratory, testing protocol and result reporting. In addition, sample transportation to the rRT-PCR central laboratory is time-consuming and thus postpones obtaining the results (Berry et al., 2016; Chu et al., 2020). This is a pivotal drawback in the COVID-19 outbreak since can lead to the rapid distribution of the disease. The need for technical expertise and being very expensive are other problems (Liaw et al., 2012; Cho et al., 2014). Also, co-detection of coronaviruses with other respiratory viruses via PCR might result in false positive cases (Fig. 1) (Cho et al., 2014).

2. Loop-Mediated Isothermal Amplification (LAMP) Assays in PoC devices

Surrogate molecular amplifications methods to overcome the limitations of RT-PCR technique are required. A novel nucleic acid amplification method with high efficiency and specificity under isothermal situations is loop-mediated isothermal amplification (LAMP) (Fig. 2). A DNA polymerase with strand displacement function and a series of four primers are used in the LAMP to fabricate several DNA copies in a short time that are stem-loop with multiple inverted repeats of the target and cauliflower-like shape (Nagamine et al., 2002). In addition to high sensitivity and specificity, simple protocol has turned it into an appealing method in the realm of molecular biology and pathogen detection. Instead of heat denaturation, LAMP used strand-displacement polymerases to synthetize a single-stranded template. The main advantage is diminishing the use of energy in thermocycler because it runs in a constant temperature. In comparison with PCR, this technology is more stable and sensitive (Labarrere et al., 2011; Galvez et al., 2020). So, there is a great hope that LAMP could be a potential candidate in point-of-care detection of COVID-19. In the project of Veterinary validation of point-of-care diagnostic instrument), LAMP has been utilized as a point-of-care device in detection of some respiratory viruses such as avian influenza virus (Galvez et al., 2020). In this project the detection time was then 60 min. also, combination of LAMP with disposable polymer chips as a lateral flow strip would be beneficial. The example for this method might be COVID-19 IgM/IgG Rapid Test of BioMedomics (Cassaniti et al., 2020) with the sensitivity of approximately 89%.

3. New technologies for SARS-CoV-2 detection

Biosensors are novel detection tools which mix the selectivity properties of a biomolecule with the sensitivity of a transducer (Kurbanoglu, 2020). Biosensors can achieve rapid, reliable, real time and sensitive diagnosis of different diseases (Sin et al., 2014). Different types of biosensors have formerly been applied in the detection of infectious diseases and pathogens (Fig. 3) (Sin et al., 2014).

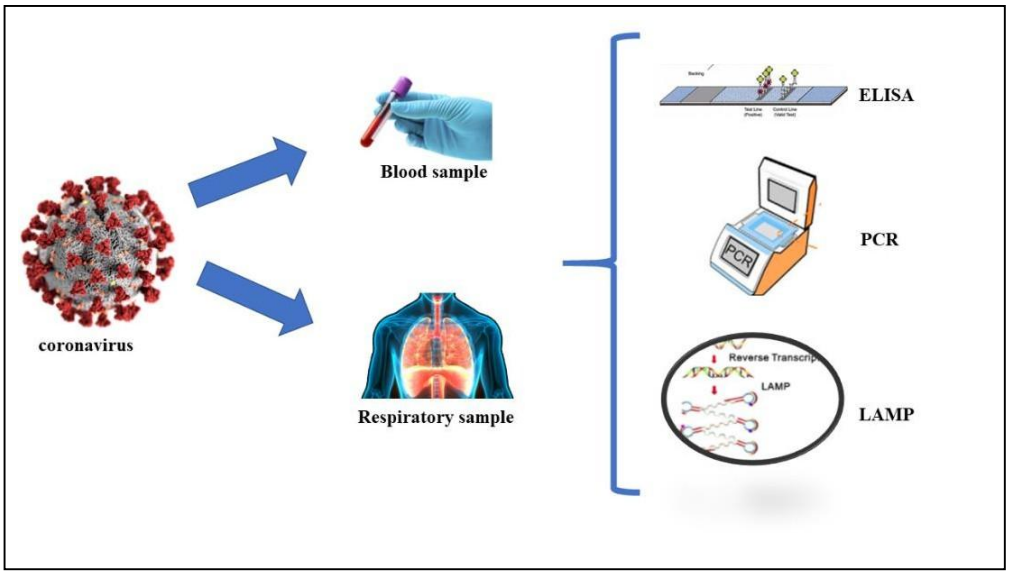

Fig. 1. Current diagnostic methods for SARS-CoV-2 detection from respiratory and serum samples.

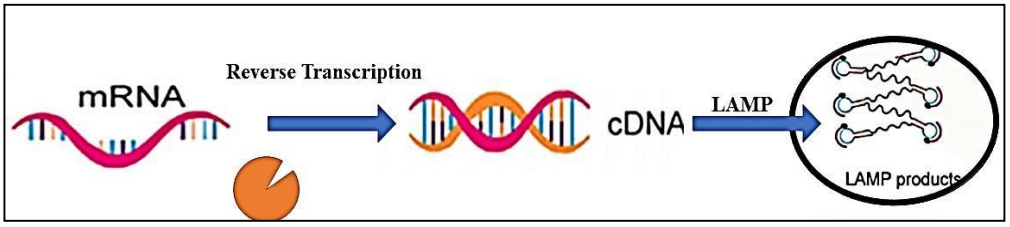

Fig. 2. Illustration of -LAMP amplification method.

The trapping of conductive nanoparticles with smaller wavelength of light is the basis of an optical sensor termed as Localized surface plasmon resonance (LSPR). Coherent localized plasmon oscillation is generated by the interaction of the incidence light and surface electrons in the conduction band. Local alterations such as the differences in the refractive indices and molecular binding change the resonance frequency (Petryayeva and Krull, 2011). Viral sequences such as ORF1ab COVID, RdRp-COVID and SARS-CoV-2 e genes havebeen detected via localized surface plasmon resonance (LSPR) sensing transduction and Dual-functional plasmonic biosensor utilizing plasmonic photothermal (PPT) impact. The in situ hybridization of RdRp of SAR S–CoV-2 and its complementary DNA has been increased using a converted PPT heat energy near the gold nano-islands. Photodermal increased LSPR produced a higher slope compared to the system destitute of photothermal effect. This biosensor could distinguish between SARS-CoV and SARS- CoV-2 viruses. However, a false positive response signal was achieved for RdRp-SARS sequence in the absence of photothermal effect. The detection limit for this fabricated device was estimated to be 0.22 pM (Qiu et al., 2020).

The basis for the field effect transistor (FET) is the regulation of carrier mobility through a biased semiconductor owed to the electrostatic field (Fig. 4). The selective diagnosis of special targets is provided with the covering of the gate surface which is covered with a layer that could be altered using different biomolecules (Ahmad et al., 2020).

The SARS-CoV-2 spike protein S1 was detected using a graphene FET modulated with an antibody of SARS-CoV-2 spike S1 subunit protein (CSAb) or angiotensin-converting enzyme 2 (ACE2).

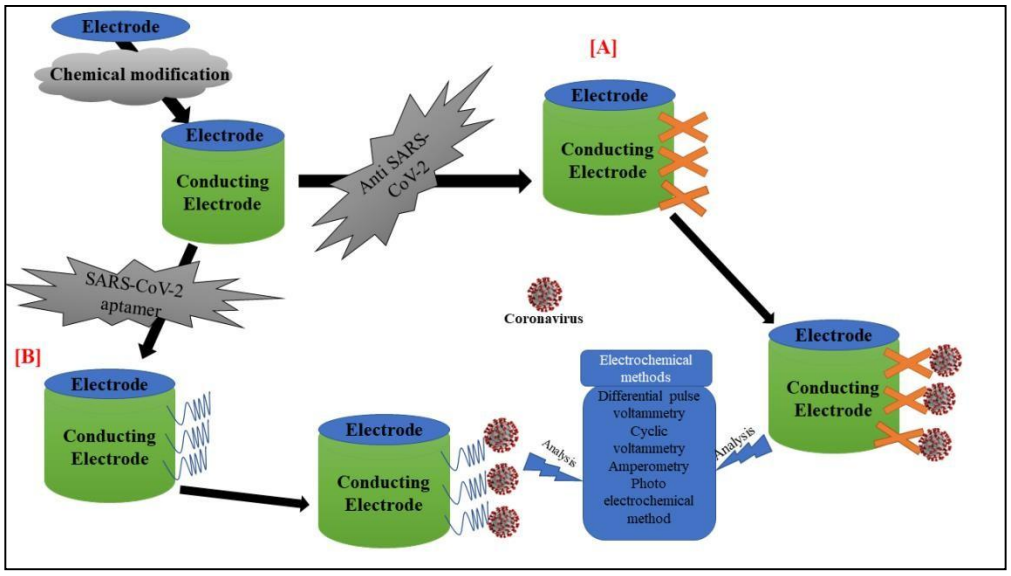

Fig. 3. Electrochemical SARS-CoV-2 sensor. Detection of COVID-19 using specific transducer system (SARS-CoV-2 specific antibodies [A] and Aptamers [B])

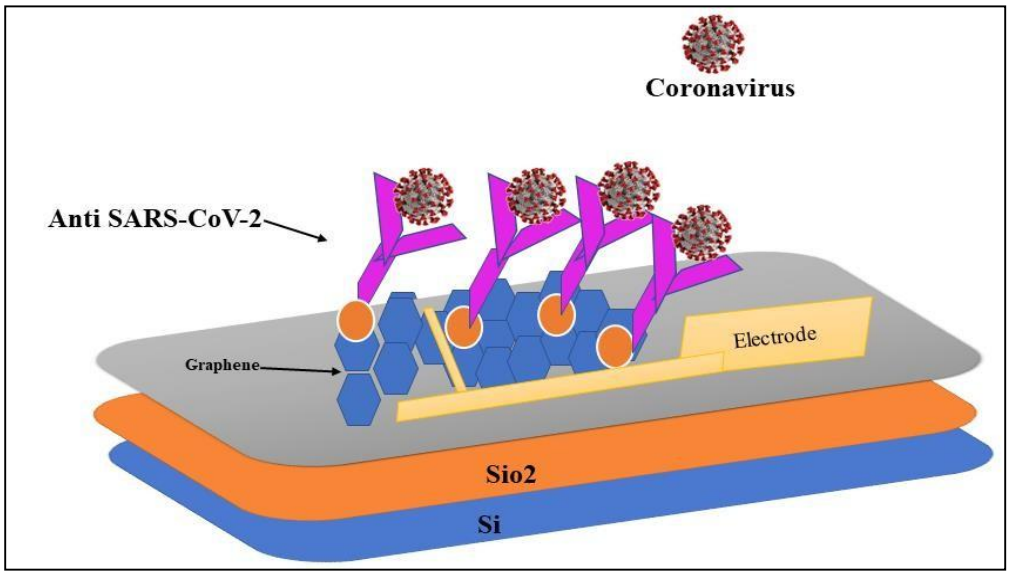

Fig. 4. Field-effect transistor biosensor

The graphen surface encompasses CDAb/ACE2 receptors and the binding of S1 protein with a positive charge mediate the conduction and resistance in the FET. In this study, the CSAb-altered graphene FET showed higher affinity to the antibody and thus a higher sensitivity with an LOD of 0.2 pM (Yüceet al., 2020).

The alterations in channel surface potential of the FET and its impact on the electrical impulse has provided the platform for detection of SARS-CoV-2. The S protein as the main transmembrane protein in the virus and acts as a great antigen but exhibits diversities in the amino acid sequences in coronaviruses.

The SARS-CoV-2 S1 antigen was also detected with a Cell-based potentiometric biosensor A membrane-engineered renal cell changed with

the SARS-CoV-2 SpikeS1 antibody. The antigen/antibody interaction changes the potential of the membrane. Eight gold screen printed electrodes along with polydimethylsiloxane (PDMS) layer with eight wells was the structure of the device. Protein adding was done subsequent to the addition of suspension of the modified membrane and the signal was measured by a potentiometer. The detection limit was estimated to be 1fg/ml which was great (Mavrikou et al., 2020).

CONCLUSION

Recent developments in the field of biosensing technology, and molecular systems including LAMP microfluidics are promising to improve the quality and efficiency of diagnostics and will replace RT-PCR**.** Additional researches are needed to improve sensitivity, reproducibility, reliability of new detection methods. Furthermore, developed systems should analyze samples from diverse routes to confirm the results. The production of POC devices is still an urgent need to sensing of pathogens on-site without the need for trained personnel.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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SARS-CoV-2–nin xəstə üzərində COVID-19 testi ilə sürətli və sadə aşkarlanması

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COVID-19 bir ictimai sağlamlıq problemi olaraq dünya miqyasında yayılmışdır və tomoqrafiya görüntüsü, əks transkripsiya-polimeraza zəncir reaksiyası (RT-PCR), fermentə bağlı immunosorbent analizi (ELISA) və hüceyrə kultivasiyası daxil olmaqla müxtəlif metodların birləşməsi SARS-CoV-2-nin aşkar edilməsi və müəyyən olunması üçün inkişaf etdirilmişdir. COVID-19-un müalicəsi üçün spesifik antiviral maddələrin və ya peyvəndlərin olmaması səbəbindən onun erkən aşkarlanması və identifikasiyası çox vacibdir.Təcili yardım əməliyyatına başlamazdan əvvəl bioloji mayeləri nümunə olaraq istifadə edən səhiyyə işçiləri tərəfindən mütəmadi olaraq istifadə edilə bilən alternativ, həssas, sürətli xəstə üzərində aşkarlama vasitəsi arzuolunandır. Döngə vasitəçiliyi ilə izotermik gücləndirmə (LAMP) testləri, antikor testləri və mikrofluik RT-PZR cihazları (çip laboratoriyası) ilə daha təsirli diaqnostika və nəzarət texnologiyalarının inkişafı üzrə işlər davam etdirilir.POC diaqnostika, SARS-Cov-2 aşkarlanmasında ümidvericidir və alimləri konsepsiya

xaricində texnologiyaları inkişaf etdirməyə təşviq edir. SARS-CoV-2 üçün inkişaf etdirilmiş əks transkripsiya LAMP (RT-LAMP) metodu, bir saatdan az müddət ərzində tüpürcək nümunələrində belə virusu aşkar edə bilər. "Lab-on-a-chip" qurğusu kiçik ölçülü mikrosxem, mikrokanal, mikroelektrodlar və mikroqızdırıcıdan ibarətdir. Hüceyrə lizisi, DNT ekstraksiyası və PZR amplifikasiyası mərhələləri bu mikroçiplərə inteqrasiya edilə bilər.

Açar sözlər: *COVID-19, RT-PZR, polimer çip, termosikl, fövqəladə hallar, biosensorlar*

Быстрое и простое обнаружение SARS-CoV-2 с тестированием COVID-19 в присутствии пациента

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COVID-19 как проблема мирового общественного здравоохранения распространился по всему миру. Для обнаружения и идентификации SARS-CoV-2 разработаны различные методы, включая томографию, полимеразную цепную реакцию с обратной транскрипцией (RT-PCR), иммуноферментный анализ (ELISA) и культивирование клеток. Из-за отсутствия специфических противовирусных агентов или вакцин для лечения COVID-19 раннее обнаружение и идентификация имеют жизненно важное значение. Желательно было бы, чтобы медицинские работники, использующие в качестве образца биологические жидкости, перед началом оказания скорой медицинской помощи (POC) имели в наличии альтернативный чувствительный инструмент быстрого обнаружения, который мог бы регулярно ими использоваться. В настоящее время предпринимаются усилия по разработке более эффективных технологий диагностики и наблюдения с использованием тестов с петлевой изотермической амплификацией (LAMP), тестирования на антитела и применением микрофлюидных устройств RT-PCR (Lab-on-a-chip). POC-диагностика перспективна в обнаружении SARS-Cov-2 и побуждает ученых, совершенствующих технологии, выходить за пределы концепции. Метод обратной транскрипции LAMP (RT-LAMP), разработанный для SARS-CoV-2, может обнаружить вирус даже в образцах слюны менее чем за час. Устройства типа «лаборатория на чипе» содержат микросхему небольшого размера, микроканал, микроэлектроды и микронагреватель. В эти микрочипы можно интегрировать этапы лизиса клеток, экстракции ДНК и ПЦР-амплификации. Благодаря быстрому обнаружению, небольшому объему образца и интеграции с ПЦР в портативной крошечной системе, эти устройства являются многообещающими для обнаружения SARS-CoV-2. Необходимо повысить достоверность и чувствительность всех вышеупомянутых методов для использования образцов слюны; в случае улучшения они могут предоставить возможность обнаружения вируса в слюне без периода ожидания и сложной аналитической инфраструктуры.

Ключевые слова: *COVID-19, ОТ-ПЦР, полимерный чип, термоциклер, аварийные ситуации, биосенсоры*