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Biosensors and Bioelectronics 173 (2021) 112777

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: http://www.elsevier.com/locate/bios

Developments in biosensors for CoV detection and future trends

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ARTICLE INFO

Keywords:
Biosensor
Coronavirus
Immunosensor
DNA-Sensor
Viral detection
COVID-19

ABSTRACT

This review summarizes the state of art of biosensor technology for Coronavirus (CoV) detection, the current challenges and the future perspectives. Three categories of affinity-based biosensors (ABBs) have been developed, depending on their transduction mechanism, namely electrochemical, optical and piezoelectric biosensors. The biorecognition elements include antibodies and DNA, which undergo important non-covalent binding interactions, with the formation of antigen-antibody and ssDNA/oligonucleotide-complementary strand complexes in immuno- and DNA-sensors, respectively. The analytical performances, the advantages and drawbacks of each type of biosensor are highlighted, discussed, and compared to traditional methods.

It is hoped that this review will encourage scientists and academics to design and develop new biosensing platforms for point-of-care (POC) diagnostics to manage the coronavirus disease 2019 (COVID-19) pandemic, providing interesting reference for future studies.

1. Introduction

Coronaviruses are a large family of viruses that usually cause mild to moderate upper-respiratory tract illnesses, like the common cold (Peiris, 2012).

There are hundreds of coronaviruses, most of which circulate among animals, such as pigs, camels, bats and cats. Sometimes those viruses jump to humans, a process called “spillover”, and cause a disease. To date, seven known human coronaviruses (HCoV) have been identified that affect the human population (Table 1). Four of them (HCoV-229E, HCoV-NL63, HCoV-OC43 and HCoV-HKU1) cause only mild disease, being responsible of approximately one-third of common flu infections in humans (Lim et al., 2016; Fung and Liu, 2019; Pene et al., 2003; Vijgen et al., 2005; Van der Hoek, 2007; Walsh et al., 2013). Three of them can cause more serious, even fatal, disease: SARS coronavirus (SARS-CoV), MERS coronavirus (MERS-CoV) and the novel SARS coronavirus (SARS-CoV-2).

SARS-CoV emerged in November 2002 in China and caused the severe acute respiratory syndrome (SARS), characterized by fever, cough and pneumonia, which might develop into acute respiratory distress. It was highly transmissible with a high mortality rate of about 10%. The virus spontaneously disappeared in 2004 (McBride and Fielding, 2012; Peiris et al., 2004).

MERS-CoV causes the Middle East respiratory syndrome (MERS). It is transmitted from an animal reservoir in camels. It was identified in September 2012 and continues to cause sporadic and localized outbreaks, mostly in the Middle East region (Assiri et al., 2013).

The third novel coronavirus, which emerged recently in this century, is called SARS-CoV-2, for its genetic similarities with SARS-CoV (Paudel et al., 2020; Wu et al., 2020). It causes coronavirus disease 2019 (COVID-19), which emerged in China in December 2019 and was declared a global pandemic by the World Health Organization (WHO) on March 11, 2020 (Udugama et al., 2020; Wang et al., 2020; WHO-World Health Organization, 2020; Zhu et al., 2020b). The COVID-19 clinical manifestations can range from very mild symptoms to life-threatening conditions (N. Chen et al., 2020; Huang et al., 2009). Some people may be asymptomatic or have only flu-like symptoms, while others may experience worsened symptoms, such as interstitial pneumonia, possibly caused or accompanied by a thrombophilic vasculitis in the lung (Boraschi, 2020).

All coronaviruses are enveloped positive single-stranded RNA viruses with a diameter of about 100 nm and a “crown-like” characteristic surface, responsible for their name (“corona” in Latin means “crown”). There are four types, depending on the sequence of entire viral genomes: α-, β-, γ- and δ-coronavirus. SARS-CoV, MERS-CoV and SARS-CoV-2 belong to the β-coronavirus family (Wang et al., 2020). Genoma analysis of SARS-CoV-2 sequences showed that the complete genome sequence recognition rates of SARS-CoV and bat SARS coronavirus were 79.5% and 96%, respectively (Wang et al., 2020). Therefore, the bat origin of SARS-CoV-2 seems to be the most probable hypothesis (Chan et al., 2013; Hu et al., 2015).

Compared to SARS-CoV and MERS-CoV, with a mortality rate of 10%

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https://doi.org/10.1016/j.bios.2020.112777
Received 8 May 2020; Received in revised form 24 October 2020; Accepted 27 October 2020
Available online 28 October 2020

and 35% respectively, the novel SARS-CoV-2 has higher transmissibility and infectivity but lower mortality rate (Huang et al., 2009; Liu, 2020), although it led to over 1,150,000 deaths globally, as of October 24, 2020.

Since MERS and COVID-19 are highly contagious diseases with the potential to cause a pandemic, in absence of a specific vaccine or effective therapeutic drugs, it is of extreme importance to find rapid and accurate detection methods for control and prevention of virus spread. The key-point to control a virus outbreak is usually the isolation of individuals presenting mild infection symptoms through a strict quarantine, thus blocking the infection transmission process. During the current COVID-19 pandemic, the Centers for Disease Control and Prevention (CDC) established the requirement of at least two negative test results in a row, at least 24 h apart, during the quarantine period before a person is declared recovered. People with more severe symptoms are normally sent to hospitals, where they receive inappropriate treatments, as no specific antiviral drugs have been developed, with the consequent overloading of the hospitals which become the main places of spread of the virus (Signorelli et al., 2020). Another important issue are the asyntomatic individuals who were diagnosed with SARS-CoV-2 infections but without any relevant clinical symptoms. Increasing evidence has shown that asymptomatic individuals can efficiently spread the virus, causing serious difficulties in the control of the epidemic (Long et al., 2020).

Therefore, early diagnostic tests, based on sensitive, specific, accurate and rapid methods, are crucial for successful outbreak containment. An adequate detection system is essential in helping to stop or decrease the spread of a virus outbreak before human and economic consequences become devastating.

There are two main biorecognition strategies for detection of virus diseases: i) detection of viral nucleic acid (DNA and RNA); ii) detection of specific viral biomarkers, such as antigens or antibodies generated against the virus by the patient's immune system response (Boonham et al., 2014; Ozer et al., 2020).

Recent traditional methods of detection of these analytes include polymerase chain reaction (PCR) (Shen et al., 2020) and enzyme-linked immunosorbent assay (ELISA), which can directly detect DNA/RNA and antibodies/antigens, respectively. Both methods show high sensitivities but present some disadvantages, as they require virus isolation and the use of sophisticated laboratory equipment. They are also difficult to use at the point-of-care (POC), requiring well-trained staff, expensive instruments and time-consuming processes. Moreover, some viruses are hard or impossible to cultivate (Krejcova et al., 2015).

Therefore, the development of rapid, accurate, miniaturized devices for virus detection useable at the POC is still needed.

Biosensors may represent a valid alternative, as they offer a rapid detection of viral diseases with high sensitivity and selectivity. A typical biosensor consists of three components: a biological element, a transducer for converting the recognition process into a quantitative signal, and an electronic system for amplification and signal processing, as schematized in Fig. 1. On the basis of the biological element being used, biosensors can be divided into four classes: enzymatic, antibody/antigen-based, nucleic acid/DNA-based and whole cells-based (Kawamura and Miyata, 2016). Depending on the transducer type being used, biosensors can be classified as: i) electrochemical biosensors (Ronkainen et al., 2010; Thévenot et al., 2001), in turn divided into amperometric, potentiometric, FET (field effect transistor)-based (Mazarin de Moares and Tatsuo Kubota, 2016; Vu and Chen, 2019) and impedimetric biosensors; ii) optical biosensors (Long et al., 2013); iii) thermal biosensors; iv) piezoelectric biosensors (Sklàdal, 2016). The classification of these biosensors is represented in Fig. 1.

Additionally, another classification can be made on the basis of the biorecognition principle: i) catalytic biosensors (typically with enzymes and cells); ii) affinity-based biosensors (ABBs) (typically with DNA, antibodies and aptamers) (Kawamura and Miyata, 2016). As schematized in Fig. 2, the principle of the biocatalytic role is the conversion of the analyte (A), during the chemical reaction by the biological element (B), to form a product (P), able to generate a signal measurable by a transducer. In case of the bioaffinity role, the analyte (A) is bound specifically and selectively by the biological element (B) to form a complex (AB), detectable by a transducer. ABBs are considered the most suitable biosensors for virus detection, especially for the reversible interaction between analyte and biological element, allowing the biodevice reuse (Antiochia et al., 2015, 2016; Pejcic et al., 2006).

Among the ABBs, biosensors employing antibodies or antigens as biological elements are referred as "immunosensors". On the basis of the detection principle, ABBs can be divided into two groups: "label-free", based on the direct measurement of the signal produced during the biochemical reaction, and "label-based or labelled" biosensors, based on the indirect measurement of the signal generating from a specific label, which makes them highly sensitive. Label-based biosensors can be, in turn, subdivided into two main formats, competitive and non-competitive ("sandwich" format), depending on their mechanism, as shown in Fig. 3.

Both label-based and label-free affinity biosensors are used for viral detection, including immunosensors, DNA-sensors and aptamer-based sensors (aptasensors) (Fig. 1) (Mollarasouli et al., 2019; Van der Kieboom et al., 2015).

The peculiar characteristics of the ABBs biosensors allow them to complement current methods of screening and monitoring for an early warning of a viral disease outbreak, especially when *in situ* and real-time analysis is required. Thanks to the recent progress in electronics, the ABBs biosensors can be miniaturized as *lab-on-chip* or handheld devices for on-site monitoring (Lafleur et al., 2016; Zhu et al., 2020a).

Moreover, the recent development of nanotechnology provides a powerful tool to improve the performances of the ABBs. Nanomaterials have been largely used as signal amplifiers to improve the sensitivity of the biosensors, thanks to their excellent conductivity and extraordinary photoelectrochemical properties (Holzinger et al., 2014; Mokhtarzadeh et al., 2017; Zhang et al., 2009; Mujawar et al., 2020).

The objective of this review is to address the developments of ABBs for coronavirus detection. The review covers papers that have been published in the last 15 years and is structured into three main sections, depending on the type of the biosensor transduction mode. Another

Table 1
Human coronaviruses.

Coronavirus	Strains	Coronaviriniae Genera	Discovery	Host	Symptoms	References
Human	HCoV-229E	α	1966	bats	mild	Pene et al. (2003) Vijgen et al. (2005)
Human	HCoV-NL63	α	2004	civets bats	mild	McBride and Fielding, 2012
Human	HCoV-OC43	β	1967	cattle	mild	Van der Hoek (2007) Walsh et al., 2013
Human	HCoV-HKU1	β	2005	mice	mild	Lau et al., 2006
Human	SARS-CoV	β	2002	civets bats	severe	McBride and Fielding, 2012
Human	MERS-CoV	β	2012	bats	severe	Assiri et al. (2013)
Human	SARS-CoV-2	β	2019	bats	severe	Udugama et al. (2020)

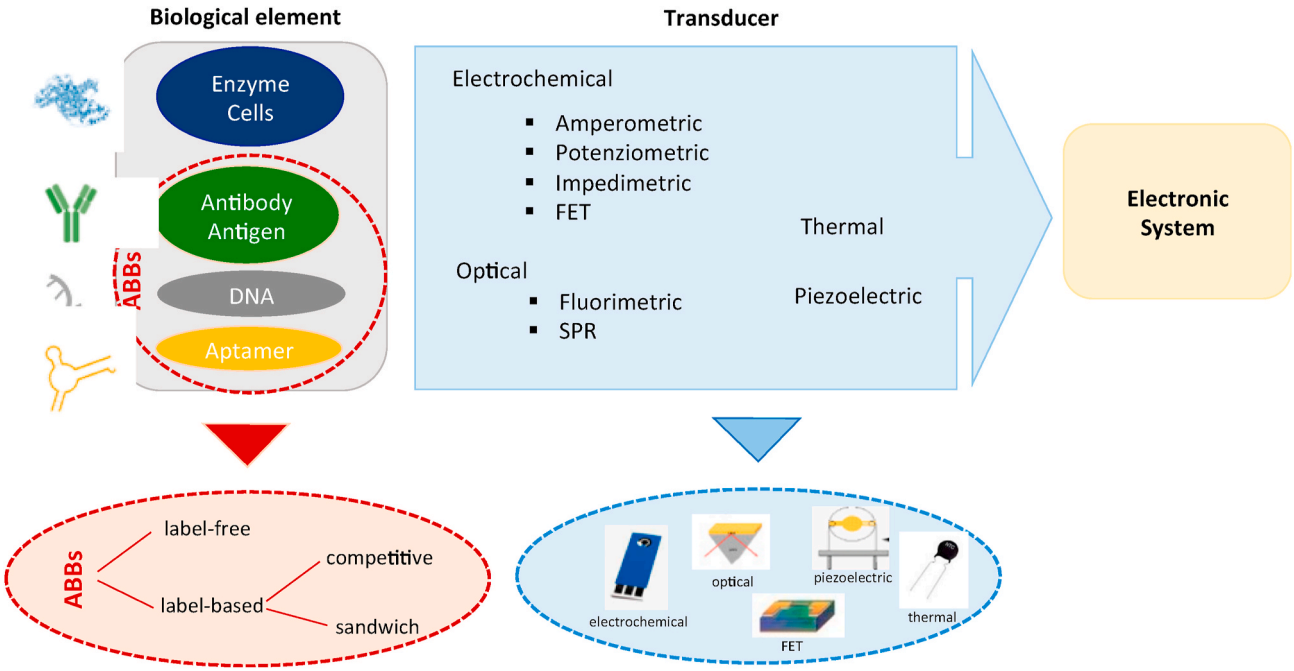

Fig. 1. Schematic illustration of typical biosensor components and biosensor classification: catalytic biosensors (blu circle); immunosensors (green circle); DNA-sensors (grey circle) and aptasensors (yellow circle). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

section has been specifically dedicated to the current methods of detection of SARS-CoV-2, with particular attention to the biosensing devices, as most of the CoV research today is focused on COVID-19 management.

2. Electrochemical biosensors

The electrochemical transduction shows several advantages compared to other transduction methods, such as low cost, high sensitivity, ease of miniaturization for POC use and relatively simple instrumentation. Biosensors involving amperometric detection usually employ an electroactive label, as both antibody/antigen and DNA hybridization reactions do not generate a significant signal on their own. Many of the studies reported in literature employ ferro/ferricyanide, as redox probe. The current signals arising from non-specific adsorption of proteins or other biomaterial and the biofouling of the electrode surface represent the main limitations of this type of biosensor. For this reason, a great deal of effort has to be devoted to control the surface structure, especially for measurements in complex matrices, such as blood (Thévenot et al., 2001). One common strategy to prevent non-specific binding (NSB) is the use of blocking reagents, such as bovin serum albumin (BSA), gelatin and casein, which occupy all the remaining NSB sites after the adsorption of the coated protein (Balcer et al., 2003). However, when using a complex biological sample such as serum, these blocking solutions might not be enough. Chemical modification and functionalization of the electrode surface is generally performed to suppress the NSB and, at the same time, to enhance the biocompatibility of the electrode surface towards antibodies or proteins and the biosensor sensitivity. Thiol terminated polyethylene glycol (PEG) has become quite popular for reducing NSB, by forming self-assembled monolayers (SAM) on the metal coated sensor electrode, providing also functional groups for surface immobilization (Contreras-Naranjo and Aguilar, 2019).

The first electrochemical biosensor for SARS-CoV detection was developed by Ishikawa and coworkers (2009). It is a FET-based immunosensor, where the change in conductance generated by the antigen-antibody binding can be measured and correlated to the analyte concentration. The virus antigen nucleocapsid N protein has been used as SARS biomarker. Instead of conventional antibodies, antibody mimic proteins (AMPs) have been utilized as affinity binding agents. These AMPs can be easily produced *in vivo* and are smaller and more stable than normal antibodies. The FET sensor has been opportunely modified with a fibronection-based protein (Fn) as AMP capture agent to selectively bind the antigen N protein. The exposed gate region of FET-based immunosensor was modified with In_2O_3 nanowires on a Si/SiO_2 substrate in order to improve the immobilization of the AMPs and the signal transducing. At the working pH = 7.4, the N proteins are positively charged and therefore their binding on a p-type channel causes depletion of charge carriers (holes) and a consequent decrease in conductance. The so-developed platform was able to detect the N protein at sub-nanomolar concentrations, with a sensitivity comparable to current immunological detection methods, but with a shorter time and without the need of labelled reagents.

In 2019, Layqah and Eissa (2019) described the first amperometric immunosensor for MERS-CoV virus detection. In particular, the spike protein S1 was utilized as MERS biomarker. The biosensor's working principle is an indirect competition between the free virus in the sample and immobilized MERS-CoV recombinant spike protein S1 for a fixed antibodies concentration added to the sample. The immunosensor was realized on an array electrodes system to allow the simultaneous detection of different types of human coronavirus. The surface of the carbon electrodes was modified with gold nanoparticles (AuNPs) in order to enhance the electrochemical properties of the electrode, providing a higher surface area and a faster electron transfer rate. Successively, MERS-CoV and HCoV antigens were immobilized onto the AuNPs/carbon electrode. The non-specific adsorptions were minimized by incubating the electrode in a BSA solution, in order to block the unreacted aldehyde groups and the free gold surface. The antibody concentration to be used for incubation of the antigen-modified electrode and the binding time were carefully optimized. The optimum conditions resulted to be 10 μg/mL and 20 min for antibody concentration and binding time, respectively. The current signal was obtained with square wave voltammetry (SWV), by measuring the peak current of the ferro/ferricyanide redox probe (label), properly added to the

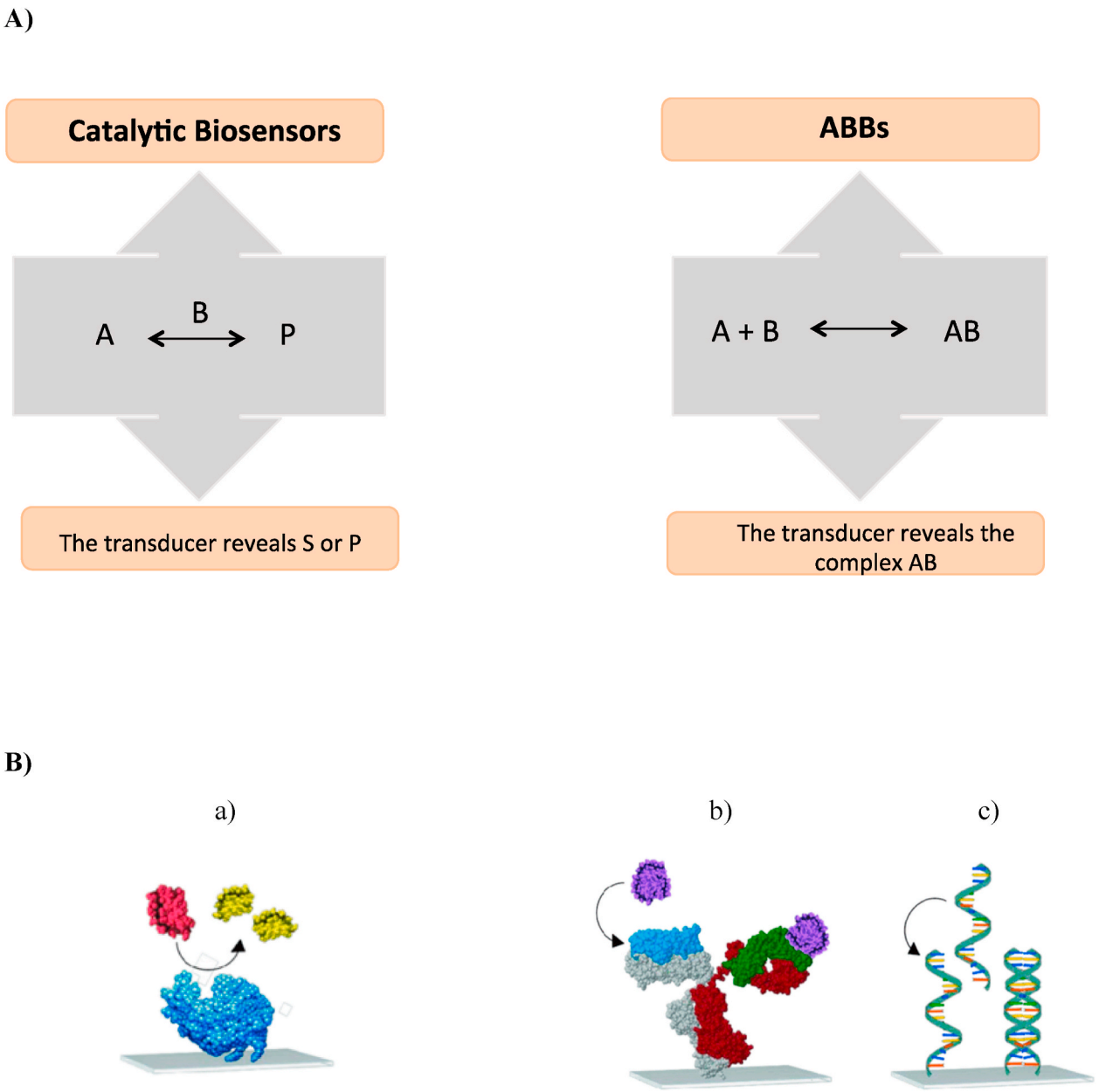

Fig. 2. Different mechanisms of catalytic and ABBs biosensors **(A)** and relative examples **(B)**: enzymatic biosensor (a), immunosensor (b) and DNA-sensor (c). A = analyte; B = biological element; P = product; AB = complex, i.e. antigen/antibody complex.

solution. When the antibodies bind to the immobilized antigens, a decrease of the SWV peak current is clearly observed, because of the "coverage" of the electrode surface by the antibody molecules. As a direct consequence, a decrease of both electron transfer efficiency and current is registered. The immunosensor showed a good linear response from 0.001 to 100 ng/mL and from 0.01 to 10.000 ng/mL for MERS-CoV and HCoV, respectively, and a very high sensitivity, with a detection limit of 0.4 and 1.0 pg/mL for MERS-CoV and HCoV, respectively, definitely lower values than those obtained with ELISA method (1 ng/mL) (Chen et al., 2015). The selectivity against virus proteins such as FluA and FluB resulted very good, attesting no cross-reactivity of the proposed biosensor. Moreover, the possibility of use of the biosensor for simultaneous multiplex detection of different types of CoV was confirmed, by mixing the two proteins MERS-CoV and HCoV on the same electrode surface. As for the sensor stability, the biosensor resulted to be stable for about 2 weeks, showing only 2% current decrease during this period. Finally, the proposed immunosensor was successfully tested in artificial nasal samples spiked with MERS-CoV and HCoV antigens, showing good recovery percentages and a good reproducibility (RSD 3–6%).

3. Optical biosensors

Although several types of optical transducers are used in affinity-based biosensing, fluorescence and surface plasmon resonance (SPR) are undoubtedly the most validated and assessed transduction techniques.

In particular, fluorescence measurements are of great interest thanks to their high sensitivity (Stefan et al., 2000). In most fluorescent-based immunosensors, molecules called fluorochromes are used to label the biomolecules and generate the fluorescent signal, as no fluorescence properties are usually exhibited by antigens and antibodies.

SPR technique can be considered the most advanced and developed optical label-free technology in recent biosensing. SPR transducers

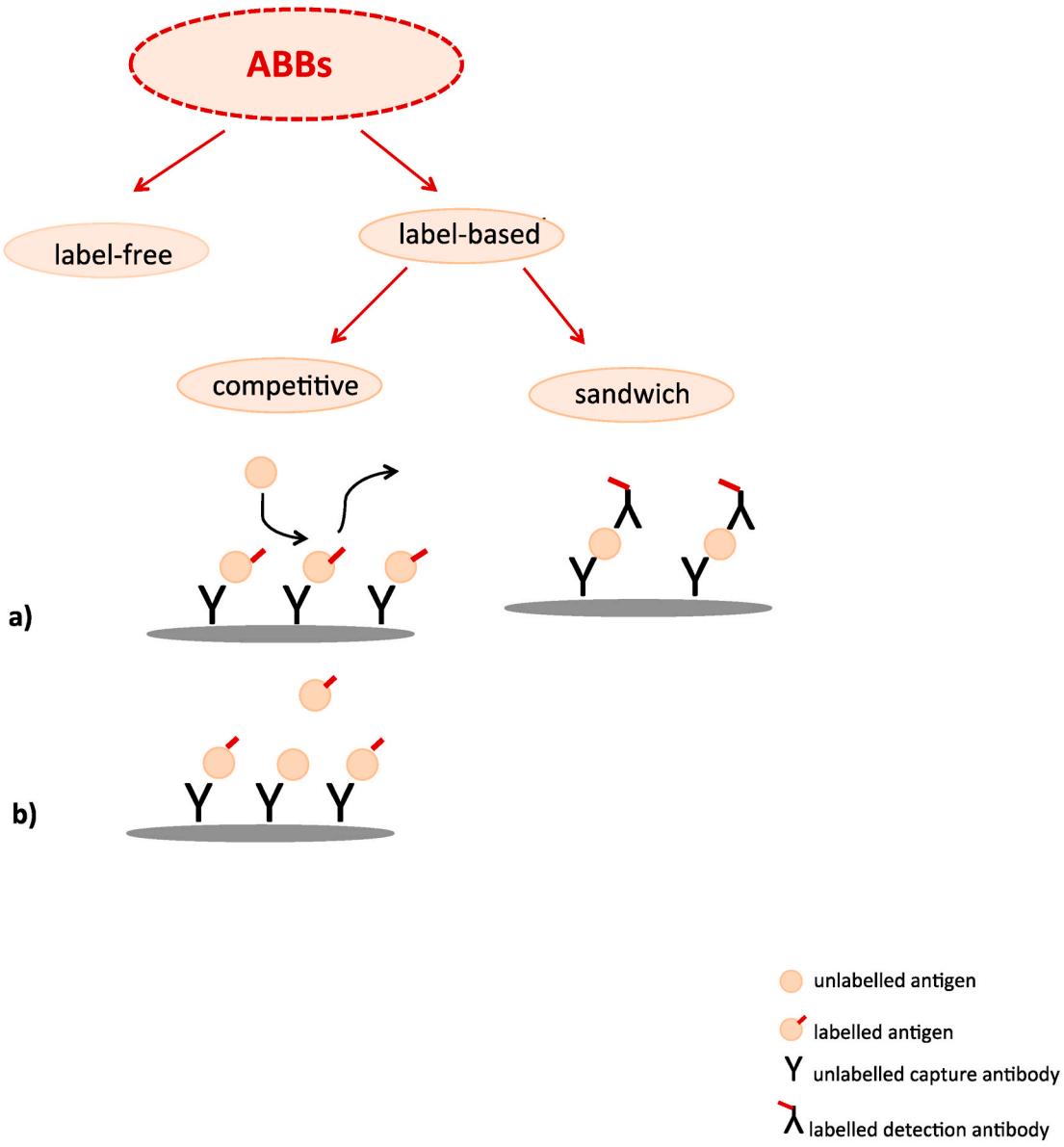

Fig. 3. Schematic classification of ABBs.

present interesting features compared to other physicochemical transducers, allowing real-time monitoring of bioanalytes, without the need of labelling. The biotransducer immobilized onto the sensor disk surface interacts with the analyte producing a local increase in the refractive index at the metal surface which promotes an SPR signal shift (Lu et al., 2000; Abdulhalim et al., 2008).

Unfortunately, also SPR based affinity biosensors present the drawback of the NSB phenomena onto the SPR disk, which can affect the accuracy of the measurements in biological fluids. Another problem is the difficulty of immobilization of large bioreceptor molecules because of their steric hindrance. The key issue to overcome these drawbacks is a proper sensor chip modification (Sarano et al., 2015). More importantly, SPR based biosensors show very high sensitivities, comparable to ELISA immunoassay (Gomara et al., 2000).

The first use of a SPR biosensor was carried out by Chen and co-workers for SARS-CoV morphological study (Chen et al., 2005). It was shown that SPR was able to verify that the N-terminal deleted proteinase dimer adopts a state different from that of the full-length proteinase dimer.

The first SPR-based biosensor for the diagnosis of SARS was developed by Park et al. (2009). The biorecognition element was the antigen SARS-CoV membrane-envelope (SCVme) protein, genetically fused to a gold binding polypeptide (GBP), able to bind a gold surface. The fusion proteins were directly self-assembled onto the SPR gold chip, thus realizing a specific sensing platform for anti-SCVme antibodies. The fusion protein-coated SPR chip showed a low detection limit of 200 ng/mL, high selectivity and a fast response time of 10 min. Selectivity studies were also carried out, using mouse IgG as negative controls, showing very low SPR responses by the NSB of mouse IgG, thus attesting no significant cross-reactivity of the proposed SPR immunosensor.

In the same year, Huang et al. (2009) described the first SPR fluorescence fiber-optic immunosensor for detection of SARS-CoV nucleocapsid protein N in human serum. Nucleocapsid protein N is one of the virus early expressed protein which can be detected one day after infection. Therefore, it is an important biomarker for an early diagnosis and for an accurate prevention of the virus spread. The biosensor combines the properties of a "sandwich immunoassay" with the localized surface plasmon (LSP) technique. Monoclonal antibodies against N (anti-N1) protein were immobilized on ELISA plates as capture antibodies for N protein and polyclonal anti-N antibodies were used as

detection antibodies. At the beginning of the assay, a rapid increase in fluorescence signal is registered as a large number of LSP coupled fluorescence (LSPCF) probes migrate into the evanescent field interaction region, close to the fiber core surface. However, some N proteins (target antigens) are captured by the immobilized anti-N1 protein antibodies (capture antibodies) on the fiber core surface and therefore not all LSPCF probes in the interaction region bind to N proteins, diffusing out of the interaction region, thus causing a decrease of the fluorescence intensity. A linear dependence between the fluorescence signal and N protein concentration was obtained over the range from 1 pg/mL to 10 ng/mL. The detection limit of the biosensor was found to be 1 pg/mL, much lower that the value reported for conventional ELISA assay (37.5 pg/mL) (He et al., 2005). A similar LOD value of 1.56 pg/mL was only obtained with a chemiluminescence enzyme immunoassay (CLEIA) based system (Fujimoto et al., 2008). The presented LSPCF fiber-optic biosensor presents many interesting and useful features, as it measures the fluorescence signal close to the reaction region resulting in a significant increase of the fluorescence efficiency (Chang et al., 1996), showing also a high specificity, thanks to the sandwich immunoassay configuration.

A fluorescence immunosensor has been realized by Weng and Neethirajan (2018) for rapid detection of the infectious bronchitis virus (IBV), an avian coronavirus which causes large economic loss in poultry industry. The biosensor is developed on a cotton thread-based microfluidic platform and utilizes the fluorescence resonance energy transfer (FRET) between the MoS_2 and a fluorescence dye labelling during the antibody-antigen interaction. MoS_2 has a strong fluorescence-quenching ability when applied to a dye-labelled antibody. The antibody probes are modified with fluorescent dye labelling and with MoS_2 (dyed-IBV-Ab and Mo_2S-IBV-Ab). In the presence of IBV, both antibody probes bind to the target IBV, forming the antigen-antibody complex. After binding, the fluorescence of the antibody probe modified with the dye is quenched, due to the transfer of energy between the closely connected dye molecules and MoS_2.

4. Piezoelectric biosensors

Piezoelectric detection is based on the principle that frequency variations of a piezoelectric quartz crystal (PQC) correspond to mass changes, as a result of an affinity interaction event, such as antibody-antigen interaction or DNA hybridization (Holford et al., 2012).

In a general way, a piezoelectric biosensor can be constructed by immobilising a receptor (antibody, nucleic acid, etc.) onto the surface of a PQC and monitoring the frequency changes, due to the binding of the specific ligand (antigen, nucleic acid, etc.). The increased mass, associated with the biorecognition reaction, results in a decrease of the oscillating frequency.

Although initially in 1990's some researchers have evaluated that the detection limit with the piezoelectric method is inferior compared to electrochemical and optical detectors (Ivnitski et al., 1999), more recently the large number of articles appeared in literature clearly demonstrated that piezoelectric immunosensors are one of the most sensitive analytical instruments developed to date, especially for detection of a wide range of viruses, being capable of detecting antigens in the picogram range. Moreover, this type of device has the potential to detect antigens in the gas phase as well as in the liquid phase without the need of a label.

The first piezoelectric immunosensor reported in literature for coronavirus detection was published by Zuo et al. (2004), regarding an immunosensor for SARS-CoV detection in sputum. The horse policlonal antibodies against SARS-CoV were immobilized onto a PQC surface. The detection of the antigen was achieved by spraying it in the form of an aerosol via ultrasonic oscillation. In particular, the antigen powder was dissolved into the sputum of a healthy person and successively the solution was sprayed into the aerosol. The frequency shift obtained was proportional to the antigen concentration in the range 0.6–4 μg/mL. The biosensor showed very good reproducibility and stability, as it can be reutilized 100 times without a significant lack of activity, remaining stable for more than 2 months, if stored at 4–6 °C.

A second piezoelectric immunosensor, based on microcantilever technology, was realized by Velanki and Ji (2006) for feline coronavirus detection. Feline coronavirus is prevalent in the cat population causing a deadly disease, called feline infectious peritonitis. The FIP (feline infectious protein) type I virus antigen was used as biomarker. The silicon microcantilever surface was coated with a thick SiO_2 layer and then modified by immobilization of FIP virus type I polyclonal antibodies. The deflection amplitudes of the microcantilever resulted in proportion to the FIP I antigen injected into the fluid cell. The proposed biosensor allowed to detect FIP I with a detection limit of 0.1 μg/mL.

4.1. Current methods for COVID-19 detection

Current methods used for screening and diagnosis of novel COVID-19 are based on three different approaches: SARS-CoV-2 antigen detection in nasopharyngeal secretions through molecular biology techniques, computed tomography, and SARS-CoV-2 antibody analysis in serum using immunoassay methods (Carter et al., 2020).

4.2. Molecular biology assays

Among the molecular techniques, reverse transcription polymerase chain reaction (RT-PCR), a well documented technique used in medicine for around 20 year to detect genetic information, has been endorsed for clinical diagnosis of SARS-CoV-2 by both the WHO and the US CDC. Many laboratories have developed real time RT-PCR assays, which have several advantages over traditional RT-PCR, such as high specificity for SARS-CoV RNA, as they use internal probes as well as amplification primers, high sensitivity, with consistent detection limits of between 1 and 10 SARS-CoV RNA copies per reaction, fast reaction time and reduced risk of contamination in the laboratory, as real-time PCR assays operate as closed systems.

However, a number of false-negative and false-positive results have been reported (Liu et al., 2020). False-negative results can arise from poor sample collection or degradation of the viral RNA during shipping or storage. Application of appropriate assay controls that identify poor-quality samples can help avoid most false-negative results. The most common cause of false-positive results is contamination with previously amplified DNA. The use of real-time RT-PCR helps mitigate this problem by operating as a contained system. A more difficult problem is the cross-contamination that can occur between specimens during collection, shipping, and aliquoting in the laboratory. Liberal use of negative control samples in each assay and a well-designed plan for confirmatory testing can help ensure that laboratory contamination is detected and that specimens are not inappropriately labelled as SARS-CoV positive. In addition, any positive specimen should be retested in a reference laboratory to confirm that the specimen is positive.

Reverse transcription loop mediated isothermal amplification (RT-LAMP) is a more recent molecular technique where the amplification is conducted at a single temperature and does not need specialized laboratory equipment. Tests for COVID-19 with RT-LAMP are still being assessed in clinical settings. Both molecular methods have the known drawback to give information only if the patient is currently infected and are not suitable for POC testing (Carter et al., 2020; Udugama et al., 2020).

4.3. Computed tomography

Due to false negative and positive results of RT-PCR, computed tomography (CT) scans started to be used in several hospitals as a clinical diagnostic tool for COVID-19 (Udugama et al., 2020). Chest CT scans are a non-invasive procedure consisting in taking many X-ray measurements at different angles to obtain cross-sectional images. This method shows

higher sensitivity but a lower specificity compared to RT-PCR, as the imaging features overlap with other viral pneumonia (Ai et al., 2020).

4.4. Serological antibody assays

Among the immunoassay methods, ELISA for detecting immunoglobulin G, M and A (IgG, IgM, IgA) from human serum of COVID-19 patients is in development. It has the advantage to be a simple, cheap and quick method to be done in a normal laboratory. It must be stressed that antibody assays are the most reliable indicators of SARS-CoV infection when applied to convalescent-phase serum specimens. In some patients, antibody becomes detectable within 8–10 days, and most have detectable antibody by 2 or 3 weeks. However, some persons do not develop detectable antibodies until 28 days after onset of illness. Overall, the medium seroconversion time for IgA, IgM, and IgG are 4–6, 4–6, and 5–10 days post symptom onset, respectively. IgA and IgM detection show the highest sensitivity during about 4–25 days after illness onset, and therefore they can provide a better diagnosis outcome in early stages compared to IgG, which reachs its peak during 21–25 days after illness onset, and stays at a relatively high reading until 31–41 days, thus being powerful for diagnostics at later stages (Ma et al., 2020). For these reasons, antibody tests have limited diagnostic use. They do not serve for an early diagnosis and can be used as a complement to the virus detection tests for patients presenting late, after symptoms onset, to healthcare facilities or when virus detection tests are negative despite strong indications of infection. Moreover, more importantly, antibody tests are utilized for sero-epidemiological surveys and studies.

According to the Food and Drug Administration (FDA) Emergency Use Authorizations (EUAs) for COVID-19 diagnostics, in addition to the most common ELISA method, there are two serological assays used for the detection of antibodies generated against SARS-CoV-2, the chemiluminescence immunoassays (CLIA) (Cai et al., 2020) and the lateral flow immunoassays (LFIA). LFIA is an immunochromatography test commonly used for pregnancy tests, utilizing the same principle as ELISA and useable at the POC. These tests are rapid (10–30 min), can be utilized directly by the patients but are more expensive for a large screening than normal ELISA tests and suffer from poor analytical sensitivity (Carter et al., 2020). Chen and co-workers have recently presented a simple and rapid LFIA that uses lanthanide doped nanoparticles for detecting *anti*-SARS-CoV-2 IgG in human serum with high diagnostic accuracy (Z. Chen et al., 2020).

There are important issues about serological antibody assays that still need to be clarified, such as to establish whether the *anti*-SARS-CoV-2 antibodies can be considered neutralizing, their persistence in blood and the possible cross-reactivity with other coronaviruses, with the risk of false-positive results. The duration of immunity after infection is a key issue for the "immunity shield", which gives protection against short-term or long-term reinfection and for taking important decisions on physical distancing and social restrictions.

Despite more than 300 serological assays for COVID-19 have been developed, the FDA has approved only 12 serological tests intended for use in clinical laboratories under the emergency use authorization (EUA), among which the most commonly used in Italy are CLIA tests, produced by Abbott Laboratories and Diasorin (Carter et al., 2020).

4.5. Biosensors for COVID-19 detection

Biosensors based on specific biomolecular interactions offer an alternative and reliable solution to current methods for clinical diagnosis of COVID-19, due to their high sensitivity, low-cost, easy to use and possibility of POC utilization.

Up to date, there are only two papers describing affinity-based biosensors for COVID-19 detection.

Seo and co-workers proposed a new FET based biosensor for SARS-CoV-2 virus detection. The sensing area of the device is a graphene sheet, modified with SARS-CoV-2 spike antibody, properly immobilized onto the graphene sheet surface, as schematized in Fig. 4. The device was able to detect the SARS-CoV-2 antigen spike protein at concentrations as low as 1 fg/mL in phosphate buffer, value much lower than that reported with ELISA platform. Moreover, the biosensor was tested in the universal transport medium (UTM), used for suspending the nasopharyngeal swabs for real clinical analysis. No reagent contained in UTM affected the measurements and therefore the COVID-19 FET biosensor can be successfully utilized to detect antigens in clinical samples without any preparation or pre-processing. Furthermore, the device exhibited no measurable cross-reactivity with MERS-CoV antigen (Seo et al., 2020).

A dual biosensor integrating the plasmonic photothermal (PPT) effect and the LSPR sensing transduction on a single chip has been proposed by Qiu and co-workers. The sensor chip was modified with a two dimensional distribution of gold nanoislands (AuNIs) and successively functionalized with complementary DNA (cDNA) receptors by forming Au–S bonds between the AuNIs and the thiolic groups of cDNA (Fig. 5). The proper surface functionalization suppresses the non-specific binding events, thus increasing the sensitivity of the biosensor. The dual-functional biosensor exhibited a linear range between 0.1 pM and 1 mM with a detection limit of 0.22 pM. The *in situ* PPT enhancement on the AuNIs chips significantly improved the hybridization kinetics and the specificity of nucleic acid detection. Similar multiple non-specific gene sequences from SARS-CoV and SARS-CoV-2 were tested and discriminated, attesting the high selectivity of the biosensor towards cross-reactive and interfering sequences (Qui et al., 2020).

Most recently, PathSensors Inc. announced the development of a "Canary" fast biosensor to detect the novel SARS coronavirus. The proposed platform utilizes a cell-based immunosensor that couples capture of the virus with signal amplification and provides a result in 3–5 min. The initial application of the PathSensors device will be for testing of environmental swabs and air monitoring in sensitive spaces, such as hospitals, offices, food services, etc. Validation data of the new biosensors will be available in July 2020 (PathSensors, 2020).

4.6. COVID-19 biomarkers

When assessing a patient with COVID-19 infection, the identification of effective biomarkers, different than immunoglobulins, can be useful to clinicians in starting treatment, monitoring the progression of the disease and closing monitoring. Moreover, on the basis of biomarkers values, patients can be classified into different risk groups for a better clinical management and prevention of serious complications.

Of course, the identification of novel biomarkers is related to the understanding of the viral pathogenetic mechanisms, as well as cellular and organ damage. The analysis of recently published studies highlights the role of systemic vasculitis and cytokine mediated coagulation disorders as the principal actors of multi-organ failure in patients with severe COVID-19 complications (Ponti et al., 2020).

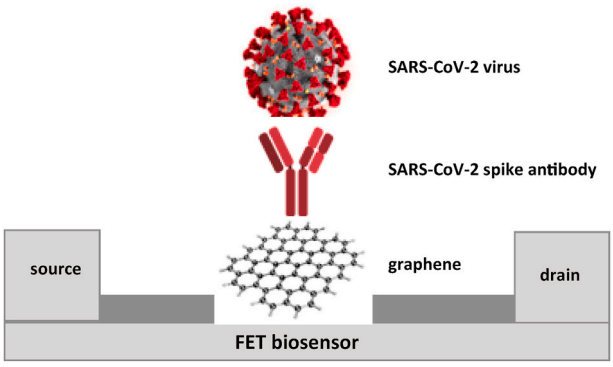

Fig. 4. Schematic representation of the FET based biosensor for SARS-CoV-2 virus detection (Seo et al., 2020).

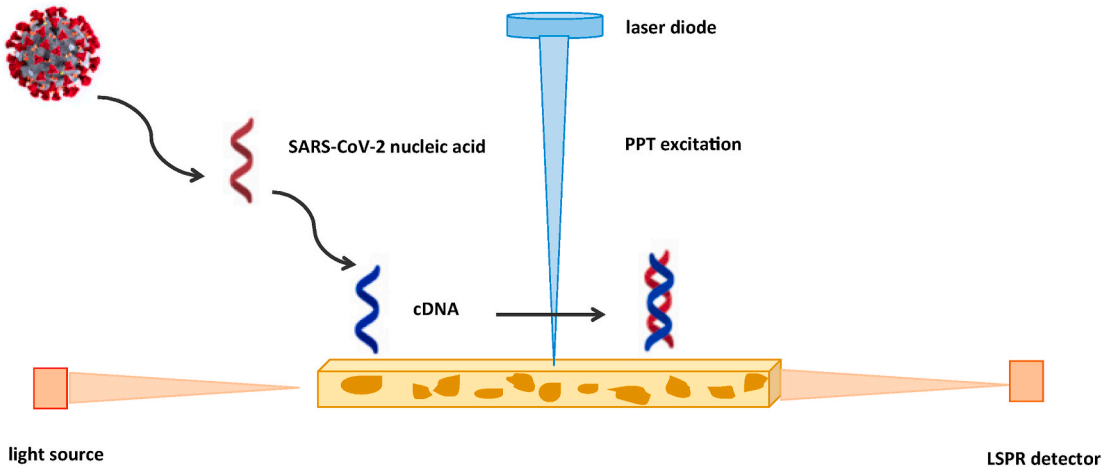

Fig. 5. Schematic representation of the dual plasmonic photothermal LSPR biosensor for SARS-CoV-2 virus detection (Qui et al., 2020).

It seems that hematological (lymphocyte count, neutrophil count), inflammatory (C-reactive protein, erythrocyte sedimentation rate, interleukin-6), and especially biochemical (D-dimer, troponins, creatine kinase) biomarkers are strongly correlated with severe prognosis or *exitus* in COVID-19 patients and can therefore be used in clinical practice as predictive biomarkers (Kermali et al., 2020; Morales-Narvaez and Diner, 2020).

In addition to the above discussed laboratory parameters, novel biomarkers are under investigation, among which homocystein, angiotensin II, -(1–7), -(1–9) and alamandine, in order to clearly determine their predictive clinical value as indicators of severe prognosis in COVID-19 patients (Ponti et al., 2020).

In this context, the development of novel biosensing devices or the modification of existing ones for the multiplexing and simultaneous detection of the above mentioned biomarkers is another challenging approach to perform effective assessment of clinical progresses or critical trends of COVID-19 infection.

5. Conclusions

Biosensors represent an attractive tool in diagnostics, as they have the potential to detect the outbreak of a virus, crucial for the control and prevention of the disease.

This review describes the recent developments in fabrication of ABBs for CoV detection. The reported papers prove that electrochemical, optical and piezoelectric biosensors offer advantages over conventional methods, such as RT-PCR and ELISA tests, due to their characteristics, such as fast response time, low cost, easy-to-use, portability, real-time and *in situ* analysis. The main characteristics of the biosensors reported in this review are summarized in Table 2. It is interesting to note that the amperometric (Layqah and Eissa, 2019) and FET-based (Seo et al., 2020) biosensors achieved detection limits lower than picomolar levels, thanks to the nanomaterials employed in their fabrication, AuNPs and graphene, respectively.

Further improvements in terms of ABBs sensitivity and selectivity will certainly be obtained by developing novel nanostructured biosensing platforms. Another interesting feature of the biosensors is the

Table 2
Characteristics of ABBs for coronavirus detection.

Disease	ABBs type	Transducer	Biomarker	Biosensor platform	Biosensor format	Linear range	LOD	Cross-reactivity	References
SARS	immunosensor	FET	antigen N-protein	Si–SiO_2/In_2O_3 nanowires/AMP fibronectin	label-free	–	sub-nM	–	Ishikawa et al. (2009)
MERS	immunosensor	amperometric	antigen spike protein S1	AuNPs/MERS-antigen/MERS Ab	label-based competitive	0.001–100 ng/mL	0.4 pg/mL	no	Layqah and Eissa (2019)
SARS	immunosensor	SPR	anti-SCVme Ab	Au/GBP-SCVme antigen	label free	–	200 ng/mL	no	Park et al., 2009
SARS	immunosensor	SPR	antigen N-protein	SARS Ab	label-based sandwich	0.001–10 ng/mL	1 pg/mL	–	Huang et al. (2009)
avian IBV	immunosensor	fluorescence	IBV antigen	cotton thread/MoS_2/IBV Ab	label-based	–	–	–	Weng and Neethirajan (2018)
SARS	immunosensor	piezoelectric	SARS-CoV antigen	PQC/SARS-CoV Ab	label free	0.6–4 μg/mL	–	–	Zuo et al. (2004)
FIP	immunsensor	piezoelectric	FIP type I antigen	MC/SiO_2//FIP type I Ab	label free	–	0.1 μg/mL	–	Velanki and Ji 2006
COVID-19	immunosensor	FET	antigen spike protein S1	graphene/SARS-CoV_2 Ab	label free	–	1 fg/mL	no	Seo et al. (2020)
COVID-19	DNA-sensor	PPT + LSPR	SARS-CoV-2 nucleid acid	AuNIs/cDNA	label free	0.1 pM −1 mM	0.22 pM	no	Qui et al., 2020

List of abbreviations: Ab = antibody; AMP = antibody mimics proteins; SCVme = SARS-CoV membrane-envelope protein; GBP = gold binding polypeptide; PQC = piezoelectric quartz cristal; IBV = infectious bronchitis virus; MC = microcantilever; FIP = felin infectious protein; PPT = plasmonic photothermal localized SPR; AuNIs = gold nanoislands; cDNA = complementary DNA.

possibility to use microarrays integrated within the device, thus allowing multiplexing simultaneous virus detections.

Although the reported biosensors demonstrated surprising characteristics, some of them still need to be validated in real samples.

6. Future perspectives

There is an urgent and growing need for reliable diagnostic solutions for early detection of viral diseases, especially COVID-19. Early detection can support important decisions in efficiently managing epidemiological and infection control measures, allowing to isolate patients in a timely manner, in order to cut off the route of transmission and take the necessary safety measures, thus facilitating the return to normal human, social and working activities.

Currently, the conventional diagnostic systems for COVID-19 are expensive and located in hospitals or specialized laboratories (Carter et al., 2020). Rapid serological tests are in development. They have been designed to give a fast result (10–30 min compared to 4–5 h for conventional methods) and for use in hospitals or near to the POC. However, they are still available for healthcare professionals, and not for patients directly.

The need of a rapid home test kit, easily useable by patients is expected to be in high demand. The fundamental concept of the POC is to carry out the test in the most comfortable and immediate way for the patient, who can take the test, obtain immediate medical reports and receive the first treatment directly at home, without having the discomfort to go to the reference hospital, where the risk of COVID-19 infection is very high. Nano-enabled ABBs possess the ideal requirements for miniaturization and therefore for POC applications.

Another important strength of ABBs is their wireless link capability, which allows the transmission of the measured data to a remote medical database or to a health care provider. The measured data could be automatically uploaded via Bluetooth to the patient's smartphone or tablet and then directly transferred to Health Centers, thus monitoring the disease outbreak (Zhu et al., 2020). Moreover, a big data "Internet of Things" (IoT) system for healthcare is emerging, so that machine learning and artificial intelligence (AI) approaches can be used to extract the maximum amount of information from the analytical responses of the developed biosensors and to allow the results to rapidly inform Health Authorities to tackle infection disease outbreak, to make epidemiological models and to prevent novel pandemic outbreaks.

Unfortunately, at present, a wireless IoT ABB for COVID-19 is not available.

In summary, as future research, it is highly recommended to scientists to invest a lot of effort in developing AI and IoT supported nano-based biosensing devices as diagnostics tools to manage COVID-19 pandemic and to prevent other possible disease outbreaks.

CRediT authorship contribution statement

Riccarda Antiochia: Conceptualization, Writing - review & editing, Visualization.

Declaration of competing interest

The author declares that she has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

In the "competitive" format (steps a and b), an analyte displaces bound labelled analyte, which is then detected or measured. In the "sandwich" format an unlabeled analyte is "sandwiched" between two antibodies, the unlabeled capture antibody and the labelled detection antibody.

Acknowledgments

The author thanks Sapienza University of Rome for financial support.

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