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Revisão

Novel electrochemical-biosensors for rapid detection of SARS-Cov-2

Novos biossensores eletroquímicos para detecção rápida de SARS-Cov-2

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ABSTRACT

This mini-review aimed to research the latest and most innovative platforms to detect SARS-CoV-2 and focused on electrochemical sensors based on virus-specific nucleic acid. The search for articles was performed using Web of Science, Scopus and Science Direct in the period of 2019-2022. The number of these researches decreased in 2022 due to the control of the pandemic, but the technologies involved in these developments have shown improvements in detection and possible application to other diseases.

Keywords: SARS-CoV-2; COVID-19; electrochemical sensors

RESUMO

Esta mini-revisão teve como objetivo pesquisar as plataformas mais recentes e inovadoras para detectar SARS-CoV-2 e focou em sensores eletroquímicos baseados em ácido nucleico específico do vírus. A busca de artigos foi realizada nas plataformas *Web of Science, Scopus* e *Science Direct* no período de 2019-2022. O número dessas pesquisas diminuiu em 2022 devido ao controle da pandemia, mas as tecnologias envolvidas nesses desenvolvimentos têm apresentado melhorias na detecção e possível aplicação para outras doenças.

Palavras-chave: SARS-CoV-2; COVID-19; sensores eletroquímicos

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread worldwide and promoted the COVID-19 pandemic. Its rapid dissemination generated this serious situation and required the urgent development of an accurate and rapid way to detect infected patients. Several systems have been developed and applied to virus detection, but several platforms have resulted in false positives or false negatives, for example those used in cassette format during the pandemic. The latter situation was more complicated because it allowed contamination to spread among healthy people and researchers were challenged to improve or create new platforms or systems to avoid false results.

Nowadays it is known that the antigen can be detected in nasopharyngeal swabs in suspected coronaviruses during the first days of infection and antibodies after a few of these days. Each case requires adequate systems for the correct diagnosis.

Reverse transcription-polymerase chain reactions (RT-PCR) has been considered the gold standard technique for diagnosing the coronavirus in the initial phase of infection by detecting its nucleic acid, the RNA of samples obtained by swabs of the nasal and oral mucosas. Any previous contact with the virus can lead to the production of specific antibodies such as immunoglobulin M or G (IgM or IgG) and demands platforms for serological tests. In the latter case, laboratories require platforms with characteristics such as simplicity, speed, sensitivity, accuracy, ease of use, price, and so on.

Due to the urgency to detect positive patients, several regulatory agencies have authorized the use of new systems for detecting antigens or antibodies after submitting analyzes from institutions with evidence of some of these characteristics to them. The coronavirus developed different mutations and then new virus lines started to spread and there were also many false positives or negatives. This fact led to the recall of several diagnostic systems, in addition to problems with the security of detection mechanisms.

Some requirements to become a popular platform for application or use in routine laboratories were previously mentioned and electrochemical biosensors were chosen for their high selectivity and sensitivity, possibility of portability, short analysis time, simplicity and low cost (1). These good features and technologies developed over the past 2 years have allowed them to be used on a number of new platforms and to detect viruses other than the coronavirus. There are two main categories of biosensors, immunological and nucleic acid based, but the latter has been considered better due to its DNA/RNA specificity and most researchers have been working based on this technology.

This work aimed to verify the best platform and new techniques for diagnosing coronavirus, as well as trends for other infections that can spread around the world.

We also emphasize that we do not intend to discuss specific details of the electronic elements for generating signals from each new type of device, but only to inform the public of general and brief characteristics of advances in diagnostic devices.

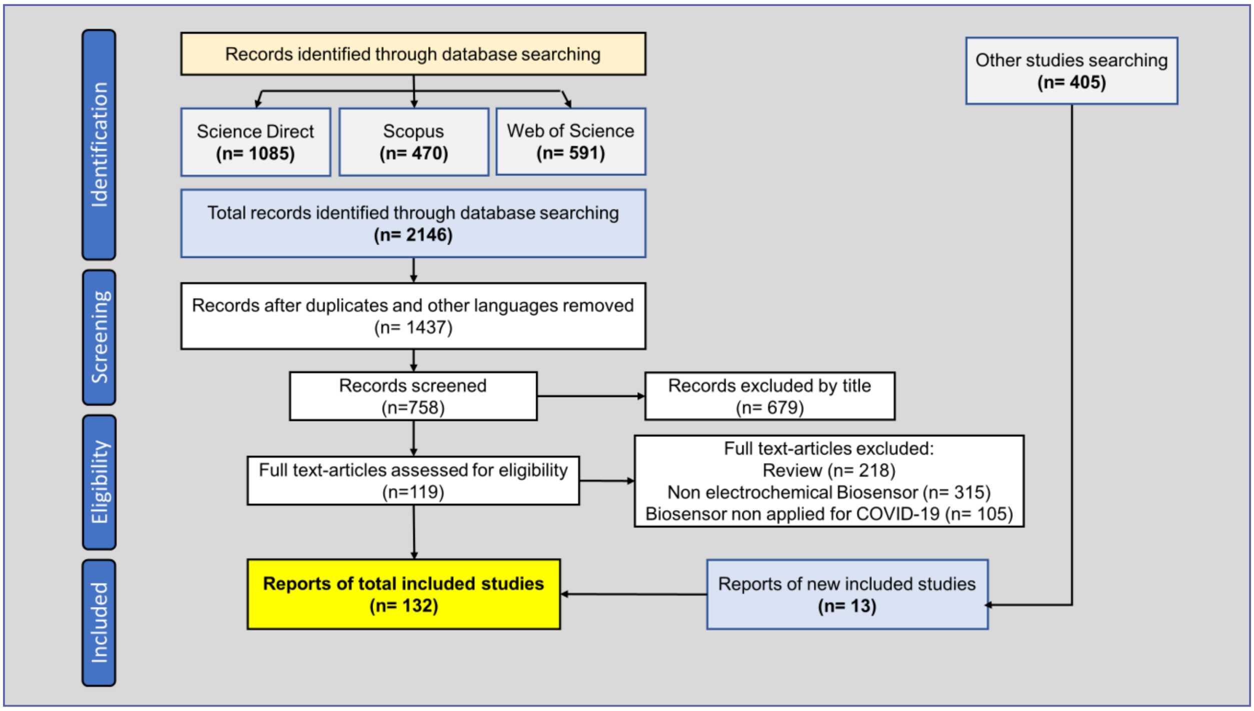
METHOD

The search for studies concerning electrochemical-biosensors was performed through the evaluation of keywords, titles, and abstracts. Articles published in English from 2019 to 2022 were considered. According to the exclusion criteria, studies that did not evaluate the applicability of electrochemical-biosensors in the detection of coronaviruses and those that used biosensors and electrochemistry, but were intended for the detection of other viruses that do not belong to the COVID-19, were disregarded. The search, organization and writing of the systematic review followed the preferred reporting elements for the systematic review and meta-analysis statement (PRISMA) (2). All available data obtained from the three scientific databases addressing electrochemical biosensors for coronavirus detection were included.

Table 1. Inclusion/exclusion criteria listed in order of application.

INCLUSION CRITERIA	EXCLUSION CRITERION
Articles in English	Non-English language articles
Electrochemical Biosensors	Short communications, reviews and thesis
Biosensors and coronavirus detection	Biosensors that do not function using an electrochemical transducer
electrochemical Immunosensors	Biosensors that were not applied for coronavirus detection

Figure 1. Scheme and results of studies included in this work with novel electrochemical biosensors.



RESULTS AND DISCUSSION

When the SARS-Cov-2 pandemic was confirmed worldwide, researchers made efforts to develop accurate and rapid tests to detect positive patients to treat them and prevent the spread of the disease by non-symptomatic individuals. Several platforms were developed, but false positives or false negatives were detected due to technical failures or emergence of new virus variants. Figure 1 presents a scheme for obtaining the main reports addressed in this mini-review and in Table 2 we summarize the main original articles published in 2022 on electrochemical sensors and present their characteristics in the subsequent paragraphs.

Table 2 – Main articles with novel techniques mentioned by Databases for year of 2022

Reference	Sensor / technique	Innovation
(3)	Electrochemical Immunosensor	Epitope based biosensor; specific to SARS-CoV-2 without interferences
(4)	Electrochemical genosensor	
(5)	Electrochemical biosensor	Use of salive
(6)	Electrochemical biosensor	Nanocrystals on paper electrode
(7)	Electrochemical immunosensor	ZnO and rGO-coated electrode and N-protein immunobiosensor

(1)	Electrochemical sensor	Novel biosensor based on gold without amplification
(8)	Impedance	NP polypirrole and gold particles nanosensors
(9)	Electrochemical sensor	CRISPR
(10)	Potentiometry	Magnetic beads and gold nanoparticles conjugated to ACE2 peptide to detection of virus in human saliva
(11)	Impedance	Compact and Low cost- sensitivity of fM
(12)	Impedance	CRISPR -Detection without amplification – sensitivity of fM
(13)	Electrochemical sensor	CRISPR - Carbon electrode
(14)	Electrochemical sensor	Aptasensor – sensitivity of fM

Legend: ACE2 –angiotensin-converting enzyme type 2; CRISPR – Clustered Regularly Interspaced Short Palindromic Repeats; fM – fentomolar; NP – nanoparticle; rGO – reduced graphene oxide; ZnO – zinc oxide.

Ameku et al. (2022) developed an electrochemical biosensor to detect serological IgG antibody in sera, specifically the spike glycoprotein epitope. The system consisted of electrodes with a surface modified with the virus peptide, the specific epitope of the SARS-Cov-2 spike glycoprotein in the case of COVID-19. This structure can capture its specific IgG antibodies and a redox molecule is generated, allowing variation of electronic signals that can be measured by the equipment. The researchers used sera obtained from 14 positive patients and 17 negative individuals for the diseases studied in order to evaluate the new system and obtained selectivity and specificity of 93% and 100%, respectively, and no cross-reaction when challenged against diseases such as Chagas disease, Chikungunya, Leishmaniasis and Dengue (3). This result is very important due to the similar symptoms between these diseases and another point demonstrated by them was the detection of infected and non-infected individuals even in samples with high serum dilutions. They also demonstrated low volume required for analysis as 2 nL by electrochemical biosensor instead of around 100 μL by ELISA (3). The authors cited the flexibility of this platform to be adapted to detect other pathogens or virus strains.

Cajigas et al. (2022) developed an electrochemical genosensor based on interaction with magnetic beads and detection by chronoamperometry. Initially, the authors evaluated the SARS-CoV-2 genome to identify some conserved bases and, based on this information, they constructed primers to use as targets and immobilization probes on magnetic particles. Since the structure was designed in the form of a sandwich, in the presence of viruses, the system can generate electrochemical signals that are detected by the instrument. The advantages of the system is its specificity and sensitivity in detecting concentrations at femtomolar (fM) levels.

The authors demonstrated also abilities to differentiate SARS-CoV-2 and other viruses such as Middle East Respiratory Syndrome (MERS), HKUI coronavirus (4). This platform can detect genetic material from infected patients even when they do not have symptoms.

Deng et al. (2022) developed an electrochemical biosensor based on target-triggered cascade signal amplification (5). The same group (Shi et al., 2021) previously developed a biosensor through an aptamer-functionalized nanochannel that detected the coronavirus in one step, and now they have improved detection by signal amplification (15). The new biosensor was tested on oropharyngeal swab samples from 5 patients and 5 healthy individuals and demonstrated high sensitivity for coronavirus RNA at low concentrations such as 45 fM levels. The authors cited the platform as low cost and easy to use, being a new option for the diagnosis of COVID-19.

Hatamluyi et al. (2022) designed a sensitive and specific electrochemical biosensor, a novel free-nucleic acid amplification and based on DNA probe specific for SARS-CoV-2 RNA immobilized on boron and gold nanostructures (1). This platform was applied to test a total of 120 samples from clinical and distinguished positive and negative groups with 100% sensitivity and 100% specificity, even in presence of interfering species in the extracted RNA matrices and required a short processing time, such as 30 min of incubation (1).

Hryniewicz et al. (2022) also worked with an electrochemical biosensor based on gold nanoparticles and detection of SARS-CoV-2 nucleocapsid protein (N) antibodies (8). In this study, the researchers tested the new system on positive and negative serum samples prepared by themselves and also on 10 SARS-CoV-2-positive samples provided by public health units. Preliminary results demonstrated good differentiation between positive and negative clinical samples. Similar results were obtained by Nascimento et al. (2022) who applied the angiotensin-converting enzyme 2 (ACE2) peptide on magnetic beads and gold nanoparticles to capture and detect the SARS-CoV-2 spike protein in human saliva (10). In this work, 32 samples were provided by the Hospital of Federal University of São Carlos (São Paulo, Brazil), 16 from healthy individuals and 16 from patients with Covid-19.

Salahandish et al. (2022) designed a binary sensing platform (BiSense) of an electrochemical immunobiosensor and demonstrated the possibility of clinical use with low cost and speed. Monoclonal N-Protein antibodies were prepared and immobilized on electrodes as a dual protein N immunobiosensor to monitor the bipotentiostat (BiSense) and measurement of impedance change associated with the immunoreaction. This apparatus could overcome the immuno-biosensor challenge regarding portability, cost and speed. According to the authors (11), the platform was 100 times cheaper than the marketed potentiostat, sensitive, fast detection (<1.5 min), very low detection limit (56 and 68 fg/mL for WE1 and WE2, respectively), less than 15 min to differentiate patients infected with COVID-19 and for early diagnosis. The researchers used the new system on 22 swabs with nasopharyngeal (NP) samples collected from 12 patients confirmed by the RT-PCR test and 10 from donors negative for SARS-Cov-2. Even with the advantages mentioned, the measurement results showed a variation of 10%, which was acceptable, but partially attributed to non-homogeneous samples collected with swabs and not due to the apparatus. This work shows the importance of reviewing several parameters to improve the system, since the advantages outweigh the difficulties experienced during the development of the sensors.

Table 3. Advantages of new technologies

References	System	Advantages
3	Electrochemical Immunosensor	- Detection and distinction of diseases such as Chagas disease, Chikungunya, Leishmaniasis and Dengue; need for low volumes for analysis, such as 2 nL.
4	Electrochemical genosensor	- Specificity and sensitivity of detection at fentomolar (fM) concentrations; abilities to differentiate SARS-CoV-2 and other viruses such as Middle East Respiratory Syndrome (MERS), HKUI coronavirus ; detection of genetic material from infected patients even without apparent symptoms.
5	Electrochemical biosensor	- High sensitivity for coronavirus RNA at low concentration as the limit of 45 fM; low cost and easy to using.
1	Electrochemical sensor	- Short processing time, such as 30 min of incubation.
11	(11) Impedance	- Low cost and speed; very low detection limit (fg/mL).

The detections of SARS-CoV-2 by gold standard methods demonstrated special and expensive equipment requirements, technicians with specific training and, consequently, high cost and time to reach accurate and safe results. Improvements must be sought and some results have been demonstrated by the use of the recent CRISPR system (clustered regularly interspaced short palindromic repeats) in electrochemical sensors. The first systems were based on DNA architecture applied to a gold electrode (9, 12) and, more recently, researchers such as Wu L et al. (2022) developed another ultrasensitive sensor with a carbon electrode (13). All these authors are looking for lower and more specific systems and have shown advantages in adapting them to detect other diseases. The number of samples tested in each article is not so large as to be validated for commercial purposes, but it can be considered for future improvements of the platform itself and extended to several viruses.

The new technologies require specific knowledge related to Immunology, Molecular Biology and Electronics. The development of biosensors associated with electronic measurements has also demonstrated the need for multidisciplinary professionals to overcome problems related to costs and speed to provide robust systems for detecting virus contamination in patients. During the peak of the pandemic, several systems were developed and tested, but antigen cassettes became more popular and faster for detecting COVID-19 and the number of articles with electronic sensors for this purpose has decreased. This fact does not invalidate the research because the new technologies can be used to improve devices for detecting other viruses.

CONCLUSIONS

When the SARS-Cov-2 pandemic was confirmed worldwide, researchers strove to develop accurate and rapid tests to detect positive patients in order to treat them and prevent the spread of the disease among non-symptomatic individuals. Several platforms have been developed, but false positives or false negatives can be detected due to technical failures or the emergence of new virus variants. RT-PCR is considered the gold standard method for detecting viral nucleic acid, but new platforms have been developed to improve sensitivity, accuracy, cost, ease of use and speed in obtaining results. Vaccines can help and decrease cases of death and, as a result, searches for diagnostic methods have also decreased. In addition, we verified a large number of new techniques for electrochemical biosensors with high sensitivity, lower cost, speed and ease than the first methods. Most of the researches were tested on real samples and almost all showed 100% sensitivity and 100% accuracy. With the control of the COVID-19 pandemic, the same platform can be adapted to detect other diseases, making the findings of these studies very significant for health science.

REFERÊNCIAS

1. Hatamluyi B, Rezayi M, Jamehdar SA, Rizi KS, Mojarrad M, Meshkat Z, Choobin H, Soleimanpour S, Boroushaki T. Sensitive and specific clinically diagnosis of SARS-CoV-2 employing a novel biosensor based on boron nitride quantum dots/flower-like gold nanostructures signal amplification. Biosens Bioelectron. 2022; 207: 114209. DOI: 10.1016/j.bios.2022.114209

2. Moher D, Liberati A, Tetzlaff J, Altman DG, Prisma Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009; 6(7): e1000097. DOI: 10.1371/journal.pmed.1000097

3. Ameku WA, Provance DW, Morel CM, De-Simone SG. Rapid detection of anti-SARS-CoV-2 antibodies with a secreen-printed electrode modified with a spike glycoprotein epitope. Biosensors. 2022;12:272. DOI: 10.3390/bios12050272

4. Cajigas S, Alzate D, Fernández M, Muskus C, Orozco J. Electrochemical genosensor for the specific detection of SARS-CoV-2. Talanta. 2022; 245:123482. DOI: 10.1016/j.talanta.2022.123482

5. Deng Y, Peng Y, Wang L, Wang M, Zhou T, Xiang L, Li J, Yang J. Target-triggered cascade signal amplification for sensitive electrochemical detection of SARS-CoV-2 with clinical application. Anal Chim Acta. 2022; 1208: 339846. DOI:10.1016/j.aca.2022.339846

6. Farzin MA, Abdoos H, Saber R.Graphite nanocrystals coated paper-based electrode for detection of SARS-CoV-2 gene using DNA-functionalized Au@carbon dot core shell nanoparticles. Microchem J. 2022;179: 107585. DOI: 10.1016/j.microc.2022.107585

7. Haghayegh F, Salahandish R, Hassani M, Sanati-Nezhad A. Highly stable buffer-based zinc oxide/reduced graphene oxide nanosurface chemistry for rapid immunosensing of SARS-CoV-2 antigens. ACS Appl Mater Interfaces. 2022; 14: 10844-10855. DOI: 10.1021/acsami.1c24475

8. Hryniewicz BM, Volpe J, Bach-Toledo L, Kurpel KC, Deller AE, Soares AL, Nardin JM, Marchesi LF, Simas FF, Oliveira CC, Huergo L, Souto DEP, Vidotti M. Development of polypyrrole (nano)structures decorated with gold nanoparticles toward immunosensing for COVID-19 serological diagnosis. Mater Today Chem. 2022; 24: 100817. DOI: **10.1016/j.mtchem.2022.100817**

9. Liu N, Liu R, Zhang J. CRISPR-Cas12a-mediated label-free electrochemical aptmer-based sensor for SARS-CoV-2 antigen detection. Bioelectrochemistry. 2022; 146: 108105. DOI: **10.1016/j.bioelechem.2022.108105**

10. Nascimento ED, Fonseca WT, Oliveria TR, Correia CRSTB, Faça VM, Morais BP, Silvestrini VC, Pott-Junior H, Teixeira FR, Faria RC. COVID-19 diagnosis by SARS-CoV-2 Spike protein detection in saliva using an ultrasentive magneto-assay based on disposable electrochemical sensor. Sens Actuators B Chem. 2022; 353: 131128. DOI:10.1016/j.snb.2021.131128

11. Salahandish R, Jalali P, Tabrizi HO, Hyun JE, Haghayegh F, Khalghollah M, Zare A, Berenger BM, Niu YD, Ghafar-Zadeh E, Sanati-Nezhad A. A compact, low-cost, and binary sensing (BiSense) platform for noise-free and self-validated impedimetric detection of COVID-19 infected patients. Biosens Bioelectron. 2022; 213 114459. DOI:10.1016/j.bios.2022.114459

12. Wu C, Chen Z, Li C, Hao Y, Tang Y, Yuan Y, Chai L, Fan T, Yu J, Ma X, Al-Hartomy OA, Wageh S, Al-Sehemi AG, Luo Z, He Y, Li J, Xie Z, Zhang H. CRISPR-Cas12a-empowered electrochemical biosensor for rapid and ultrasensitive detection of SARS-CoV-2 delta variant. Nanomicro Lett. 2022; 14: 159. DOI:10.1007/s40820-022-00888-4

13. Wu L, Wang X, Wu C, Cao X, Tang T, Huang H, Huag X. Ultrasensitivite SARS-CoV-2 diagnosis by CRISPR-based screen-printed carbon electrode. Anal Chim Acta. 2022; 1221: 340120. DOI:**10.1016/j.aca.2022.340120**

14. Yu M, Zhang X, Zhang X, Zahra Q, Huang, Z, Chen Y, Song C, Song M, Jiang H, Luo Z, Lu Y. An electrochemical aptasensor with N protein binding aptamer-complementary oligonucleotide as probe for ultra-sensitive detection of COVID-19. Biosens Bioelectron. 2022; 213: 114436. DOI:10.1016/j.bios.2022.114436

15. Shi L, Wang L, Ma X, Fang X, Xiang L, Yi Y, Li H, Luo Z, Li G. Aptamer-functionalized nanochannels for one-step detection of SARS-CoV-2 in samples from COVID-19 patients. Anal Chem. 2021; 93(49):16646-16654. DOI: 10.1021/acs.analchem.1c04156