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EFICÁCIA DIAGNÓSTICA DO BIOSSENSOR ELETROQUÍMICO COMPARADO AO RT-PCR PARA DIAGNÓSTICO DE COVID-19: UMA REVISÃO SISTEMÁTICA

Uberaba

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Fisiológicas, área de concentração I: Bioquímica, Fisiologia e Farmacologia, da Universidade Federal do Triângulo Mineiro, como requisito parcial para obtenção do título de mestre em Ciências Fisiológicas.
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RESUMO

Em dezembro de 2019, foi identificado na China, o novo coronavírus que recebeu o nome de SARS-CoV-2. O vírus se disseminou rapidamente e em março de 2020 foi decretada pandemia pela Organização Mundial da Saúde. Com a inicial falta de diagnósticos rápidos e tratamentos eficazes houve sobrecarga dos sistemas de saúde devido ao alto número de infectados e casos graves. As técnicas diagnósticas disponíveis atualmente possuem limitações e assim, a busca por novos métodos com técnicas sensíveis, rápidas, baratas e de uso local, como os biossensores eletroquímicos, vem sendo amplamente explorada. Diante das vantagens do desenvolvimento de biossensores eletroquímicos para diagnóstico sensível e seletivo, esta revisão sistemática teve como objetivo buscar as publicações referentes ao tema e responder a seguinte pergunta: O diagnóstico de COVID-19 realizado através de biossensor eletroquímico, nos indivíduos com suspeita da doença, é tão eficiente quanto o realizado por RT-PCR? Foi elaborado um protocolo de estudo seguindo as diretrizes PRISMA-DTA e registrado no PROSPERO sob o código de aprovação CRD42021282561. As buscas foram realizadas em seis bases de dados eletrônicas, foram aplicados os critérios de inclusão e exclusão e dezessete publicações foram selecionadas para esta revisão. Foi realizada extração dos dados e a análise do risco de viés foi feita através do QUADAS-2. Os resultados foram apresentados de forma qualitativa descritiva, não foi possível a elaboração de metanálise.

Palavra-chaves: Técnicas Biossensoriais; eletroquímica; diagnóstico; COVID-19.

ABSTRACT

In December 2019, the new coronavirus that was named SARS-CoV-2 was identified in China. The virus spreaded quickly and in March of 2020 it was declared a pandemic by the World Health Organization. Due to the initial lack of rapid diagnoses and effective treatments, the health systems were overloaded as a consequence of the high number of infected and severe cases. The diagnostic techniques currently available have limitations, for this reason, the search for new methods with sensitive, fast, cheap and locally used techniques, such as electrochemical biosensors, has been widely explored. Given the advantages of developing electrochemical biosensors for sensitive and selective diagnosis, this systematic review aimed to search for publications on the subject and answer the following question: The diagnosis of COVID-19 performed through an electrochemical biosensor, in individuals with suspected disease is it as efficient as that performed by RT-PCR? A study protocol was developed following the PRISMA-DTA guidelines and registered with PROSPERO under the approval code CRD42021282561. Searches were carried out in six electronic databases, inclusion and exclusion criteria were applied and seventeen publications were selected for this review. Based on the data, the analyse of risk was done using QUADAS-2. The results were presented in a descriptive qualitative manner it was not possible to carry out a meta-analysis.

Keywords: Biosensing Techniques; electrochemistry; diagnosis; COVID-19.

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SARS-CoV-2 - Coronavirus 2 da Síndrome Respiratória Aguda Grave

OMS – Organização Mundial de Saúde

RNA – Ácido Ribonucéico

ACE_2 – Enzima Conversora de Angiotensina 2

RT-PCR - Reação da transcriptase reversa seguida pela reação em cadeia da polimerase

LFIA - *Lateral flow immuno assay*

SUMÁRIO

1. INTRODUÇÃO

A pandemia de COVID-19, doença causada pelo vírus SARS-CoV-2 (sigla do inglês, que significa coronavirus 2 da síndrome respiratória aguda grave), impactou o mundo e trouxe consequências aos sistemas de saúde e economia. O elevado número de casos graves e óbitos fez com que pesquisadores buscassem formas de diagnósticos rápidos, tratamentos eficazes e o desenvolvimento de vacinas para conter a disseminação do vírus com brevidade.

1.1 Doenças Virais Emergentes

As questões relacionadas à saúde e a cura de doenças que garantam a sobrevivência da espécie humana é uma demanda histórico-social. Dessa forma, é sabido que as doenças infecciosas emergentes, causadas por vírus, ameaçam à saúde humana há vários séculos e pandemias globais já assombraram a humanidade. De acordo com Morens e Fauci (2020), em 1918 houve uma pandemia de gripe que levou mais de 50 milhões de pessoas à óbito. Recentemente passamos por vários surtos de doenças virais, como a gripe causada pela cepa H1N1 do vírus Influenza, Zika e Chikungunya (CHAIBUN et al., 2021).

O aumento das doenças infecciosas emergentes se deve a diferentes fatores, como por exemplo crescimento populacional e aproximação do ser humano a animais selvagens. Esse rápido aparecimento de novas doenças virais fez com que governos e estabelecimentos de saúde iniciassem adequações em seus serviços para melhor gerenciamento dos casos (CHIDIAC; FERRY, 2016; GRAHAM; SULLIVAN, 2018).

No entanto, vírus são micro-organismos com capacidade de mutação genética, o que permite uma adaptação rápida a diferentes ambientes, facilitando sua evolução e transmissão. A capacidade de driblar o sistema imune e de tropismo celular, também são fatores importantes que dificultam a identificação de tratamentos e vacinas (MORENS; FAUCI, 2020).

Neste sentido, a realização de pesquisas que busquem compreender mecanismos ecológicos e evolutivos destes organismos, representa um passo importante na tentativa de lidar melhor com surtos de enfermidades causadas por eles.

O mais recente surto de doença viral relacionado a infecção aguda do trato

respiratório foi o da COVID-19, assim denominada por ser causada pelo coronavírus e ter os primeiros casos relatados no ano de 2019. A COVID-19 causou impacto na saúde, economia e educação, mostrando nossa fragilidade em relação ao controle dos micro-organismos.

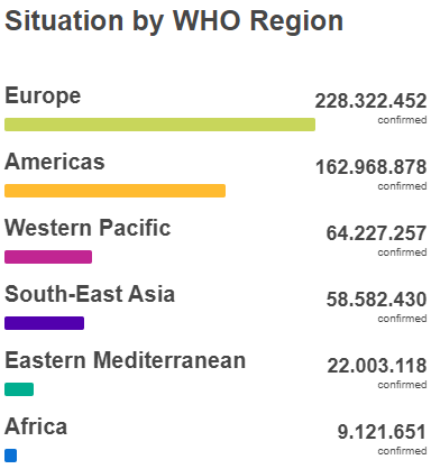
1.2 COVID-19

Em dezembro de 2019, foi identificado na cidade de Wuhan, em Hubei na China, o novo coronavírus que recebeu o nome de SARS-CoV-2. Conforme seu próprio nome diz, o SARS-CoV-2 é responsável por causar a síndrome respiratória aguda grave, com alta taxa de transmissão e contágio. A doença causada pelo SARS-CoV-2, foi denominada COVID-19 e se espalhou rapidamente. Antes do final de janeiro de 2020, já havia relatos de contaminação em outras localidades do mundo (RAHMAN et al., 2021; SHAHRIAR et al., 2021).

A Organização Mundial da Saúde (OMS) declarou a COVID-19 como pandemia global em março de 2020 e o mundo sofreu as consequências dessa rápida disseminação. (OPAS, 2022). A inicial falta de diagnósticos rápidos, tratamentos eficazes e contenção dos infectados, gerou um alto número de internações, o que aumentou os gastos e sobrecarregou os sistemas de saúde de diversos países (BÖGER et al., 2021).

De acordo com informações disponíveis pela OMS, até 01 de julho de 2022, o número de casos confirmados de COVID-19 em todo o mundo foi de 545.226.550, incluindo 6.334.728 mortes. A Europa era líder no número de casos, conforme apresentado na figura 1.

Figura 1. Situação de casos de COVID-19 por região mundial.

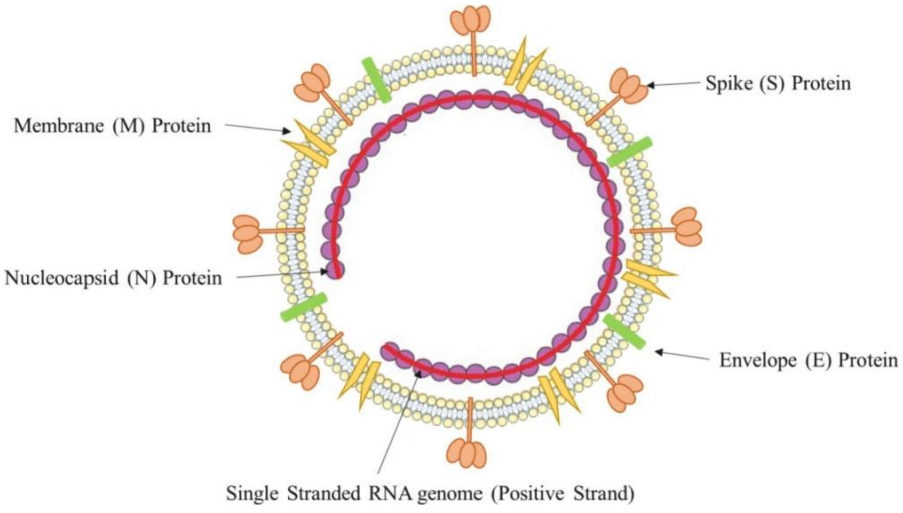


Fonte: WHO, 2022, acesso em 01/07/2022.

O número elevado de ocorrências em contexto global, se deve a alta taxa de transmissão da COVID-19. O vírus é disseminado através de gotículas expelidas por pessoas infectadas ao tossir, falar, espirrar e até mesmo rir, a saliva e o escarro contém grande quantidade do micro-organismo infeccioso. A COVID-19 ainda possui um agravante no que diz respeito a propagação, o patógeno pode ser espalhado por portadores sintomáticos e assintomáticos (SALIAN et al., 2021).

O SARS-CoV-2 pertence à família *Coronaviridae*, possui genoma de RNA fita simples positiva, é um vírus envelopado com proteínas estruturais como spike (S), envelope (E), membrana (M) e nucleocapsídeo (N) (Figura 2) (V'KOVSKI et al., 2021).

Figura 2. Estrutura do vírus SARS-CoV-2



Fonte: SHARMA ET AL. , 2021

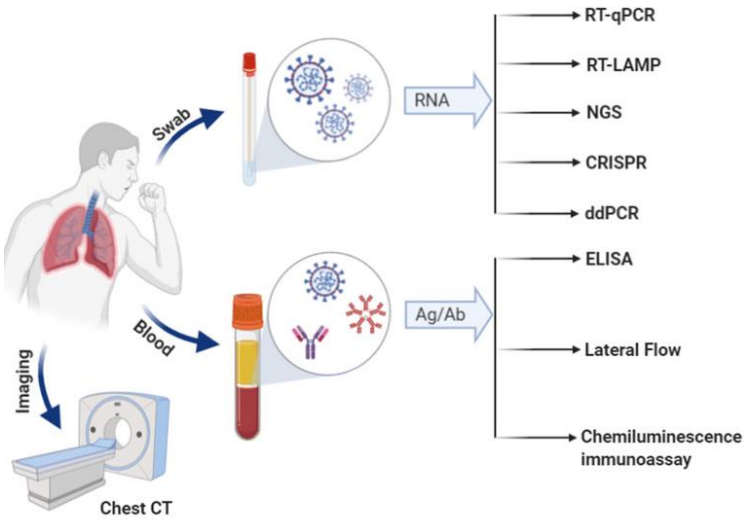
A entrada e fixação nas células do organismo humano é o início da patogenia. O SARS-CoV-2 entra na célula hospedeira a partir do receptor da enzima conversora de angiotensina 2 (ACE2) que está presente em vários tipos de células humanas, como mucosa nasal e oral, pulmões, fígado e coração. A proteína spike (S) do SARS-CoV-2 se liga ao ACE2 da célula humana e o vírus entra por fusão direta de membranas celulares ou por endocitose (CROOK et al., 2021; ŞİMŞEK YAVUZ; KOMŞUOĞLU ÇELİKYURT, 2021).

1.3 Diagnósticos de COVID-19

A primeira sequência do genoma do SARS-CoV-2 foi descoberta em janeiro de 2020 e desde então buscam-se formas de diagnosticar a patologia causada por este vírus (LAI; LAM, 2021).

Identificar a doença é a melhor forma de controle da disseminação e por isso a procura por métodos eficazes e rápidos é uma constante para os pesquisadores. A figura 3 representa vários métodos de detecção para o SARS-CoV-2.

Figura 3. Representação esquemática de vários métodos para detecção de SARS-CoV-2



Fonte: BANERJEE; RAI, 2020)

As ferramentas mais utilizadas na identificação do SARS-CoV-2 são testes moleculares, testes sorológicos e imunoensaios de fluxo lateral (SHARMA et al., 2021).

O RT-PCR é um teste molecular, considerado o padrão-ouro para o diagnóstico

de COVID-19, entretanto, apresenta limitações como alto custo, necessidade de pessoal treinado para coleta e realização do teste, longo tempo de execução e demora na liberação do resultado. Os testes sorológicos são boas opções, todavia, esse tipo de exame depende da produção de anticorpos pelo organismo, geralmente produzidos apenas após duas semanas de contaminação, contraindicando o uso desse tipo de método nos primeiros dias de suspeita da infecção (SHARMA et al., 2021).

Ademais, os testes de sorologia podem apresentar baixa especificidade e imprecisão, pois pode haver reação cruzada entre o SARS-CoV-2 e outros tipos de vírus com estrutura semelhante (TALEGHANI; TAGHIPOUR, 2021).

Os ensaios de fluxo lateral (LFIA, sigla em inglês para *Lateral flow immuno assay*), são os chamados "testes rápidos" e foram criados devido ao alto número de pessoas infectadas que causou a necessidade de avaliações rápidas, de custo reduzido e auto coleta de amostra (SHARMA et al., 2021). Porém, esse tipo de ensaio é considerado de baixa sensibilidade e pode apresentar reação cruzada devido a semelhança do SARS-CoV-2 com outros coronavírus (LAI; LAM, 2021).

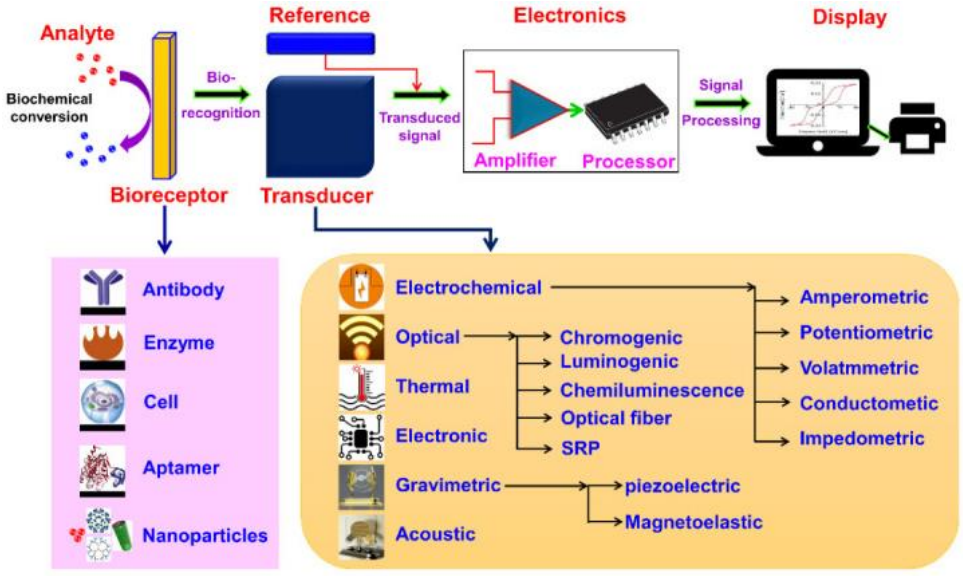
Diante do exposto, a busca por novos métodos diagnósticos com técnicas sensíveis, rápidas, baratas e de uso local, vem sendo explorada por diversos cientistas de todo o mundo. Os biossensores eletroquímicos apresentam essas características e podem permitir diagnósticos mais precisos e eficientes.

1.4 Biossensores

Biossensores são dispositivos que medem reações biológicas ou químicas, possuem receptor e transdutor integrado com capacidade de converter uma resposta biológica em sinal elétrico e mensurá-lo (Figura 4) (NARESH; LEE, 2021).

Inúmeros constituintes biológicos podem ser aplicados no desenvolvimento de biossensores, podendo ser baseados na ligação antígeno-anticorpo, hibridização de enzimas, aptâmeros, ácidos nucléicos e biorreceptores. Os dispositivos são utilizados em diversas atividades como monitoramento do meio ambiente, controle de qualidade da água e de alimentos, controle antidoping, acompanhamento e diagnóstico de doenças (BHALLA et al., 2016).

Figura 4. Diagrama esquemático de biossensor típico consistindo de biorreceptor, transdutor, sistema eletrônico e display e vários tipos de biorreceptores e transdutores usados nos biossensores



Fonte: (NARESH; LEE, 2021)

Os biossensores podem utilizar diferentes biorrecptores, como anticorpos (imunossensores), enzimas, ácidos nucléicos (genossensores e aptasensores), células e materiais sintéticos. Os mais utilizados são os imunossensores e sensores enzimáticos (MEHROTRA, 2016).

De acordo com os transdutores, os biossensores podem ser eletroquímicos, ópticos, térmicos, eletrônicos, gravimétricos e acústicos. A classificação ainda pode ser realizada pelo sistema de detecção e tecnologia utilizada no equipamento (NARESH; LEE, 2021).

Os biossensores eletroquímicos são os mais utilizados e estudados por serem dispositivos sensíveis e seletivos. Seu funcionamento é baseado na reação eletroquímica entre o biorreceptor e a amostra a ser analisada (AMADOR SALOMÃO, 2018).

Em relação as técnicas eletroquímicas utilizadas, os biossensores podem ser classificados em amperométricos, potenciométricos, voltamétricos, condutimétricos e impedimétricos. Assim são produzidos sinais eletroquímicos em voltagem, corrente, impedância e capacitância, que são detectados e quantificados (NARESH; LEE, 2021).

Adaptações e melhorias das plataformas diagnósticas ofertadas por biossensores eletroquímicos podem ser necessárias, e para isso, nanomateriais podem ser acoplados visando oferecer melhor performance diagnóstica destes dispositivos.

1.4.1 Nanomateriais

Os nanomateriais, em especial as nanopartículas, são frequentemente utilizados em sensores eletroquímicos com o objetivo de aumentar a área superficial e detecção do dispositivo e melhorar seu desempenho. Os nanomateriais a base de carbono, como por exemplo o grafeno, são ótimos condutores elétricos e térmicos, possuem estabilidade química, flexibilidade e além disso, tem excelente área de superfície (VERMISOGLOU et al., 2020).

As nanopartículas facilitam a transferência de elétrons entre as biomoléculas e/ou superfície dos eletrodos, melhoram conformação e aumentam atividade biológica, o que resulta em uma melhor performance do sensor. A utilização de nanopartículas incorporadas aumenta a condutibilidade eletrônica e consequentemente a sensibilidade (PRAKASH et al., 2013).

Os benefícios do uso de nanomateriais são inúmeros, dentre os quais podemos citar vantagens ópticas e elétricas únicas que os tornam relevantes para a incorporação, aumentando a sensibilidade, concedendo níveis reduzidos de detecção e diminuindo os efeitos da matriz. Algumas nanopartículas apresentam condutividade, atividade catalítica, biocompatibilidade ou estabilidade que podem influenciar no crescimento da área de superfície eletroativa, além de favorecer a transferência eletrônica, amplificando sinais eletroquímicos (REVERTÉ; PRIETO-SIMÓN; CAMPÀS, 2016).

Diante das vantagens do desenvolvimento de biossensores eletroquímicos para diagnóstico, esta revisão sistemática teve como objetivo buscar as publicações referentes ao tema e responder a seguinte pergunta nordeatora: O diagnóstico de COVID-19 realizado através de biossensor eletroquímico, nos indivíduos com suspeita da doença, é tão eficiente quanto o realizado por RT-PCR (reação da transcriptase reversa seguida pela reação em cadeia da polimerase)?

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APÊNDICE A – ARTIGO EM INGLÊS

Diagnostic efficacy of electrochemical biosensor compared to RT-PCR for diagnosis of COVID-19: A systematic review.

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ABSTRACT

In December 2019, the new coronavirus that was named SARS-CoV-2 was identified in China. The virus spreaded quickly and in March of 2020 it was declared a pandemic by the World Health Organization. Due to the initial lack of rapid diagnoses and effective treatments, the health systems were overloaded as a consequence of the high number of infected and severe cases. The diagnostic techniques currently available have limitations, for this reason, the search for new methods with sensitive, fast, inexpensive and locally used techniques, such as electrochemical biosensors, has been widely explored. Given the advantages of developing electrochemical biosensors for sensitive and selective diagnosis, this systematic review aimed to search for publications on the subject and answer the following question: The diagnosis of COVID-19 performed through an electrochemical biosensor, in individuals with suspected disease is it as efficient as that performed by RT-PCR? A study protocol was developed following the PRISMA-DTA guidelines and registered with PROSPERO under the approval code CRD42021282561. Searches were carried out in six electronic databases, inclusion and exclusion criteria were applied and seventeen publications were selected for this review. Based on the data, the analyse of risk was done using QUADAS-2. The results were presented in a descriptive qualitative manner it was not possible to carry out a meta-analysis.

Keywords: Electrochemical biosensor. Electrochemistry. Diagnosis. COVID-19.

1. INTRODUCTION

In December 2019, the new coronavirus was identified in China, which was named SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). The virus is responsible for causing severe acute respiratory syndrome, with a high rate of transmission and contagion. The disease caused by SARS-CoV-2, called COVID-19, spread quickly. Before the end of January 2020, there were already reports of contamination in different locations around the world (Rahman et al., 2021; Shahriar et al., 2021).

The World Health Organization (WHO) declared COVID-19 a global pandemic in March 2020 and the world has suffered the consequences of this quick spread (OPAS, 2022) The initial lack of rapid diagnoses, effective treatments and containment of those infected, generated a high number of hospitalizations, which increased expenses and overloaded the health systems of several countries (Böger et al., 2021).

The first case of genome SARS-CoV-2 was discovered in January 2020 and since then, a lot of research has been done to diagnose the pathology caused by the virus (Lai & Lam, 2021). The best way to control the spread is identifying the disease, therefore the search for effective and fast methods is a constant routine for researchers. The tools most used in the identification of SARS-CoV-2 are molecular tests, serological tests and lateral flow immunoassays (Sharma et al., 2021).

The technique of lateral flow, called as “rapid tests”, are considered to have low sensitivity and may cross-react due to the similarity of SARS-CoV-2 to other coronaviruses (Lai & Lam, 2021).

The RT-PCR (reverse transcriptase polymerase chain reaction) is a molecular test, considered the gold standard for the diagnosis of COVID-19, however, it has limitations such as high cost, it is required a qualified person to collect and proceed the test, the test take a long period of time to be completed, and also, the results can take long to be released (Sharma et al., 2021).

A very good option is the serological tests, however, this type of test depends on the production of antibodies by the body, usually they are produced by our organism only after two weeks of contamination, contraindicating its use in the first days of suspicion of infection (Sharma et al., 2021). They may also have low

specificity and imprecision, due to cross-reaction between SARS-CoV-2 and other types of viruses with a similar structure (Taleghani; Taghipour, 2021).

Thus, the search for new diagnostic methods with sensitive, fast, inexpensive and locally used techniques has been explored by scientists around the world. Electrochemical biosensors have these characteristics and can allow accurate and efficient diagnosis. They are devices that measure biological or chemical reactions, they have an integrated receptor and transducer capable of converting a biological response into an electrical signal and measuring it (Naresh & Lee, 2021).

The electrochemical biosensors are the most used and studied because they are sensitive and selective devices. Its operation is based on the electrochemical reaction between the bioreceptor and the sample to be analyzed (Amador Salomão, 2018).

Due to the advantages of developing electrochemical biosensors for diagnosis, this systematic review aimed to answer the following question: The diagnosis of COVID-19 performed through an electrochemical biosensor, in individuals with suspected disease, is as efficient as that performed by RT- PCR?

2. MATERIALS AND METHODS

2.1 Protocol and Registration

The question in this review was based on the acronym PIRO (P= Population, I= *Index test*, R= *Reference Standard*, O = *Outcome).*

P = Individuals with suspected COVID-19;

I = Electrochemical Biosensor;

R = RT-PCR;

O = Sensitivity and specificity of the index test;

A protocol was developed following the Preferred Reporting Items for a Systematic Review and Meta-analysis of Diagnostic Test Accuracy Studies 2020 (PRISMA-DTA) guidelines and registered in the Prospective Register of Systematic Reviews (PROSPERO) under approval code CRD42021282561.

2.2 Eligibility Criteria

Studies that met the following inclusion criteria were included: articles published in the last 4 years (2019 to 2022), which compared the diagnosis of COVID-19

using an electrochemical biosensor with the RT-PCR test in samples from individuals with suspected of the disease. The period of search for publications was between 2019 and 2022 due to the emergence of COVID-19 in 2019. The first search in the databases was carried out on 09/26/2021 and the last on March 17, 2022.

Exclusion criteria for articles: 1) They did not use samples of individuals; 2) They did not compare the index test to RT-PCR; 3) Lack of comparator method; 4) Matters unrelated to the topic of the review (diagnosis of other diseases, other matters related to COVID-19 other than diagnosis); 5) Review studies; 6) They used a technique other than electrochemistry; 7) Full-text articles and author contact available in Chinese only; 8) Full text not available.

2.3 Database and search strategy

The searches were performed in six electronic databases (PUBMED, SCOPUS, WEB OF SCIENCE, LILACS, EMBASE and LIVIVO). For each database was structured a strategy of searching (supplementary material 1). A gray literature search was also performed on Google Scholar and Open Gray. Endnote Web software was used to manage references and check for duplication, and for organization and selection of publications, we used Rayyan (Intelligent Systematic Review).

2.4 Selection of studies

The selection was performed blindly and independently in two phases. In the first one, two reviewers R1 and R2 (R.F.B and B.R.M) made the selection by reading the title and abstract and identified the studies that they considered to meet the inclusion criteria. The second stage of selection included the reading of the full texts selected in the first stage by the same pair of reviewers, applying the same eligibility criteria. In case of disagreements, a discussion was held between the two reviewers to define the inclusion of the publication. If the disagreement persisted, a third reviewer (R3 – R.P.A.) was invited for the final definition.

2.5 Data extraction

The data extraction was performed by R1, through a spreadsheet, where the following information from the included articles was described: number of clinical

samples tested, type of biosensor, sample used, test execution time, sensitivity, specificity, detection limit and cost. Then, after the R1 has completed all the data, the R2 has checked the information, and if found a disagreement between the information collected by R1 and R2, a third reviewer would be required again. In addition, the data on authorship, the year of publication, the objective and then conclusion of the articles were also collected.

2.6 Risk of Bias Analysis of the included articles

The risk of bias was assessed using the QUADAS-2 tool. The analysis was performed by R1 and R2 independently and the results were evaluated, in the case of different answers, R3 was activated to solve the question.

2.7 Synthesis and analysis of results

The results were presented in table format and qualitative description. Due to the variability of publications, it was not possible to perform a meta-analysis.

3 RESULTS

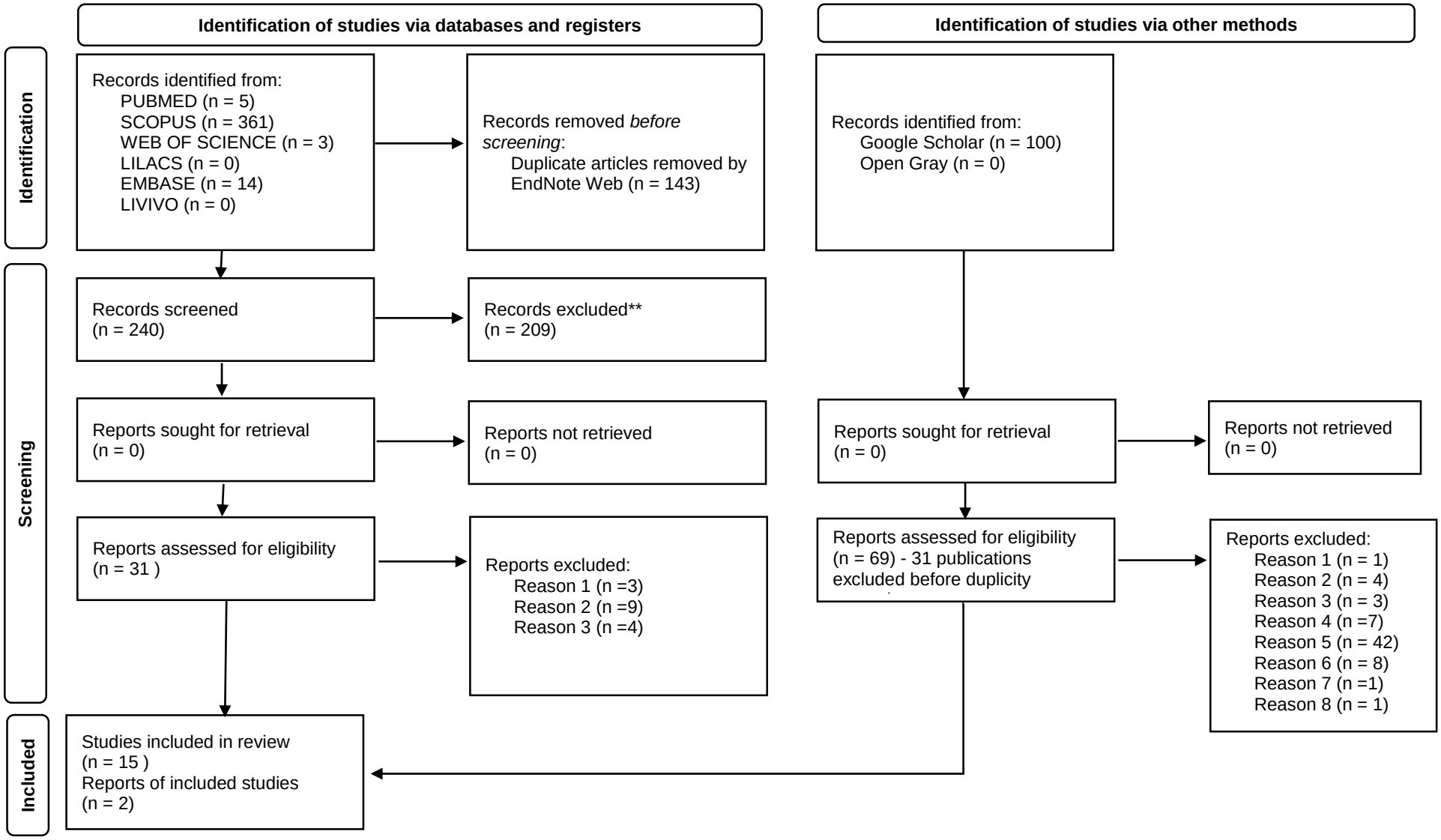
3.1 Selection of Studies

The Figure 1 shows the steps of slection that was included on the studies, for this revision were included 17 publications in total.

Initially, 383 publications were found, of which 143 were excluded due to duplicity and 240 entered the evaluation by reading the title and abstract, with 209 articles excluded and 31 selected for reading the full text. After completing the reading, 15 articles were included in the systematic review.

It was found 3920 citations searching on gray literature, with the first 100 being evaluated. Of these, 31 were excluded due to duplicity and 69 were included for evaluation, 67 of which met exclusion criteria and 2 were included. The appendix 2 of the supplementary material presents the author's name and the reason for excluding articles selected for full reading.

Figure 1. Flowchart of identification and selection of studies (PRISMA, 2020).



3.2 Characteristics of the included studies

The articles did not describe the characteristics of the participants and most of the samples used were provided by health institutions. All of them presented the development of the sensor and the technique used.

The articles included showed variability in the data. The types and number of samples tested were heterogeneous, in addition to the different measurement units to present the detection limit of each sensor.

Not all the publications included were able to describe the sensitivity information, specificity information and detection of time.

All the variables extracted from the studies included are described in table 1.

Table 1. Characteristics of included studies.

IDENTIFICATION			METHODS				RESULTS				
AUTHOR	**PLACE/ YEAR**	**OBJECTIVE**	**BIOSENSOR TYPE**	**SAMPLE USED**	**TEST EXECUTION TIME**	**Nº TESTED SAMPLES**	**SENSITIVITY**	**SPECIFICITY**	**LIMIT OF DETECTION**	**COST**	**CONCLUSION**
Alafeef et al.	United States 2020	To develop a rapid, low-cost, easy-toimplement, and quantitative paper-based electrochemical sensor chip to enable the digital detection of SARS-CoV-2 genetic material.	Genosensor	Nasopharyngeal swab	Not available	48	Almost 100% 231 (copies/µL) -1	Almost 100%	6.9 copies/µL	Not available	It was developed herein an electrochemical platform made up of graphene and gold nanoparticles conjugated with suitably designed antisense oligonucleotides for the rapid, accurate, selective, and ultrasensitive detection of SARS-CoV-2 viral RNA within a time period of less than 5 min. The developed test can detect the early stage of infection. The sensor chip showed a significant change as a response to COVID-19 positive samples, whereas an insignificant change has been observed as a response to the healthy subjects' samples, with a classification accuracy of nearly 100%.

Ayankojo et al.	Estonia 2022	To develop electrochemical sensor for rapid detection of SARS-CoV-2 S protein, where the disposable thin-film metal electrodes (Au-TFME) chip was modified with a molecularly imprinted polymer (MIP) film endowed with the selectivity for S protein subunit S1 (ncovS1) and used as a recognition element.	Genosensor	Nasopharyngeal swab	20 minutes	8	Not available	Not available	64fM (4.8pg/ml)	Not available	The work demonstrated the electrochemical ncovS1 sensor armed with a molecularly imprinted polymer synthetic receptor (ncovS1-MIP). The sensor has show a possibility rapid diagnostic. The electrochemical characteristics of the sensor could be readily handled by a portable potentiostat allowing on-site measurements thus holding a great potential as a point-of-care testing platform for rapid and early diagnosis of COVID-19 patients.

Beduk et al.	Turkey 2021	A method for the quantification of SARSCoV-2 levels in blood serum by recognizing its protein host cell receptor domain. They proposed a laser-scribed graphene (LSG) and electrodeposited gold nanostructures (AuNSs) in a disposable electrochemical immunoassay.	Immunosensor	Blood Serum	Not available	23	Not available	Not available	2.9 ng/mL	Not available	It was describe a smart antibody sensor based on electrodeposition of electrodeposited gold nanostructures (AuNS) on LSG electrodes for SARS-CoV-2. Was tested the correlation between our proposed test and commercially available test results, and our test yielded the best agreement with the RT-PCR test. The results show that the proposed sensor has the possibility to be an alternative detection method with a convenient detection time. The authors concluded though further improvements are required for LSG/AuNS electrodes to develop fully optimized POC diagnostic tools, the proposed sensing system offers a good and stable alternative platform for future applications.
Tepeli Büyüksünetçi et al.	Turkey 2021	Develop a diagnostic technique for SARS-COV-2, immobilizing angiotensin-converting enzyme 2 (ACE2) and CD147 on different electrodes and monitoring their electrochemical interactions with protein S.	Immunossensor	Nasopharyngeal swab and Oropharyngeal swab	Not available	82	Not available	Not available	Receptor CD147: 38.99 ng mL−1. Receptor ACE2: 299.30 ng mL -1	Not available	An electrochemical approach based on the mechanism of SARS CoV-2 infection was developed. This system has been shown to work for ACE2 and CD147 receptors. Very accurate and effective results were also obtained for analyzes of real samples that were validated with RT-PCR. The authors believe that this system would be effective in detecting existing and new mutations.

Chaibun et al	Thailand 2021	In this study, we describe an electrochemical biosensor based on multiplex isothermal rolling circle amplification (RCA) for the rapid detection of the N and S genes of SARS-CoV-2 from clinical samples.	Genosensor	Nasal swab	< 2 hours	106	99%	Not available	1 copy/µL para genes N e S	Not available	It was developed an electrochemical biosensor coupled with RCA for the highly sensitive and specific detection of SARS-CoV-2. The high amplification capability of RCA and sensitivity of the electrochemical detection method enabled the detection of the viral N and S genes in synthetic linear targets as well as clinical samples. The whole assay took under 2 h to complete, from RNA extraction to the detection step, and does not require the use of a thermal cycler. The performance of the assay with clinical samples was comparable to that of RT-qPCR, which is currently the standard for SARS-CoV-2 detection, whereas also demonstrating zero false-positive results. This approach may have a significant impact in places where rapid detection is required to minimize emerging SARS-CoV-2 outbreaks.

Daniels et al	France 2021	Propose a face mask o collect exhaled breath condensate (EBC); Show the utility of an aptamer-based electrochemical biosensor and to report electrochemical sensing format targeting the spike protein (S) which is embedded in a lipidic membrane forming the SARS-CoV-2 viral outer wall.	Genosensor	Exhaled breath condensate (EBC)	Not available	14	Not available	Not available	3 pfu mL^{-1}	Not available	The sensor selectively detected SARS-CoV-2. Additionally, was shown that converting exhaled breath vapor into EBC provides a convenient and accessible sample source for SARSCoV-2 viral particles. The work highlighted that EBC can identify SARSCoV-2 by RT-PCR in patients identified as SARS-CoV-2 positive using nasopharyngeal swab samples. Some optimization would need to be implemented to make the sampling step more robust to overcome some false negative issues. Eventual integration of the sensor into the mask itself would likely make the method even more robust and user-friendly. Nevertheless, the results validate the concept that the detection of SARS-CoV-2 in the breath of COVID-19 patients using a rapid aptasensor is feasible.
Ehsan et al.	Saudi Arabia 2021	Present an impedance biosensor for the detection of the SARS-CoV-2 spike protein utilizing the IgG anti-SARS-CoV-2 spike antibody.	Immunosensor	Nasopharyngeal swab	Not available	5	Not available	Not available	0.25 fg/mL	Not available	The fabricated sensors show promised in direct, rapid, and low-cost diagnosis without sample pretreatments. Moreover, the sensor fabrication process could be automated, and such developments were underway in the authors lab.

Eissa & Zourob	Saudi Arabia 2021	Develop a cotton-tipped electrochemical immunosensor that could perform both collection and detection. To report for the first time the combination of cotton fibers and electrochemical assays for the detection of the SARS-CoV-2 antigen.	Immunosensor	Nasopharyngeal swab	Not available	3	Not available	Not available	0.8 pg/mL	Not available	The cotton-tipped electrochemical immunosensor integrated the sample collection and detection tools into a single platform by coating screen-printed electrodes with absorbing cotton padding. The biosensor did not show cross-reactivity with antigens from other viruses, implying high selectivity of the method. Moreover, the biosensor was successfully applied for the detection of the virus antigen in spiked nasal samples. The signal measurements can be realized using a handheld potentiostat and easily monitored using a smartphone device. The developed cotton based electrochemical immunosensor is a promising diagnostic tool for the direct, low cost, and rapid detection of the COVID-19 virus which requires no sample transfer or pretreatment.
Eissa et al	Saudi Arabia 2021	Develop of a label-free voltammetric-based immunosensor for the determination of SARS-CoV-2 N antigen using gold nanoparticles-modified screen-printed carbon electrodes.	Immunosensor	Nasopharyngeal swab	Not available	Not available	Not available	Not available	0.4 pg.mL −1	Not available	The immunosensor was considered a rapid, low-cost, selective and sensitive diagnostic method capable of being integrated into a portable potentiostat and controlled via a common cell phone for point-of-care testing. Work has focused on detection of the nucleocapsid protein, but future work should focus on comparing sensitivities using other target antigens.

Fabiani et al	Italy 2021	Develop a sensitive electrochemical biosensor for the detection of SARS-CoV-2 in saliva using an electrochemical assay based on magnetic beads (MBs) and screen-printed electrodes (SPEs) based on carbon black as a sensor combined with a PALM SENS portable potentiostat as a reader. Both SARS-CoV-2 proteins, namely protein S and protein N, were used as a target analyte by developing a sandwich assay with immobilized antibodies to proteins S or N on MBs.	Immunossensor	Saliva	Not available	24	Not available	Not available	Protein S: 19 ng/mL - Protein N: 8 ng/mL	Not available	Developed a smart immunosensor for SARS-CoV-2 detection in saliva by combining the use of MBs as support for immunological chain and carbon black-based SPEs for sensitive and reliable detection. This sensor configuration demonstrated the capability to detect S and N proteins in untreated saliva, without any crossreactivity when tested with others virus. The satisfactory analytical features found in terms of sensitivity, accuracy, and selectivity with the time of analysis, easiness to use, and the requirement of portable instrumentation boost this biosensor to acquire a relevant position in SARS-CoV-2 device scenario, taking into account also the easy sampling of saliva.

Jo et al	South Korea 2021	To evaluate the clinical performance of the MARK-B test (intended for the qualitative and semi-quantitative detection of the SARS-CoV-2 nucleocapsid antigens) based on magnetic force-assisted electrochemical immunoassay (MESIA), compared with RT-PCR and a commercially available rapid antigen (Ag) test.	Immunossensor	Nasopharyngeal swab	15 minutes	170	90%	99%	1×10^{2} pfu/mL	Not available	The MARK-B test, a MESIA-based rapid Ag test, showed higher sensitivity compared to commercial rapid Ag tests for the detection of SARS-CoV-2. Furthermore, the MESIA technique and automated portable device provided results with improved clarity in 15 min as well as reliable semi-quantitative measurement. These results indicate that these rapid Ag tests can be useful for preventing the spread of COVID-19 via timely diagnosis and subsequent containment measures.

Kashefi-Kheyrabadi et al	South Korea 2021	Develop a nucleic acid amplification-free electrochemical biosensor based on four-way junction (4-WJ) hybridization is developed, which can simultaneously detect SARS-CoV-2 spike (S) and open reading frame (Orf1ab) genes within 1 hour.	Genosensor	RNA samples isolated from nasopharyngeal swab samples	Not available	21	Not available	Not available	For the S and Orf1ab genes, 5.0 and 6.8 ag/μL were determined, respectively.	Not available	The S and Orf1ab genes were detected in both synthetic and clinical samples thanks to signal amplification capability provided by nanotextured electrodes and high sensitivity of the 4-WJ based electrochemical detection method. This approach has the following advantages: (i) multiplexed detection that avoids the generation of falsenegative results; (ii) high specificity and ability to differentiate between closely related RNA target sequences down to single nucleotide substitution; (iii) a single step procedure and short assay period; (iv) low LOD that satisfies sensitivity requirement and could potentially be used to detect SARS-CoV-2 RNA targets in the early stages of the disease while the viral genes load is low.
Lasserre et al	United Kingdom 2021	To present an impedimetric SARS-CoV-2 biosensor using SARS-CoV-2 truncated aptamers, compatible with lowcost electrode systems	Immunossensor	Nasopharyngeal swab	Not available	Not available	Not available	Not available	Not available	Not available	Showed the development of a specific aptamer sequence for the SARS-CoV-2 spike protein and its subsequent use for the detection of the virus from complex clinical samples. The detection system as presented has several key advantages including a simple impedance measurement to determine target binding, a high stability aptamer receptor to detect the spike protein, and the use of low-cost gold electrodes, similar to blood glucose sensors.

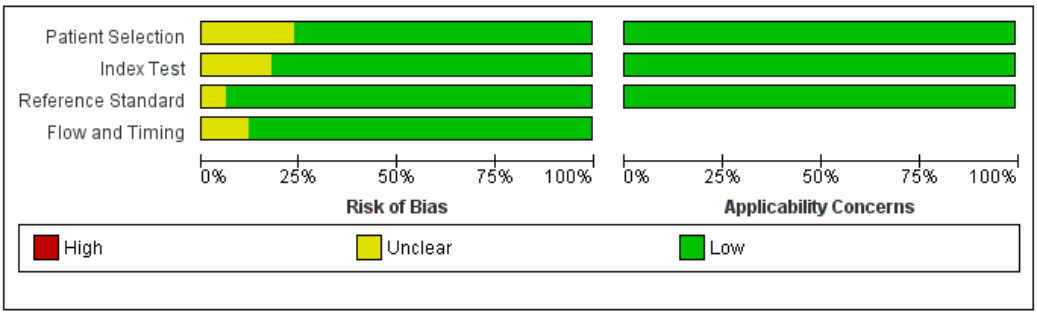
Liv et al	Turkey 2021	To develop a newly designed, easy-to-prepare, and more sensitive graphene oxide-modified glass carbon electrode sensor to voltammetrically determine SARS-CoV-2 spike antigen protein.	Immunosensor	Gargle and mouthwash samples	Not available	110	93.30%	92.50%	Not available	Not available	The developed method showed excellent reliability and precision for the diagnosis of COVID-19 in real samples and a perfect agreement with the RT-PCR results. The sensor could be used even long after preparation and a sensor could be used three times on positive samples which would minimize the cost of testing. Easily manufactured and supplied as a ready-to-use kit on a commercial scale.
Rahmati et al	Iran 2021	To suggest a new ultrasensitive electroanalytical nanobiodevice made using Screen-printed carbon electrode (SPCE) modified by Staphylococcal Protein A and Cu_2O nanotubes as a substrate for the orderly orientation of IgG antibodies as a specific receptor.	Immunosensor	Nasopharyngeal swab	Less than 20 minutes	8	Not available	Not available	0.04 fg mL−1	Not available	Was developed an electrochemical nanobiodevice which was applied for rapid screening of people suspicious to SARS-CoV-2 with the aim of facilitating the point-of-care diagnosis. In this sense, the disposable SPCEs were modified with Cu2O NCs, and, then, the ProtA layer was used to immobilize the IgG antibody, as a receptor element, in the regular direction. This sensor was able to be used in clinical samples to detect the SARS-CoV-2 virus in less than 20 min, without any cross-reactivity when tested with influenza viruses 1 and 2.

Raziq et al	Estonia 2021	Report development of a molecularly imprinted polymers (MIP) based electrochemical sensor for detection of SARS-CoV2 nucleoprotein (ncovNP).	Immunosensor	Nasopharyngeal swab	Not available	8	Not available	Not available	0.7 pg/mL	Not available	Was developed first time a portable electrochemical sensor integrated with a molecular imprinted polymer (ncovNP-MIP) as a synthetic recognition element capable of selective detection of SARS-CoV-2 antigen (ncovNP). The results of the sensor performance validation in the clinical samples of the nasopharynx swabs of patients were promising, confirming the capability of the sensor to detect ncovNP in the complex biological media.
Torres et al	Estados Unidos 2020	To describe a simple, inexpensive, and rapid test for the detection of SARS-CoV2. The RAPID 1.0 that transforms biochemical information from a specific molecular binding event between the SARS-CoV2 spike protein (SP) and angiotensin-converting enzyme-2 (ACE2) into an electrical signal that can be easily detected.	Genosensor	Saliva and Nasopharyngeal/ oropharyngeal swab	4 minutos	Saliva: 50 Nasopharyngeal/ oropharyngeal swab: 139	Saliva: 100% Nasopharyngeal/ oropharyngeal swab: 83.5%	Saliva: 100% Nasopharyngeal/or opharyngeal swab: 100%	1.16 PFU mL−1	$4.67	The biosensor that has been described is inexpensive and portable, allowing for decentralized diagnosis at the point of care. The detection time (4 min) was significantly shorter than existing diagnostic tests and could be further reduced using engineered versions of human ACE2 with improved selective binding to SARS-CoV-2 SP. The use of these ACE2 variants would also help to reduce the false positive rate in complex biofluids such a saliva.

3.3 Risk of bias analysis

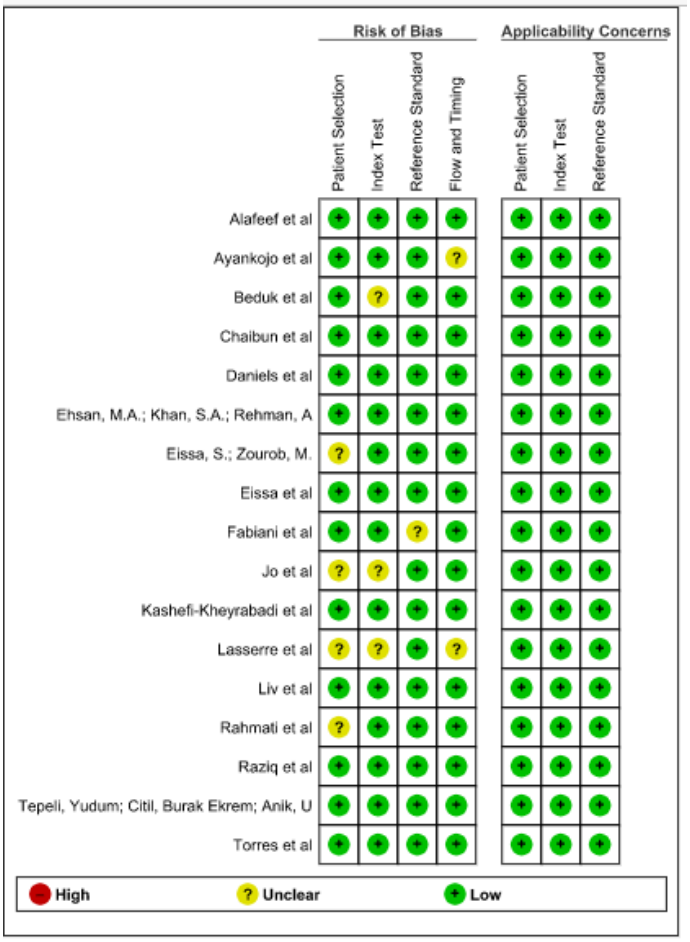
The assessment of the methodological quality of the included studies was performed according to the QUADAS-2 tool.

Figure 2. Summary of the QUADAS-2 methodological quality assessment:



Source: The author - through the Review Manager 5.4 program.

Figure 3. Methodological quality of studies included in QUADAS-2 (individual assessment).



Source: The author - through the Review Manager 5.4 program.

The QUADAS-2 was adapted for the evaluation of studies included in this review, as it is generally used to evaluate methods already used in patients, which is not the case with the biosensors described here. All the devices presented are still in the experimental phase and they were tested only on samples provided. We realized that this may have interfered with our analysis, as some QUADAS-2 questions had no answers available in the articles.

It is noted that 58.8% of the studies had a low risk of bias in all domains. Approximately 41.2% of the articles present uncertain risk in one or more of the one method`s domains.

3.4 Individual study results

Alafeef et al., 2020 aimed to develop a graphene-based electrochemical biosensor. The device's performance was tested on nasopharyngeal swab and saliva samples and the authors concluded that the device outperformed serological tests, had an excellent detection limit, quick response, good shelf life, and reasonable cost.

Ayankojo et al., 2022 reported the development of an electrochemical sensor modified with molecular imprint polymer with selectivity for one of the Spike protein subunits. The results coincided with those of RT-PCR, however, there was a difference in the intensity of the positive response and the authors discussed the possibility of dissimilarity in viral load and disease stages at the time of sample collection. The sensor was considered possible for rapid diagnosis, compared to available antigen tests.

Beduk et al., 2021 used blood serum as a sample. The objective of this work was to describe a graphene immunosensor enriched with gold nanostructures. The samples tested showed agreement with RT-PCR. The sensor was considered a good alternative for detection, however, it was mentioned that modifications and improvements are needed.

Tepeli Büyüksünetçi et al., 2022 developed an immunosensor based on the electrochemical interactions of angiotensin II converting enzyme and CD147 with the Spike protein of SARS-CoV-2. The device was tested on real oropharyngeal and nasopharyngeal swab samples and compared to RT-PCR results. The authors concluded that the response was accurate and effective.

Electrochemical biosensor for rapid isothermal rolling-circle amplification (RCA) based detection of N and S genes through nasopharyngeal swab was described by

Chaibun et al., 2021. The tested samples showed results in agreement with the RT-PCR tests, without false-positive results.

Daniels et al., 2021 developed an aptamer-based biosensor using a face mask to collect exhaled breath condensate as a sample for Spike protein detection. The results showed that the detection of SARS-CoV in the breath of infected patients is feasible, however, they reported that some adjustments should be made to avoid false-negative problems.

The publication by Eissa & Zourob, 2021 integrated the collection method with the detection method. The development of an immunosensor for identification of the SARS-CoV-2 nucleocapsid virus antigen on cotton tip was described. Test results on clinical specimens showed compliance with RT-PCR tests.

For detection of Spike protein, Ehsan et al., 2021 reported an impedance immunosensor using the anti-SARS-CoV-2 IgG antibody. The sensor was tested on real samples, showing agreement with the RT-PCR tests. The results showed high selectivity and good reproducibility.

Eissa et al., 2021 described an immunosensor for detection of the nucleuscapsid antigen, using carbon electrodes enriched with gold nanoparticles. Nasopharyngeal swabs were used as clinical samples. The research shows a strong correlation between test and RT-PCR results.

Fabiani et al., 2021 developed an immunoassay to detect Spike protein or nucleocapsid protein in saliva. The test was compared to RT-PCR, showing high agreement.

Jo et al., 2021 developed an immunosensor for SARS-CoV-2 antigen detection. The sensor was tested on nasopharyngeal swabs from patients with symptoms of COVID-19. ROC curve analysis was performed to assess device sensitivity and specificity. The authors confirmed that the test results had high agreement with RT-PCR and concluded that the sensor can be useful in rapid diagnosis.

Kashefi-Kheyrabadi et al., 2022 described a biosensor for detecting spike and open reading frame genes (Orflab). To test the detection, the RNA samples were isolated from nasopharyngeal swabs obtained from medical laboratories. The sensor was able to identify positive and negative samples according to RT-PCR.

An antigen-based aptamer sensor was developed by Lasserre et al., 2022. Oropharyngeal and nasal swab samples were used to test the sensor that distinguished positive and negative samples.

Liv et al., 2021 presented the detection of Spike SARS-Cov-2 protein antigen in gargle and mouthwash samples. Compared to RT-PCR, the sensor showed 91.7% of correct results. The authors concluded that the method described had good reliability, accuracy and would be an option for point-of-care diagnosis.

Rahmati et al., 2021 developed an immunosensor to detect Spike protein. The device was tested on real nasopharyngeal swab samples and the results proved to be reliable.

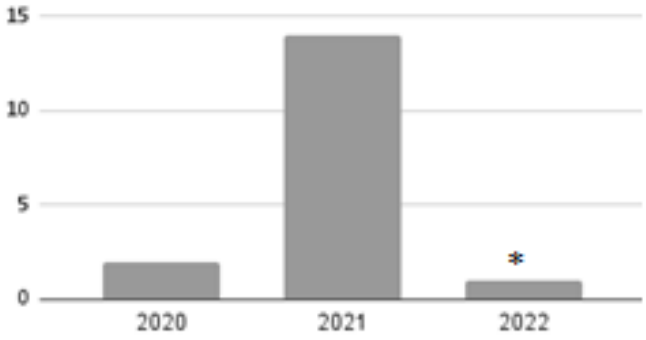
Raziq et al., 2021 reported a sensor based on molecularly imprinted polymers for SARS-CoV-2 nucleoprotein antigen detection. Nasopharyngeal swabs from patients were previously tested by RT-PCR and then by the developed device, which showed promising results.

Torres et al., 2021 described the development of a genosensor for the detection of SARS-CoV-2. The device was tested on real naso/oropharyngeal swab and saliva samples. Compared to RT-PCR, the sensor demonstrated high sensitivity, specificity and accuracy for both types of samples.

3.5 Summary of results

In this review, the data were summarized in a qualitative and descriptive manner.

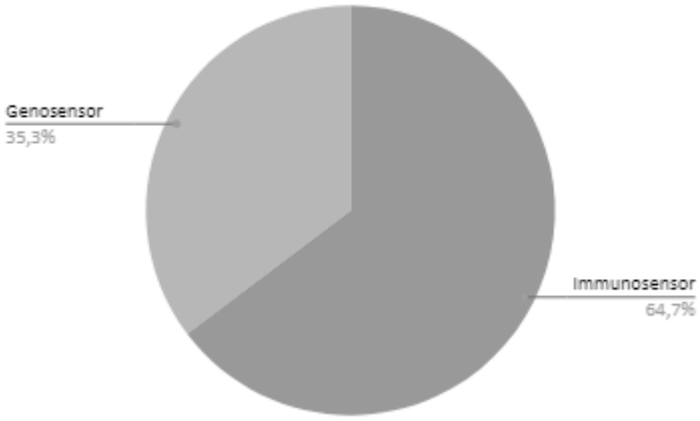
Figure 4. Number of publications per year



*Searches were performed until March 17, 2022.

Source: The author

Figure 5. Percentage of immunosensors and genosensors



Source: The author

Table 2. Detection target of devices described in publications included in the review

Author	Detection target
Alafeef et al.	Viral RNA
Ayankojo et al.	Spike protein subunit S1
Beduk et al.	Spike Protein
Tepeli Büyüksünetçi et al.	Spike Protein
Chaibun et al.	N and S genes
Daniels et al.	Spike Protein
Ehsan et al.	Spike Protein
Eissa & Zourob	N Protein (nucleocapsid)
Eissa et al.	Nucleocapsid Antigen
Fabiani et al.	Spike Protein and N Protein
Jo et al.	Nucleocapsid Antigen
Kashefi-Kheyrabadi et al.	S Gene and Orflab Gene
Lasserre et al.	Spike protein subunit S1
Liv et al.	Spike Protein
Rahmati et al.	Spike Protein
Raziq et al.	Nucleoprotein (ncovNP)
Torres et al.	Spike Protein

4 DISCUSSION

As mentioned and described, there was heterogeneity in the publications included in this review, from the sensor development methodology, to the number of samples tested and description of sensitivity and specificity. According to Cronin et al, 2018, when there is heterogeneity, it is appropriate to perform only the systematic review and not perform the meta-analysis.

The first descriptive analysis of the results was performed in relation to the year of publications included (Figure 4). The COVID-19 pandemic was decreed in March 2020 (OPAS, 2022) and due to uncertainties regarding the transmission, diagnosis and treatment of the disease, social isolation was the first measure to try to contain the spread of the virus (Banerjee & Rai, 2020).

According to Banerjee & Rai, 2020, at the beginning of the pandemic, health and educational institutions were greatly affected. Most universities stopped their activities, including research, and many professionals turned their occupations to assistance on the front lines of fighting the disease, which explains the small number of publications found by our search this year. In 2021, restrictions were relaxed and activities resumed, significantly increasing the number of studies.

Another point to be discussed in our review is the types of biosensors presented in the included studies. Rahman et al., 2021 report that since the beginning of the pandemic, rapid and effective detection of the virus has become essential in order to identify the disease and contain its spread.

According to Lai & Lam, 2021 after the sequencing of the first genome of SARS-CoV-2, several tests were developed for the diagnosis of COVID-19, including rapid tests. These diagnostic methods varied in technology and detection target (Table 2). As per our research we noticed with the use of sensonr the outcome was the same. Separating the tests only by the type of sensor developed, we observed 35.3% of the publications describing a genosensor and 64.7% presenting the description of an immunosensor (Figure 5).

Genosensors are biosensors that use DNA or RNA as recognition elements, based on the hybridization of the chains that occur in the electrode (Liu et al., 2012; Yang et al., 1997). On the other hand, immunosensors are based on the binding between antigen and antibody, which form a stable complex (Burcu Bahadır & Kemal Sezgintürk, 2015).

Although genosensors have advantages over immunosensors such as lower cost and the need for less biomolecule in detections, immunosensors were predominant in the studies included in this review. The smaller number of genosensors reflects some difficulties in working with nucleic acids. According to Wang et al., 2021, techniques based on DNA/RNA samples have challenges such as the difficulty of maintaining structural integrity and the need to work with controlled and elevated temperatures.

We also found a lot of diversity in the detection targets of the developed biosensors. This variability is not just a characteristic of sensor development methods. The range of different diagnostic tests and targets provides the option of choosing the best protocol, evaluating the patient's clinical conditions and the advantages and disadvantages of each technique (Mohamadian et al., 2021).

We note that most studies chose the spike protein as a detection target, an external protein that mediates between the virus and the host cell receptors, playing an essential role in the entry and replication of the same, in addition to the junction of the virus with the membranes of the cells (Harvey et al., 2021).

We also observed the use of different clinical samples (nasopharyngeal, oropharyngeal and nasal swab, saliva, blood serum, exhaled breath condensate, gargle and mouthwash samples and isolated DNA). Böger et al., 2021 reported in a systematic review of the diagnostic accuracy of COVID-19 tests that the choice of sample type is important for successful diagnosis and concluded in their meta-analysis that respiratory samples were the ones that achieved the best sensitivity rates, despite of not being the most easily collected samples. Even so, these results explain the greater use of respiratory samples in the sensors presented in our studies.

We noticed the use of nanomaterials and nanoparticles in the presented sensors. Graphene, a type of nanomaterial, has been used in several devices, in agreement with the data available in the literature, which report that the use of the material has been widely diffused for improving the sensor surface, having high electrical conductivity, facilitating the transfer of electrons and excellent biocompatibility with different biomolecules (Peña-Bahamonde et al., 2018; Szunerits & Boukherroub, 2018).

The use of nanoparticles in biosensors, mainly made up of noble metals, is widespread in the literature. We verified the use of these nanostructures in some of the studies included in the review in order to amplify the detection signal, conductivity in electron transfer, consequently improving the performance and sensitivity of the device (Ibrahim et al., 2021; Tan et al., 2020). In addition, the use of aptamers was also observed in some of the works included in this review. Aptamers are single-stranded RNA or DNA molecules designed to bind to specific substrates, having high affinity. They can be composed of proteins, nucleotides and oligonucleotides and, like nanoparticles, their use aims to improve sensor

performance due to their specificity, low cost and great compatibility (MacKay et al., 2014; Mo et al., 2022; Xiang et al., 2020).

Data regarding the detection limits of the tests presented were also collected, there was variability in the values and measurement units described. The measurement units are all sensitive and vary between plaque forming units (pfu/mL), copies/µL, nanogram (ng/ml), picogram (pg/mL), phentogram (fg/mL), attogram (ag/mL).

The sensitivity and specificity values of the publications included in the review were presented by only 29.41% and 23.53%, respectively, hindering the comparison of information and the performance of statistical analysis.

5 CONCLUSION

The diagnosis of COVID-19 performed through an electrochemical biosensor is on the way to being as efficient as that performed by RT-PCR. The articles found in this review show that electrochemical biosensors are effective in diagnosing the disease, have advantages such as speed, good sensitivity and use at the point of care, and most of the results were convergent with RT-PCR. However, there are some limitations for the proper comparison and affirmation of the devices' effectiveness, among them: variability in the units of detection limits, lack of description of sensitivity and/or specificity and low number of clinical samples tested in most articles. Tests with standardize methodology and a larger number of samples are needed to validate the sensor and thus make a safe statement that it is as effective as the RT-PCR test.

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APÊNDICE B - MATERIAL SUPLEMENTAR

Apêndice 1. Chaves de busca para cada base de dados

Chave de busca - Base de dados LILACS (descritores DeCS, em português, inglês e espanhol + sinônimos + código (para COVID-19 foi somente o descritor principal + códigos): Título, resumo e assunto:

(mh: ((COVID-19)) OR (C01.748.214$) OR (C01.748.610.763.500$) OR (C01.925.705.500$) OR (C01.925.782.600.550.200.163$) OR (C08.381.677.807.500$) OR (C08.730.214$) OR (C08.730.610.763.500$) AND (mh:(Diagnóstico)) OR (Diagnose) OR (mh:(Diagnosis)) OR Antemortem Diagnoses OR (Antemortem Diagnosis) OR (Diagnoses) OR (Diagnoses and Examinations) OR (Diagnoses, Antemortem) OR (Diagnoses, Postmortem) OR (Diagnosis, Antemortem) OR (Diagnosis, Postmortem) OR (Examinations and Diagnoses) OR (Postmortem Diagnoses) OR (Postmortem Diagnosis) OR (Diagnosticar) OR (E01$) OR (SP4.051.512$) AND (mh:(Eletroquímica)) OR (mh:(Electrochemistry)) OR (Electrochemistries) OR (mh:(Electroquímica)) OR (H01.181.529.307$) OR (SP4.097.060$) AND (mh:(Reação em Cadeia da Polimerase)) OR (PCR) OR (PCR Ancorado) OR (PCR Aninhado) OR (PCR Reverso) OR (Reação da Polimerase em Cadeia) OR (Reação de Polimerase em Cadeia) OR (Reação em Cadeia de Polimerase) OR (mh:(Polymerase Chain Reaction)) OR (Anchored PCR) OR (Anchored Polymerase Chain Reaction) OR (Inverse PCR) OR (Inverse Polymerase Chain Reaction) OR (Nested PCR) OR (Nested Polymerase Chain Reaction) OR (PCR) OR (PCR, Anchored) OR (PCR, Inverse) OR (PCR, Nested) OR (Polymerase Chain Reactions) OR (Reaction, Polymerase Chain) OR (Reactions, Polymerase Chain) OR (mh:(Reacción en Cadena de la Polimerasa)) OR (PCR Anclado) OR (PCR Anidado) OR (PCR Inverso) OR (E05.393.620.500$)

Chave de busca - Base de dados PUBMED: descritores MeSH + sinônimos (exceto COVID-19): All Fields

"COVID-19"[Mesh] AND "Diagnosis"[Mesh] OR (Antemortem Diagnoses) OR (Antemortem Diagnosis) OR (Diagnoses) OR (Diagnoses and Examinations) OR (Diagnoses, Antemortem) OR (Diagnoses, Postmortem) OR (Diagnosis,

Antemortem) OR (Diagnosis, Postmortem) OR (Examinations and Diagnoses) OR (Postmortem Diagnoses) OR (Postmortem Diagnosis) AND "Electrochemistry"[Mesh] OR (Electrochemistries) AND "Polymerase Chain Reaction"[Mesh] OR (Anchored PCR) OR (Anchored Polymerase Chain Reaction) OR (Inverse PCR) OR (Inverse Polymerase Chain Reaction) OR (Nested PCR) OR (Nested Polymerase Chain Reaction) OR (PCR) OR (PCR, Anchored) OR (PCR, Inverse) OR (PCR, Nested) OR (Polymerase Chain Reactions) OR (Reaction, Polymerase Chain) OR (Reactions, Polymerase Chain)

Chave de busca - Base de dados SCOPUS: descritores MeSH + sinônimos (exceto COVID-19): All fields

"COVID-19" AND "Diagnosis" OR "Antemortem Diagnoses" OR "Antemortem Diagnosis" OR "Diagnoses" OR "Diagnose" OR "Diagnoses and Examinations" OR "Diagnoses, Antemortem" OR "Diagnoses, Postmortem" OR "Diagnosis, Antemortem" OR "Diagnosis, Postmortem" OR "Examinations and Diagnoses" OR "Postmortem Diagnoses" OR "Postmortem Diagnosis" AND "Electrochemistry" OR "Electrochemistries" AND "Polymerase Chain Reaction" OR "Anchored PCR" OR "Anchored Polymerase Chain Reaction" OR "Inverse PCR" OR "Inverse Polymerase Chain Reaction" OR "Nested PCR" OR "Nested Polymerase Chain Reaction" OR "PCR" OR "PCR, Anchored" OR "PCR, Inverse" OR "PCR, Nested" OR "Polymerase Chain Reactions" OR "Reaction, Polymerase Chain" OR "Reactions, Polymerase Chain"

Chave de busca - Base de dados WEB OF SCIENCE - descritores MeSH + sinônimos (exceto COVID-19): Todos os campos

"COVID-19"AND "Diagnosis" OR "Antemortem Diagnoses" OR "Antemortem Diagnosis" OR "Diagnoses" OR "Diagnose" OR "Diagnoses and Examinations" OR "Diagnoses, Antemortem" OR "Diagnoses, Postmortem" OR "Diagnosis, Antemortem" OR "Diagnosis, Postmortem" OR "Examinations and Diagnoses" OR "Postmortem Diagnoses" OR "Postmortem Diagnosis" AND "Electrochemistry" OR "Electrochemistries" AND "Polymerase Chain Reaction" OR "Anchored PCR" OR "Anchored Polymerase Chain Reaction" OR "Inverse PCR" OR "Inverse Polymerase Chain Reaction" OR "Nested PCR" OR "Nested Polymerase Chain Reaction" OR "PCR" OR "PCR, Anchored" OR "PCR,

Inverse" OR "PCR, Nested" OR "Polymerase Chain Reactions" OR "Reaction, Polymerase Chain" OR "Reactions, Polymerase Chain"

Chave de busca - Base de dados EMBASE: descritores EMTREE + sinônimos (exceto coronavirus disease 2019): Busca avançada
'coronavirus disease 2019'/exp AND 'diagnosis'/exp OR (bacteriologic diagnosis) OR (diagnostic screening) OR (diagnostic screening programs) OR (diagnostic sign) OR (diagnostic tool) OR (diagnostics) OR (disease diagnosis) OR (medical diagnosis) OR (physical diagnosis) AND 'electrochemistry'/exp AND 'polymerase chain reaction'/exp OR PCR (polymerase chain reaction)

Chave de busca - Base de dados LIVIVO: descritores principais
COVID-19 AND Diagnosis AND Electrochemistry AND Polymerase Chain Reaction

Apêndice 2. Estudos excluídos e as razões para exclusão

Author, Year	Reason For Exclusion
Berkenbrock, Greco-Machado and Achenbach, 2020	5
Krishnan et al, 2021	5
Chauhan et al, 2020	5
Kaushik and Rawtani, 2022	5
LeMieux, 2021	5
Sadique et al, 2021	5
Rong et al, 2021	5
Ulucan-Karnak, Kuru and Yilmaz-Sercinoglu, 2021	5
Cui and Zhou, 2020	5
Verma et al, 2020	5
Madurani et al, 2021	5
Kotru et al, 2021	5
Mukhopadhyay et al, 2020	5
Sekar et al, 2020	5
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ANEXO A – COMPROVANTE SUBMISSÃO NA REVISTA CIENTÍFICA

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