Diagnostic accuracy of clinically applied nanoparticle-based biosensors at detecting SARS-CoV-2 RNA and surface proteins in pharyngeal swabs compared to RT-qPCR as a reference test

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ABSTRACT

Introduction: Nanoparticle-based biosensors (NPBs) are point-of-care diagnostic platforms that can be used for detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) with high accuracy. Areas covered: EBSCOhost Web, Embase, ProQuest, PubMed/MEDLINE, Scopus, Web of Science, and WHO Global Literature on Coronavirus Disease 2019 (COVID-19) were searched for relevant records published from 1 November 2019 to 30 April 2022. Records reporting original data on the accuracy of clinically applied nanoparticle-based biosensors at detecting SARS-CoV-2 RNA and surface proteins from pharyngeal swab specimens were considered. Findings were reported based on the PRISMA 2020 statement. The QUADAS-2 tool was used for assessment of quality and risk of bias among the included studies. Expert opinion: A total of 50 relevant records were identified, of which 13 were included. The included studies explored the diagnostic performance of 13 clinically applied distinct nanoparticle-based biosensors in a total of 789 pharyngeal swabs collected from 376 COVID-19 patients and 413 otherwise healthy individuals. The mean sensitivity, specificity, and accuracy were 97.07%, 94.43%, and 96.91%, respectively, in comparison to RT-qPCR as the reference test. Considering their ease-of-operation, portability, low-cost manufacturing, NPBs could be considered suitable candidate diagnostic platforms for substituting RT-qPCR.

1. Introduction

With as many as 1050 and 120 patents registered in the US and Europe within the last decade, nanoparticle-based biosensors (NPBs) were anticipated to make substantial contributions to diagnosis of human pathologies in 2020s [1]. Having been recognized as the most impactful crisis in global health in the 21st century [2], the coronavirus disease 2019 (COVID-19) would soon become a target of exceedingly high interest for investigations focused on the diagnostic accuracy of NPBs, as confirmed by the soaring number of studies aimed at developing and evaluating the efficacy of such NPBs for identification of the genetic material or surface proteins of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), or anti-SARS-CoV-2 antibodies, in different clinical samples collected from individuals suspected to have COVID-19 [3]. As point-of-care (PoC) molecular diagnostics, NPBs were initially speculated to provide an alternative for more time and expertise-demanding assays, such as quantitative reverse transcription PCR (RT-qPCR), and thus, investigative collaborations from all around the globe have been developing their customized iterations of NPBs ever since the emergence of COVID-19.

In short, a biosensor is a device consisting of bioreceptors (e.g. antibody, DNA, protein receptors, etc.) and transducers, and is considered the backbone of biosensing platforms that are commonly used for detection of various molecules such as infective agents and disease-specific biomarkers. The transducer is an integrated part of any biosensor and is responsible for converting a biological response; for instance, the presence of SARS-CoV-2 particles in a patient sample, to electrical, optical, fluorescent or any other type of signal that can be measured visually. Nanobiosensors or nanosensors are, in effect, biosensors with integrated nanomaterials. These nanomaterials include a very extensive range of nanocomposites like nanotubes (NTs), nanorods (NRs), nanowires (NWs) and nanoparticles (NPs), the latter of which have garnered widespread attention, owing to their high carrier capacity and stability [4].

The earliest account of a clinically validated NPB for diagnosis of COVID-19 is perhaps the one reported in 2020 by Shan B, who developed a multiplex gold nanoparticle (AuNPs)-based sensor array that was capable of detecting...
SARS-CoV-2 particles in human exhaled breath. The device was clinically tried on 49 confirmed cases of COVID-19 and 58 healthy individuals, returning a minimum accuracy of 76% in distinguishing SARS-CoV-2⁺ patients from non-infected individuals [5]. Though, this relatively unsatisfactory precision was significantly improved with iron oxide nanoparticles (Fe₂O₃-NPs) up to 94% within only a year, as demonstrated by Büyüksünetçi YT in an investigation on 82 clinical samples collected from 41 COVID-19 patients and 41 healthy individuals [6]. Considering that it has now been over two years since the first report of COVID-19 in China [2], one might speculate that the field of NPB-based molecular diagnostics has advanced so much that it could outperform other diagnostic assays that are currently being used for clinical identification of SARS-CoV-2 infection. Though, before coming to that conclusion, we should first compare the sensitivity and specificity, the two most important indicators of diagnostic accuracy, of these NPBs with those of RT-qPCR, which is universally regarded as the gold standard for diagnosis of COVID-19 with high reliability based on patient nasopharyngeal (NPS) or oropharyngeal (OPS) swab specimens [7].

As such, we sought to perform a systematic review on the accuracy of nanoparticle-based biosensors as index molecular diagnostics in detection of SARS-CoV-2 infection, compared with RT-qPCR as the current clinical gold standard for this purpose. To this end, we synthesized a set of highly customized search query strings to look for relevant original records, published from 1 November 2019 (the onset of COVID-19 outbreak) to 30 April 2022, on 7 literature databases to deliver the most conclusive evidence-based review on the subject matter. The protocol for the present systematic review was registered on PROSPERO in May, 2021 [8].

2. Methods

2.1. Defining the review question
To come up with the proper question for our prospective systematic review, we used to PICO model, standing for Population, Intervention/Index, Condition/Comparator and Outcome [9]. These four determinants for the present systematic review were: 1) population: COVID-19 patients, 2) index: nanobiosensor-based testing, 3) comparator: RT-qPCR testing, and 4) outcome: diagnostic accuracy of nanobiosensor-based molecular assays at detecting SARS-CoV-2 particles in nasopharyngeal/oropharyngeal swab specimens.

2.2. Validating and registering the study protocol
In order to avoid duplication of efforts, we then searched a number of well-known systematic review registries to make sure that the proposed question had not been reviewed by previous studies. To this end, Database of Abstracts of Reviews of Effects (https://www.crd.york.ac.uk/CRDWeb), the Cochrane Database of Systematic Reviews (https://www.cochranelibrary.com), JBI Systematic Review Register (https://jbi.global), Open Science Framework (https://osf.io) and PROSPERO (https://www.crd.york.ac.uk/prospero) were screened for the proposed question. Ultimately, the proposed protocol was registered on PROSPERO in May 2021 (ID: CRD42021254021).

2.3. Synthesizing search query strings
Seven literature databases, including: 1) EBSCOhost, 2) Embase, 3) ProQuest, 4) PubMed/MEDLINE, 5) Scopus, 6) Web of Science and 7) WHO Global Literature on Coronavirus Disease (https://search.bvsalud.org/global-literature-on-novel-coronavirus-2019-ncov) were selected to be searched using systematically synthesized search query strings that can be viewed in Tables 51-S6. Each combined search query comprised six major keywords, namely, 1) COVID-19 Diagnosis, 2) Diagnostic Accuracy, 3) RT-qPCR, 4) Pharyngeal swab, 5) Nanoparticle, and 6) Biosensor. Synonyms for each keyword were extracted from Embase Emtree (https://www.embase.com/#emtreeSearch/default). The query strings used for searching PubMed/MEDLINE are listed in Table 1. When searching, queries pertaining to each major keyword was augmented with the exact search interval (1 November 2019 to 30 April 2022) to enhance reproducibility of the search. The 6 query strings were then combined into one final string that included two major filters to corroborate those original reports were included (AND Article) and non-original review material (NOT Review) were excluded.

2.4. Systematic search and data extraction
The search queries mentioned above were then looked up independently on the included literature databases by two reviewers (MS and RS). Citation information of records returned by each database were downloaded and imported into Mendeley Desktop Version 1.19.18 (Elsevier, Amsterdam, The Netherlands). After updating meta information and merging duplicates, titles and abstracts of the imported records were screened by the two reviewers against the eligibility or inclusion criteria, which is discussed later. Potentially eligible records were then highlighted and sought for their full texts. After screening the full text of potentially eligible records, studies that met all criteria were included, while records that did not meet at least one criterion were excluded. Reasons for exclusion of each record were provided by the reviewers, who then screened the included studies for key data, which were
Table 1. Query strings used for searching PubMed/MEDLINE.

| Search Number | Category               | Query String |
|---------------|------------------------|--------------|
| #1            | COVID-19 Diagnosis     | ‘COVID-19’[MeSH Terms] OR ‘coronavirus disease 2019’[Title/Abstract] OR ‘sars-cov-2’[MeSH Terms] OR ‘Severe Acute Respiratory Syndrome Coronavirus 2’[Title/Abstract] OR ‘nCoV’[Title/Abstract] OR ‘2019 nCoV’[Title/Abstract] AND ‘(covid-19 testing’[MeSH Terms] OR ‘diagnosis’[MeSH Terms] OR ‘detect’[Title/Abstract] OR diagnostic equipment’[MeSH Terms] OR diagnostic services’[MeSH Terms] OR ‘diagnosis’[MeSH Subheading] AND (2019/11/01[PDAT]: 2022/04/30[PDAT]) |
| #2            | Diagnostic Accuracy    | sensitivity[Title/Abstract] OR specific[Title/Abstract] OR ‘sensitivity and specificity’[MeSH Terms] OR ‘diagnostic accuracy’[Title/Abstract] OR ‘diagnostic efficacy’[Title/Abstract] OR ‘diagnostic value’[Title/Abstract] OR ‘predictive value’[Title/Abstract] OR ‘limit of detection’[MeSH Terms] AND (2019/11/01[PDAT]: 2022/04/30[PDAT]) |
| #3            | RT-qPCR                | ‘reverse transcriptase polymerase chain reaction’[MeSH Terms] OR ‘reverse transcription polymerase chain reaction’[Title/Abstract] OR ‘rt-pcr’[Title/Abstract] OR ‘rt-qpcr’[Title/Abstract] OR ‘rt’[Title/Abstract] OR ‘rna’[MeSH Terms] AND (2019/11/01[PDAT]: 2022/04/30[PDAT]) |
| #4            | Pharyngeal Swab        | ‘opharynx’[MeSH Terms] OR ‘nasopharynx’[MeSH Terms] OR ‘oropharynx’[MeSH Terms] OR ‘nasopharyngeal’[Title/Abstract] OR ‘opharyngeal’[Title/Abstract] OR ‘opharynx’[Title/Abstract] OR ‘throat’[Title/Abstract] OR ‘nasal’[Title/Abstract] OR ‘swab’[Title/Abstract] OR ‘nose’[MeSH Terms] AND (2019/11/01[PDAT]: 2022/04/30[PDAT]) |
| #5            | Nanoparticles          | ‘nanoparticles’[MeSH Terms] OR ‘nanoparticle’[Title/Abstract] OR ‘nano-scale particle’[Title/Abstract] OR ‘nano-sized particle’[Title/Abstract] OR ‘nano-structured particle’[Title/Abstract] OR ‘nanoparticle’[Title/Abstract] OR ‘nanoparticulate’[Title/Abstract] OR ‘nanoparticle’[Title/Abstract] OR ‘nanoscale particle’[Title/Abstract] OR ‘nasopharyngeal’[Title/Abstract] AND (2019/11/01[PDAT]: 2022/04/30[PDAT]) |
| #6            | Biosensors             | ‘biosensing techniques’[MeSH Terms] OR ‘biosensor’[Title/Abstract] OR ‘aptasensor’[Title/Abstract] OR ‘biochip’[Title/Abstract] OR ‘immunosensor’[Title/Abstract] AND (2019/11/01[PDAT]: 2022/04/30[PDAT]) |
| #7            | Combined Search        | (#1 AND #2 AND #3 AND #4 AND #5 AND #6) NOT ‘review’[Title/Abstract] AND [journalarticle[Filter]] |

prospectively entered into a spreadsheet on Google Sheets (Alphabet Inc., CA, USA).

2.5. Inclusion criteria

As part of every systematic review, we defined a set of inclusion criteria that would help us with proper identification and inclusion of potentially eligible records. Records meeting the criteria below were included:

(1) **Study type**: original
(2) **Language**: English or any other language that can be translated to English
(3) **Access**: full-text available
(4) **Index test**: nanoparticle-based biosensor with clearly stated details
(5) **Reference test**: RT-qPCR
(6) **Primary outcome**: sensitivity and specificity (or data required for calculation of each variable)
(7) **Molecular basis of diagnosis**: SARS-CoV-2 RNA and/or surface proteins
(8) **Sample type**: oropharyngeal/nasopharyngeal swab specimens

Any records not meeting the criteria above were duly excluded.

2.6. Assessment of methodological quality and risk of bias

Selected reports will be assessed for their methodological quality based on a modified version of QUADAS-2 introduced in 2013 [10]. QUADAS-2 features a series of signaling questions in four major categories answered with ‘yes, no, or unclear.’ These categories include: 1) Patient Selection, 2) Index Test, 3) Reference Test, and 4) Flow and Timing. We used robvis [11] to generate risk-of-bias figures.

2.7. Evidence synthesis

As the majority of the included studies failed to report standard deviation (SD) for sensitivity and specificity, we adopted a narrative approach for synthesizing evidence based on the quantitative data reported by each study. The only way to synthesize evidence regarding sensitivity and specificity was to calculate positive predictive agreement (PPA) and negative predictive agreement (NPA) based on the number of RT-qPCR-verified positive and negative specimens, and the number of specimens deemed positive and negative by the NPBs under investigation. PPA and NPA are two indicators used for assessment of sensitivity and specificity of an index test in comparison with a reference test [12]. Accuracy, on the other hand, shows the overall performance of an index test compared with that of the reference test. In the present systematic review, PPA, NPA, and accuracy were calculated accordingly:

\[
1.\text{PPA}(\text{Sensitivity}) = \frac{\text{Number of samples identified as positive by NPB}}{\text{Number of samples identified as positive by PCR}}
\]

\[
2.\text{NPA}(\text{specificity}) = \frac{\text{Number of samples identified as negative by NPB}}{\text{Number of samples identified as negative by PCR}}
\]
Number of samples identified as negative or positive by NPB
3. Accuracy = Number of samples identified as negative or positive by PCR

3. Results

3.1. Systematic search

Our systematic search returned 50 records in total (Table S9). Only 19 records remained once duplicates (n = 31) were removed. Of these, one record was, in fact, a review article by Panahi A [13] that was deemed not eligible. The remaining 18 records, all of which were original investigations, were screened for their full texts by two independent reviewers. After exclusion of 5 studies, the reasons for which are listed in Table 2, a total of 13 studies were included (Table 3). We reported the entire process of identification of new studies based on the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) 2020 statement [14], which is illustrated in Figure 1.

3.2. Risk of bias assessment

We developed a personalized version of the QUADAS-2 tool that can be viewed in Table S7-S8. Of the 13 studies included, 5 (38.46%) were found to confer a low risk of bias, while the remaining 8 studies were suspected to be of concern to some extent (see Figure 2). Based on the questions under the first domain (D1), which is concerned with patient selection, 4 (30.76%) investigations were determined to have a high risk of bias, due to their low sample size. The minimum sample size for sensitivity and specificity analysis for a disease with a prevalence of 90, 50, 10, and 5% is 34, 62, 310, and 620, respectively [21]. Regardless of prevalence, which is different for COVID-19 based on the location from which patients under the study come from, investigations by Eissa S [22], Li J [23], Zhao H [24] and López-Valls M [25] all had sample sizes below the minimum, giving rise to a presumed high risk of bias.

In D2, index test, 10 (77%) studies were found to confer moderate risk of bias, since these investigations had interpreted the results of their index test with knowledge of the results of their reference test (RT-qPCR).

While all of the included studies successfully passed the risk of bias assessment in D3, some investigations in D4, patient flow and timing, were suspected to be moderately biased, due to the fact that they had failed to give all their patients the same reference standard, viz., they had collected different types of samples (serum, saliva, feces, or mixed NPS/OPS), and we only used a fraction of their clinical specimens that were, in fact, pharyngeal swab samples.

3.3. Demographic evidence: nanoparticle-based biosensor characteristics

Table 3 provides a biosensor-specific overview of the included studies. Based on the extracted data, the most common type of biosensors was electrochemical (38%), followed by optical (31%) and fluorescent (15%) biosensors. The remaining 15%, labeled as other, refers to biosensors that could not be categorized based on conventional classification of biosensors (Figure 3A). These NPBs developed by Li S [26] and Zhu X [27] were different in that they did not rely on electrochemical, optical or fluorescent signals for detection of SARS-CoV-2 particles, but rather, delivered a platform that could be used for visual identification of COVID-19 samples with naked eye based on lateral movement of the diluted NPS/OPS sample on the sensor, a platform known as lateral flow assay (LFA), which is most commonly adopted for development of rapid antigen test kits [28].

Aside from transducer, which is the part responsible for conversion of signals, bioreceptors integrated into a biosensor are tasked with biological detection of target molecules, e.g. SARS-CoV-2 RNA or surface proteins. Of the 13 NPBs reviewed here, 6 (46%) adopted antibodies as their bioreceptors (Figure 3B). This was followed by DNA (23%), angiotensin-converting enzyme 2 or ACE2 (15%) – the pivotal receptor for SARS-CoV-2 located on the surface of many human cells [29], and RNA (15%).

The building pillars of NPBs, nanoparticles are generally used to enhance conduction of signals in nanobiosensors. These nanoscale particles are often incorporated into biosensors while attached to another molecule termed conjugate, which is responsible for improving the stability of NPs. Based on the information presented in Figure 3C, it can be easily inferred that gold nanoparticles (AuNPs) are the most popular type of nanoparticles, as they were used in 7 of the 13 NPBs reviewed in this article, followed by Polymer NPs (PNPs, 15%) and other types of nanoparticles.

We can see the distribution of target SARS-CoV-2 molecules used for COVID-19 diagnosis in Figure 3D. As expected, spike or S protein (22%) is a frequently used target molecule for detection of SARS-CoV-2, sharing place with the N gene (22%), which codes for the nucleocapsid protein. These two fragments of SARS-CoV-2 are

| Study        | Year | Country     | Biosensor   | Nanomaterial            | Reason for exclusion                                               | Ref. |
|--------------|------|-------------|-------------|-------------------------|-------------------------------------------------------------------|------|
| Eissa S      | 2021 | Saudi Arabia| Electrochemical | Carbon nanofibers       | Application of nanofibers instead of nanoparticles                 | [15] |
| Jian ZW      | 2021 | China       | Photoelectrochemical | AuNPs               | Artificially spiked NPS specimens                                  | [16] |
| Rahmati Z    | 2021 | Iran        | Electrochemical | Copper oxide nanocubes | Artificially spiked NPS specimens                                  | [17] |
| Sampa MHN    | 2021 | USA         | Optical      | Nanoporous material     | Application of nanoporous material instead of nanoparticles        | [18] |
| Tang Z       | 2022 | USA         | Optical      | Quartz capillaries      | Application of quartz capillaries instead of nanoparticles         | [19] |
Table 3. List of studies included in the systematic review, along with biosensor-specific data extracted from their full-texts.

| Study     | Year | Country         | Biosensor         | Detection                  | Nucleic Acid Amplification Type | Bioreceptor | Nanoparticle | Target SARS-CoV-2 Molecule |
|-----------|------|-----------------|-------------------|----------------------------|--------------------------------|-------------|--------------|---------------------------|
| Li S      | 2020 | China           | Other             | Visual                     | LAMP                           | Antibody    | PNs          | Streptavidin              |
|           |      |                 |                   |                            |                                |             | 129          | Nucleocapsid ORF1ab NPS  |
| Ventura BD| 2020 | Italy           | Optical           | Colorimetric               | –                              | Antibody    | AuNPs        | Anti-E/M/S protein antibody |
|           |      |                 |                   |                            |                                |             | 20           | Envelope Membrane NPS OPS |
| Zhu X     | 2020 | China           | Other             | Visual                     | LAMP                           | Antibody    | PNs          | Streptavidin              |
|           |      |                 |                   |                            |                                |             | 129          | Nucleocapsid ORF1ab NPS  |
| Eissa S   | 2021 | Saudi Arabia    | Electrochemical   | Voltammetric               | –                              | Antibody    | AuNPs        | MUA                       |
| Feneira AL| 2021 | Brazil          | Optical           | Colorimetric               |                                | Protein (ACE2) | AuNPs     | Cysteamine NPS OPS       |
|           |      |                 |                   |                            |                                |             | 7            | –                         |
| Li J      | 2021 | China           | Electrochemical   | Voltammetric               | Amplification-free            | DNA         | AuNPs        | PMO                       |
|          |      |                 |                   |                            |                                |             | 10           | RdRp NPS               |
| Liang J   | 2021 | China           | Fluorescent       | Fluorescence               | Amplification-free            | DNA         | AgNPs        | 4ATP Nucleocapsid NPS     |
| Zhao H    | 2021 | Singapore       | Electrochemical   | Voltammetric               | RT-RAA                        | DNA         | DNA-NPs       | Biotin Spike               |
|           |      |                 |                   |                            |                                |             | –            | –                         |
| Beduk D   | 2022 | Turkey          | Electrochemical   | Voltammetric               | –                              | Protein (ACE2) | AuNPs     | Cysteamine – Spike NPS   |
|           |      |                 |                   |                            |                                |             | –            | –                         |
| Dighe K   | 2022 | USA             | Optical           | Colorimetric               | Amplification-free            | Antibody    | AuNPs        | Cysteamine Nucleocapsid   |
|           |      |                 |                   |                            |                                |             | 29           | –                         |
| Durmus C  | 2022 | Turkey          | Electrochemical   | Voltammetric               | –                              | Antibody    | MNPs         | ~ 50 EdC/NHS Spike NPS   |
| López-Valls M | 2022 | Spain          | Optical           | Colorimetric               | NASBA                          | RNA         | AuNPs        | ~ 12 ssRNA – Spike – OPS |
|           |      |                 |                   |                            |                                |             | ~ 22         | ~ 34            |
| Zhang Q   | 2022 | China           | Fluorescent       | Fluorescence               | RT-RAA                        | RNA         | QDMs         | Streptavidin              |

AuNPs: gold nanoparticles; MUA: 11-mercaptoundecanoic acid; ACE2: angiotensin-converting enzyme 2; PMO: phosphorodiamidate morpholino oligos; AgNPs: silver nanoparticles; 4ATP: 4-aminophenol; MNPs: magnetic nanoparticles; PNs: polymer nanoparticles; EdC/Nhs: (N-ethyl-N-(3-dimethyloxypropyl)carbobdilimide/N-hydroxy succinimide); NPS: nasopharyngeal swab; OPs: oropharyngeal swab; LAMP: loop-mediated isothermal amplification; NASBA: Nucleic Acid Sequence Based Amplification; RT-RAA: reverse transcription recombinase-aided amplification.
followed equally by the spike-coding S gene and ORF1ab (17%). Both ORF1ab and the N gene are adopted for RT-qPCR-based diagnosis of COVID-19 in the clinic [30].

A lesser-appreciated constituent of NPBs, conjugates ensure the stability of NPs in test conditions, while providing a surface onto which other molecules such as SARS-CoV-2-targeting antibodies can be lodged. This will be discussed in further detail later in the manuscript. Figure 3E is a pie chart of the prevalence of conjugate molecules in NPBs surveyed in this review, with Cysteamine (23%) and Streptavidin (23%) being the most routinely applied conjugate molecules. According to Figure 3F, the majority of studies preferred NPS as their sample type, with very few collecting their samples in the form of OPS.

3.4. Synthesized evidence

3.4.1. Sensitivity, specificity, and accuracy

Table 4 represents the diagnostic accuracy of NPBs in terms of PPA (sensitivity), NPA (specificity), accuracy, and limit of detection (LOD). In the present systematic review, we surveyed 13 original studies investigating diagnostic accuracy of NPBs on a total of 789 NPS/OPS samples collected from 376 COVID-19 patients (confirmed by RT-qPCR as reference test) and 413 otherwise healthy/COVID-19- individuals. Of the 376 positive samples, 365 were identified as positive by NPBs, indicating a PPA or sensitivity of 97.07%. Of the 413 negative samples, 390 were correctly identified by NPBs as negative, indicating an NPA or specificity of 94.43%. Of the 789 clinical samples tested with RT-qPCR, 755 were correctly identified as positive/negative by NPBs, resulting in an accuracy of 96.91%.

According to Table 5, which summarizes the diagnostic performance of NPBs in three major subgroups defined based on biosensor type, nanoparticle type, and the target molecule being detected or analyzed, biosensors labeled as other, developed by Li S [26] and Zhu X [27], deliver the highest sensitivity and specificity, with detection times as low as 2 minutes. Another important point to be considered here, is the relatively lower specificity of optical biosensors (91.79%), designed by Ventura BD [31] and Ferreira AL [32], that despite being appreciably high, may render this type of biosensors prone to a higher rate of false positives compared with other biosensor types. In terms of nanoparticle type, the least satisfactory accuracy was reported for DNA nanostructures (DNA-NPs), a type of particulate nanosensor. These short strands of 3-deoxyribonucleic acid conjugated with biotin were incorporated by Zhao H [24] into an electrochemical biosensor ultimately yielded a sensitivity of 85% and specificity of 88%, which are considerably lower than those of its counterparts.

The bubble charts in Figure 4 and Figure 5 represent the sample size of each study against its sensitivity, with the size of each bubble indicating the weight of that study in terms of evidence quality. Upon comparing the findings of studies with one another, we noted that the evidence provided by Zhu X [27] was of the highest quality, followed by Ferreira AL [32] and Liang J [33] (see Figure 4A). When compared based on biosensor category, optical biosensors were found to confer the highest quality of evidence, while the other types of biosensors lay close to one another (see Figure 4B). In terms of nanoparticle type, AuNP-based NPBs delivered the highest quality of evidence, followed by PNP-based NPBs (see Figure 5A).

Lastly, we categorized the included NPBs, according to the target SARS-CoV-2 molecule and the application of nucleic acid amplification (NAA) into three subgroups including: 1) protein, 2) RNA with NAA, and 3) RNA without NAA. In general, NPBs designed for detection of SARS-CoV-2 surface proteins are not based on the amplification of DNA or RNA. However, those NPBs working with the genetic material of SARS-CoV-2 may use or skip NAA as a pretreatment procedure, with the latter being known as amplification-free biosensors. Although protein-detecting NPBs demonstrate relatively diagnostic accuracy compared to those developed for detection of SARS-CoV-2 RNA, the ultimate quality of evidence, in the case of protein-detecting NPBs was the highest, followed by RNA-detecting NAA+ and RNA-detecting NAA-free NPBs (see Figure 5B).
3.4.2. Cycle threshold

To provide a better picture of the accuracy of these diagnostic nanobiosensors, we also extracted data regarding the lowest and highest cycle threshold (Ct) values of the correctly identified samples, either positive or negative. Ct value is a well-known indicator of sensitivity in RT-qPCR and is defined as the amplification cycle number that is required for a certain gene, e.g. the N gene of SARS-CoV-2, to exceed the threshold of positivity, and thus, result in a positive RT-qPCR test result. Generally, clinical samples with Ct values occurring in the range of 25–40 are considered to be positive or infected, though, this can be subject to change based on a number of other factors. There is an inverse relationship between Ct value and viral load, meaning that higher Ct values indicate lower concentrations of viral particles in the sample [34]. In this sense, the lower limit of Ct is an indicator of sensitivity, i.e. the performance of a diagnostic test at distinguishing patients based on a given viral load, which is technically desired to be lower to make the test more sensitive. Accordingly, the upper limit of Ct range delineates specificity, i.e. the performance of a diagnostic test at discerning patients with trace or minimal viral loads from healthy individuals.

Of the 13 studies included, only 10 had provided details regarding the Ct values of their samples either in the article or supplementary material. The lowest and highest Ct values of each study are listed in Table 4. The lowest Ct value to have
Figure 3. Demographic illustration of the characteristic features of nanoparticle-based biosensors based on data extracted from the included studies: (a) distribution pattern of NPBs based on the type of biosensor, (b) bioreceptor, (c) nanoparticle, (d) target SARS-CoV-2 molecule, (e) conjugate and (f) clinical sample.

Table 4. List of diagnostic performance indicators based on data extracted from the included studies.

| Study   | Total | COVID+ | CIPS | COVID− | CINS | Patient Status | PPA (%) | NPA (%) | Accuracy (%) | Detection Time (min) | Ct Value | Limit of Detection |
|---------|-------|--------|------|--------|------|----------------|---------|---------|---------------|---------------------|----------|-------------------|
| Li S    | 37    | 14     | 14   | 23     | 23   | Symptomatic (Acute Phase Convalescence) | 100     | 100     | 100           | 2                   | -        | -                 |
| Ventura BD | 94  | 45     | 42   | 49     | 46   | Symptomatic | 93.33   | 93.87   | 93.61         | 3                   | 7        | 36.5              |
| Zhu X   | 129   | 33     | 33   | 96     | 96   | Symptomatic   | 100     | 100     | 100           | 2                   | -        | 12                |
| Eissa S | 6     | 5      | 5    | 1      | 1    | Symptomatic   | 100     | 100     | 100           | 15                  | 21       | 33                |
| Ferreira AL | 100 | 50     | 48   | 50     | 42   | Symptomatic   | 96      | 98      | 90            | 5                   | 19.2     | 35.4              |
| Li J    | 30    | 20     | 20   | 10     | 9    | Symptomatic   | 100     | 90      | 96.66         | 2                   | -        | 223               |
| Li J    | 112   | 24     | 21   | 88     | 88   | Symptomatic   | 87.5    | 100     | 97.32         | 2                   | 24       | 37                |
| Zhao H  | 21    | 13     | 11   | 8      | 7    | Symptomatic   | 84.67   | 87.5    | 85.71         | 0.5                 | -        | 7                 |
| Beduk D | 63    | 55     | 55   | 8      | 8    | Symptomatic   | 100     | 100     | 100           | 1                   | 14.6     | 35                |
| Dighe K | 60    | 30     | 30   | 30     | 30   | Symptomatic   | 100     | 100     | 100           | 10                  | 7        | 29                |
| Durmus C | 50   | 40     | 40   | 10     | 10   | Symptomatic   | 100     | 100     | 100           | 7                   | 11.3     | 35                |
| López-Valls M | 15 | 10    | 9    | 5      | 5    | Symptomatic   | 90      | 100     | 93.33         | 15                  | -        | 16.35             |
| Zhang Q | 62    | 37     | 37   | 25     | 25   | Symptomatic   | 100     | 100     | 100           | 15                  | 24       | 35                |
| Total   | 779   | 376    | 365  | 413    | 390  |                | 97.07   | 94.43   | 96.91         | -                   | 16.95    | 34.75             |

PPA: positive predictive agreement; NPA: negative predictive agreement; CIPS: correctly identified positive samples; CINS: correctly identified negative samples
† This symbol indicates that the clinical specimens were hospital-derived, indicating the possibility that the patients from whom the samples were taken might have been hospitalized.
been detected by NPBs was 7, whereas the highest Ct value was 37. The lowest and highest mean Ct value, averaged from 10 studies, were 16.95 and 34.75, respectively. It should be noted that these Ct values were determined by RT-qPCR for the patient samples analyzed by each investigation, i.e. these values pertain to patient samples, not the absolute performance of NPBs.

### 4. Conclusions

Nanoparticle-based biosensors have been continuously investigated for their accuracy at detecting pathogens since early 2000s, with tremendous efforts being made by a great many of scientists to optimize these emerging molecular diagnostics for clinical purposes. This is confirmed by the considerably high number of NPBs to have been tested for detection of SARS-CoV-2 RNA and surface proteins within the last two years, delivering minimum and maximum sensitivity/specificity of 84.61/87.5 and 100/100%, in comparison with those of RT-qPCR as the current reference test, which is sufficient to warrant new research and development initiatives for mass production and distribution of these nanoparticle-powered biosensing platforms. Although we, in no way, suggest that NPBs must be replaced with RT-qPCR, or any other diagnostic assay, our systematic review mostly seeks to build a proper basis upon which the hidden potentials of NPBs can be appraised.

### 5. Expert opinion

#### 5.1. RT-qPCR; the reference test

Owing to the global spread of COVID-19, today, a good majority of the public are actually familiar with the fact that RT-qPCR is standardly used for ruling out SARS-CoV-2 infection [35]. This is only appropriate, as the PCR testing platform is quite reliable for this purpose with an average specificity of 95%, and a sensitivity ranging from 70 to 98% [36]. As any other diagnostic assay would mandate, RT-PCR or its quantitative counterpart, RT-qPCR, is performed on patient clinical specimens, the most commonly used of which are NPS and OPS specimens. Although other types of samples such as saliva [37], sputum [38] and anal swabs [39] are also frequently used in many parts of the world, NPS/OPS are still considered the reference clinical specimen for COVID-19 diagnosis [40]. In the case of saliva, which is occasionally preferred to NPS/OPS, due its patient-wise convenient method of collection, an overall sensitivity of 86.5% was indicated in a recently published meta-analysis, rendering saliva a less suitable specimen than NPS/OPS that are 92% sensitive [41]. Hence, we set our inclusion criteria in a way to only include studies using NPS/OPS samples as the input for both NPBs (index) and RT-PCR (reference) tests.

Regardless of the sample type, time and time again, RT-PCR is either replaced or complemented with other diagnostic modalities, including reverse transcription loop-mediated isothermal amplification (RT-LAMP), IgM/IgG antibody assays, and CRISPR/Cas, the latter of which is less studied than the former [42]. As an independent platform, RT-LAMP is still subject to a two-fold higher rate of false negatives than RT-PCR [43]. Anti-SARS-CoV-2 antibody assays, on the other hand, are far from being perfect as a result of temporal changes in serum levels of antibodies, they can be used for ruling out COVID-19 in individuals suspected to have false negative RT-PCR results or follow-up of patients who had the disease in the past [44]. Nevertheless, it should be noted that even RT-PCR itself is not 100% reliable. According to a number of investigations, RT-PCR might falsely identify a COVID-19 patient as a healthy individual 0.2–5.8% of instances, depending on the regional prevalence of disease. The rate of misdiagnosis may go as high as 29% when the prevalence is 50%, i.e. one out of two individuals is infected [45]. Despite certain limitations, PCR is a highly flexible technology that can be incorporated into newer platforms like droplet digital PCR (ddPCR), which is believed to be more accurate than its conventional counterpart [46].

One clinically important feature of RT-qPCR is the Ct value assigned to each sample, providing a quantitative measure of viral load that can be used for comparing consecutive test results. Based on a clinical investigation on over 18,000 COVID-19 cases in Spain, the Ct value is usually increased from 23.4 to 35.5 within 19 weeks from the onset of SARS-CoV-2 infection [47]. Another wide-scope study on 254,744 NPS/OPS samples attained a mean lowest Ct value of 25.64 in COVID+ individuals [48]. It is interesting to know that the range of Ct value in asymptomatic patients (15–32) is

| Subgroup | Total | COVID+ | CIPS | COVID− | CINS | PPA (%) | NPA (%) | Accuracy (%) |
|----------|-------|--------|------|--------|------|---------|---------|--------------|
| **Biosensor Type** |       |        |      |        |      |         |         |              |
| Electrochemical | 170   | 133    | 37   | 35     | 98.49 | 49.59   | 97.64   |              |
| Fluorescent       | 174   | 61     | 58   | 113    | 95.08 | 100     | 98.27   |              |
| Optical            | 269   | 135    | 129  | 134    | 95.55 | 91.79   | 93.68   |              |
| Other              | 166   | 47     | 47   | 119    | 100   | 100     | 100     |              |
| **Nanoparticle Type** |     |        |      |        |      |         |         |              |
| AgNPs             | 112   | 24     | 21   | 88     | 87.5  | 100     | 97.32   |              |
| AuNPs             | 368   | 215    | 209  | 153    | 97.20 | 92.15   | 95.18   |              |
| DNA-NPs           | 21    | 13     | 11   | 8      | 84.61 | 87.5    | 85.71   |              |
| MNPs              | 50    | 40     | 40   | 10     | 100   | 100     | 100     |              |
| PNP               | 166   | 47     | 47   | 119    | 100   | 100     | 100     |              |
| QDMS              | 62    | 37     | 37   | 25     | 100   | 100     | 100     |              |
| **Target Molecule** |     |        |      |        |      |         |         |              |
| Protein           | 313   | 195    | 190  | 110    | 97.43 | 90.67   | 94.88   |              |
| RNA with NAA      | 264   | 107    | 104  | 157    | 97.19 | 99.36   | 98.48   |              |
| RNA without NAA   | 202   | 74     | 71   | 128    | 95.94 | 99.21   | 98.01   |              |

PPA: positive predictive agreement; NPA: negative predictive agreement; NAA: nucleic acid amplification.
narrower than symptomatic patients (13–34) [49], with 34 being generally considered the cutoff value for SARS-CoV-2 infectivity [50]. For comparison, SARS-CoV-2 cannot usually be cultured in RT-PCR+ samples with a Ct value above 30 [51]. The significance of Ct value is that it can be used for predicting the likelihood of endotracheal intubation, a method of invasive ventilation support, and the risk of in-hospital mortality [52].

5.2. Nanoparticle-based biosensor; the index test

Within the last two decades, numerous experimental iterations of biosensors based on gold (AuNPs) and silver nanoparticles (AgNPs) have been developed for detection of viral genes, particularly those of RNA nature like SARS-CoV-2 [53]. The earliest report of such lab-based experimental nanobiosensors is perhaps the one published by Grant SA in 2006 that described successful application of an optical AuNP-based biosensor for...
identification of porcine reproductive and respiratory syndrome virus (PRRSv, an enveloped RNA virus causing disease in pigs) with sensitivity of 25 viral particles per mL [54]. Within the next few couple of years to come, alternative versions of AuNP-based optical biosensors were developed for detecting influenza viruses with a minimum LOD of 0.1 pg/mL [55]. One particular AuNP-based RT-LAMP biosensor designed by Ge Y, in 2017, was capable of detecting 101 and 102 RNA copies of influenza A and B viruses per μL, delivering a sensitivity and specificity of 98.3% and 100%, respectively [56]. Most recently, scientists from Brazil

Figure 5. Comparison of the sensitivity and specificity of NPBs. Bubble charts in this figure deliver a specificity-weighted comparison of the sensitivity of the included NPBs, with respect to their sample sizes, divided based on (a) nanoparticle type and (b) mode of detection, i.e. target molecule and the application of the nucleic acid amplification technique as part of the procedure. The size of each colored pin on the bubble charts reflects the specificity of NPB, i.e. the bigger the size, the higher the specificity.
applied a silsesquioxane-functionalized gold nanoparticle-based biosensor for identification of Zika virus (containing RNA genome) particles at concentrations nearing 0.82 pM [57].

Beyond this, NPBs can also be utilized for tracing of pathogenic protein molecules, e.g. surface antigens of several viruses, in a way similar to the NPBs tested by Ventura BD [31], Eissa S [22], Ferreira AL [32], Beduk D [58], and Durmus C [59], all of which were part of our included studies. Another good example of protein-based detection of viruses is the label-free AuNP-decorated biosensor, which was shown to detect the influenza virus hemagglutinin (HA) protein at concentrations as low as 1 pM [60].

Application of NPBs in the case of COVID-19 has not solely been aimed at isolation of viral particles in humans, as there is a whole bigger picture encompassing diagnostic implementation of these devices as part of itself. It was in late 2021 that Alafeef M came up with their customized carbon nanoparticle-based fluorescent biosensor meant to recognize SARS-CoV-2 particles in still waterbodies as a measure for predicting waterborne transmission of virus, capable of detecting 1.5 RNA copies per μL [61]. Preventive application of NPBs was taken to a whole level by Vaquer A, in 2021, when they devised an AuNP-based optical biosensor for recognition of SARS-Cov-2 surface proteins trapped in surgical face masks, which was determined to be 96.2% sensitive and 100% specific for SARS-CoV-2 proteins [62].

With a relative sensitivity, specificity, and accuracy of 97.07%, 94.43%, and 96.91%, respectively, compared to that of RT-qPCR as a 100% sensitive, specific and accurate diagnostic test (in theory), NPBs can be suitable substitutes and even successors to RT-qPCR, given that they are significantly inexpensive to produce. The NPB developed by Ferreira AL only costed 15€ (Euro) at the time of its preparation [32]. The nanobiosensor tested by Dighé K was designed with a total cost of 12 USD (USD), making it an even more affordable option [63]. This is an advantage that should not be overlooked, particularly when considering the fact that the majority of these NPBs are self-contained standalone assays that do not require specialized devices, take for instance the LFA-based biosensors developed by Li S [26] and Zhu X [27]. For comparison, the cost of a basic RT-qPCR device is at least 30 USD [64], and the mean cost of RT-qPCR-based COVID-19 diagnosis was around 50 USD in 2020 [65].

5.3. Challenges associated with NPB-based diagnostics

Nevertheless, in spite of all merits associated with NPBs, these diagnostic nanodevices come with their own set of disadvantages, which might prove too challenging of an issue to overcome at times. An important issue associated with NPB-based diagnosis of nanoscale pathogens such as viruses is that they require simultaneous and sufficient binding of recognition sites in a highly restricted space, with a surface area of a few nanometers, to produce fluorescent or colorimetric signals that can be read by the sensor [66]. A good majority of NPBs reviewed here are based on a protein – target binding system for diagnosis of COVID-19, in which the protein is an antibody as bioreceptor, and the target is the genetic material or surface antigens of SARS-CoV-2. Though, the mere presence of these two sets of molecules may not result in effective detection of the pathogen, as certain conjugates such as streptavidin should also be readily available to functionalize and stabilize the nanoparticles being incorporated into the system, while also facilitate the binding of, say, SARS-CoV-2 RNA to the bioreceptor [26]. Generation of such bioreceptors to confer the optimal bioaffinity might be a challenging task in the mass-production of a highly sensitive and specific nanobiosensor, resulting in the lack of a uniform platform capable of delivering comparable levels of accuracy compared to the reference test [67].

The signal transduction element is another feature of NPBs that may challenge the clinicians handling these devices. The principal purpose of a signal transduction element is to translate the biorecognition event (e.g. successful binding of SARS-CoV-2 RNA to its bioreceptor) to a readable electrical, optical, thermal or magnetic signal that can be interpreted by the clinician [67]. While a number of NPBs based on lateral flow assay (LFA) provide a visual means of detecting SARS-CoV-2, other NPBs that give out an electrochemical or optical signals might require a certain level of expertise, as does the standard RT-PCR method, that would lead to lesser inclination of clinicians to use such cumbersome devices. In this regard, it is recommended that research and development programs associated with NPBs be focused on designing certain iterations that provide enhanced readability for rapid clinical application.

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