

# Identification of SARS-CoV-2 by gold nanoparticles

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**Abstract:** The SARS-CoV-2 outbreaks highlighted the need for effective, reliable, fast, easy-to-do and cheap diagnostics procedures. We pragmatically experienced that an early positive-case detection, inevitably coupled with a mass vaccination campaign, is a milestone to control the COVID-19 pandemic. Gold nanoparticles (AuNPs) can indeed play a crucial role in this context, as their physicochemical, optics and electronics properties are being extensively used in photothermal therapy (PTT), radiation therapy (RT), drug delivery and diagnostic. AuNPs can be synthesized by several approaches to obtain different sizes and shapes that can be easily functionalized with many kinds of molecules such as antibodies, proteins, probes, and lipids. In addition, AuNPs showed high biocompatibility making them useful tool in medicine field. We thus reviewed here the most relevant evidence on AuNPs as effective way to detect the presence of SARS-CoV-2 antigens. We trust future diagnostic efforts must take this ‘old-fashioned’ nanotechnology tool into consideration for the development and commercialization of reliable and feasible detection kits.

## Introduction

### *Focus on the SARS-CoV-2*

The Coronaviruses, belonging to the *Coronaviridae* family, are characterized by an enveloped, non-segmented, positive-sense RNA (Yang *et al.*, 2020; Liu *et al.*, 2020b). In humans, they can induce severe symptoms at respiratory, hepatic, and enteric level. In December 2019, a novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) quickly spread from China to the whole world (Mi *et al.*, 2020), inducing the well-known Covid-19 disease. As of 4 April 2022, time of this article drafting, there were approximately 486 million confirmed infection cases worldwide (<https://covid19.who.it>; Dong *et al.*, 2020). The easy human-to-human transmission is ascribed to the virus airborne nature, which makes it easy to spread through air droplets especially in the last two virus variants, namely Omicron and Omicron 2 (Rowe *et al.*, 2022; Dance, 2022). Also, the biomolecular interaction has been characterized straight after the beginning of the pandemic outbreak and explained by the selective binding of the virus spike protein to the angiotensin-

converting enzyme 2 (ACE2) receptors, which are over-expressed by type 2 pneumocytes in human airways (Kumar *et al.*, 2021). The further virus uptake is due to the lysis of spike proteins induced by specific proteases of pneumocytes, which favors the entry by endocytosis or membrane fusion (Mirastschijski *et al.*, 2020). Once within cells, the SARS-CoV-2 releases its positive-sense RNA within the host cytoplasm, which triggers the production of pp1a and pp1ab polyproteins, and promotes the replication and transcription of viral RNA. The further transcription of viral mRNA induces the production of viral structural components like membrane, spike and nucleocapsid proteins (Zhang *et al.*, 2021). The viral particles are transported to the proximity of the host plasma membrane through the Golgi, and the new formed viruses escape by exocytosis, a specific mechanism promoting the infection of neighboring cells (Nakagawa *et al.*, 2016; Mason, 2020) (Fig. 1).

The massive inoculation of different kinds of vaccines worldwide has provided a reduction of Covid-19 outbreaks; in addition to the more conventional vaccines containing a weakened or killed virus, mRNA vaccines, such as the BNT162b2 vaccine, produced by BioNTech (in collaboration with Pfizer) delivers a small segment of mRNA loaded in lipidic nanovesicles into the cells (Kitchin *et al.*, 2020). This mRNA portion has the particularity to have the instructions to produce the spike proteins activating immune cells

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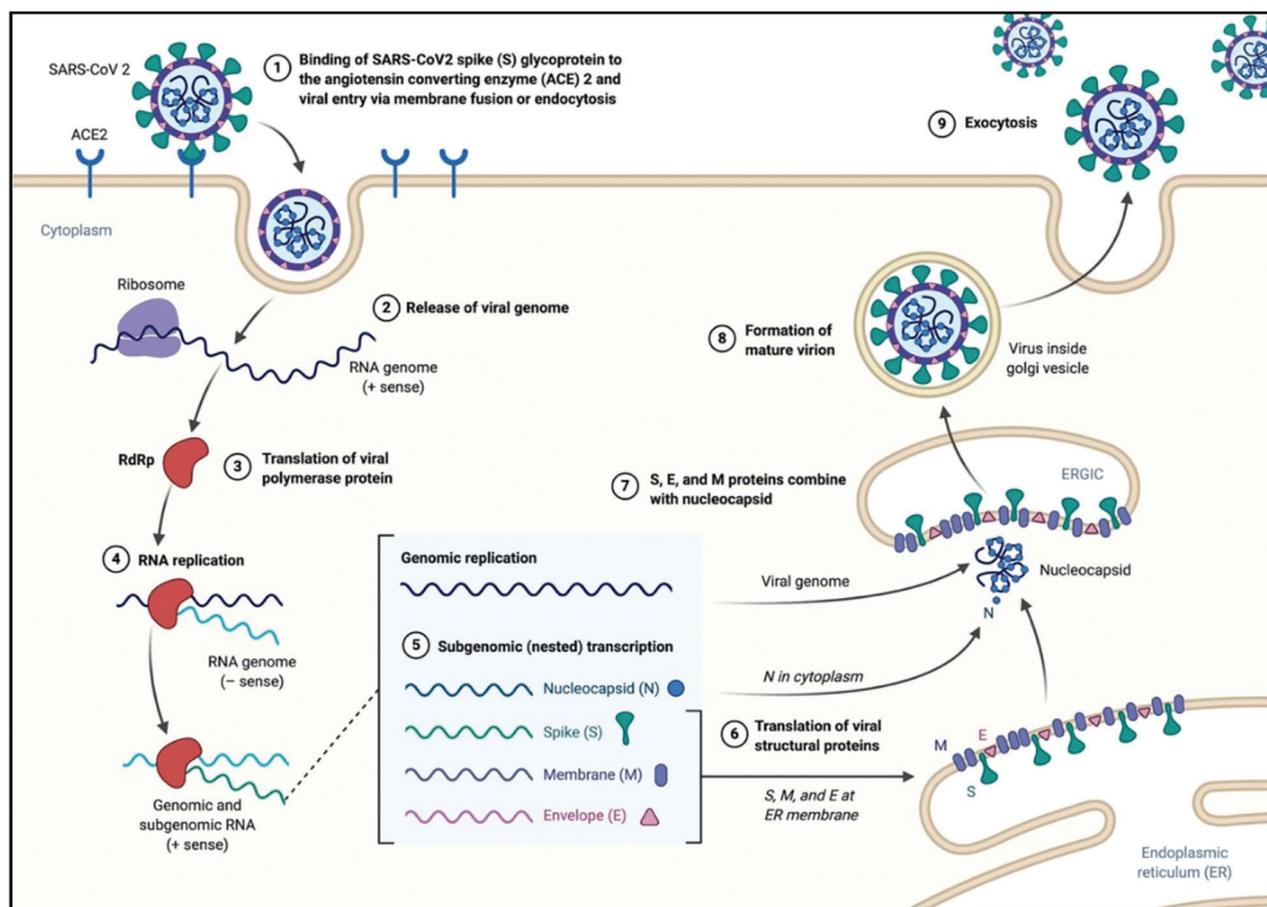


FIGURE 1. Mechanism of infection and life cycle of SARS-CoV-19 (Alanagreh et al., 2020).

producing antibodies (Tejaro and Farber, 2021). The lipidic nanostructures are constituted by different types of lipidic molecules, but one of these is positive charged. Then, these molecules bind mRNA (negatively charged) but lose their positive charge when the basic conditions of the bloodstream occurred to reduce their adverse effects in human body (Dolgin, 2021). However, scientists were developing new strategies for enhancing the endosomal release of therapeutic nucleic acids, such as the use of new surfactants that enhance the delivery of mRNA into the cells (Roise et al., 2022). Nevertheless, the scientific community also focused on the early diagnosis of the virus, to develop remedies that can support the active vaccination campaign. Indeed, the diagnostic assays can be a powerful tool to facilitate the surveillance after the vaccination as vaccinated people can also be infected by the virus (Fresco-Taboada et al., 2022). In this scenario, nanotechnologies could be an optimal alternative for early SARS-CoV-2 diagnostic, especially using metallic NPs. Among these, gold (Au) based nanomaterials are probably the best choice for its unique physicochemical properties as it allows a combination of reliable detection (for diagnostic) together with a low toxicity (de Matteis and Rizzello, 2020; Silva Lopes et al., 2014; de Matteis et al., 2017).

#### Physicochemical properties of AuNPs

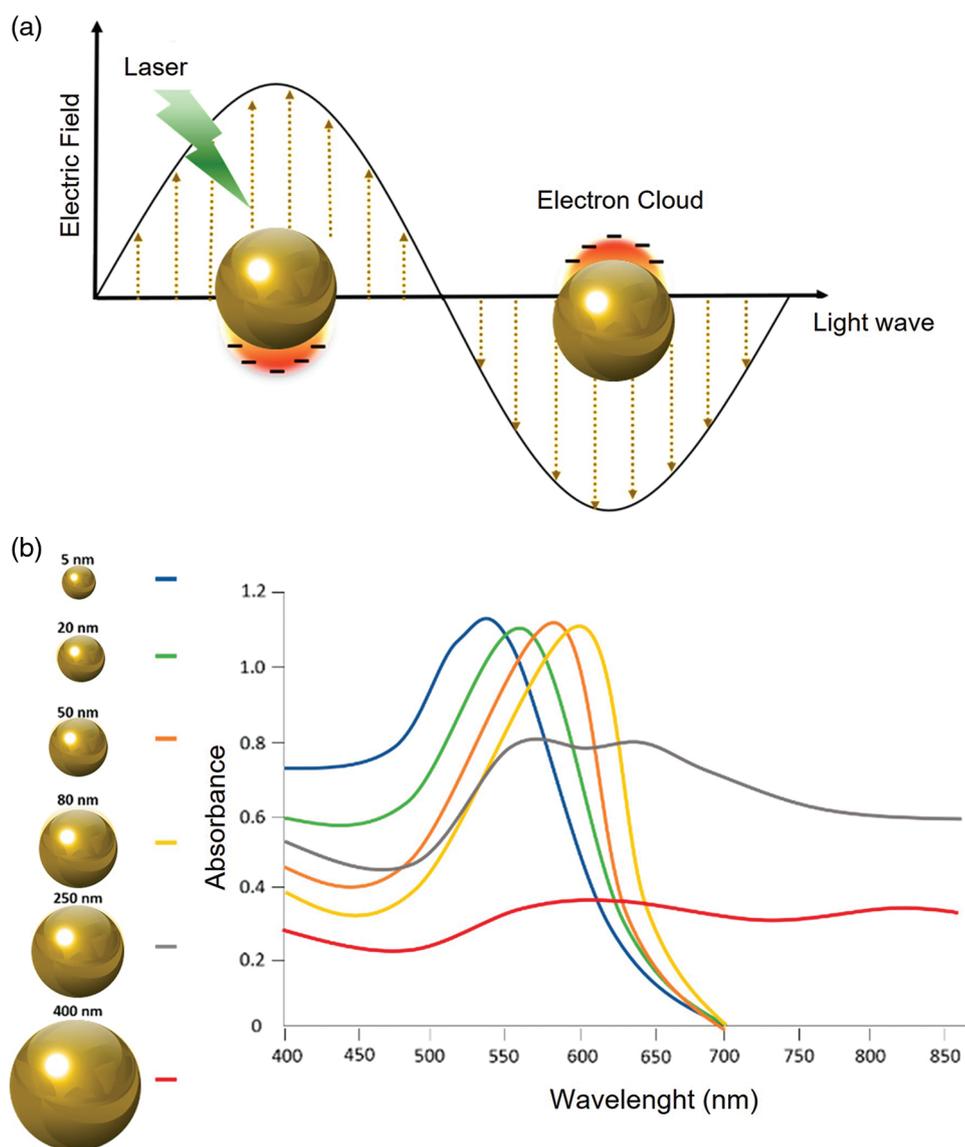
The physicochemical properties of AuNPs are several. However, some of them turn out to be suitable with a view to using these nano-objects as a diagnostic or therapeutic agent

(Dykman and Khlebtsov, 2011; Yeh et al., 2012). First, the Localized Surface Plasmon Resonance (LSPR) is by far the 'hallmark' among all the AuNPs physical properties. LSPR is an optical phenomenon occurring when polarized light triggers the oscillation of free conduction electrons on the AuNPs surface (de Matteis et al., 2019). This specific feature of AuNPs makes possible a strong improvement of the optical extinction compared to the conventional organic molecules.

In general, the position of the LSPR peak is strongly dependent on the nanoparticle's physical properties such as such shape and size. In addition, the dielectric properties (Saison-Francioso et al., 2015) as well as the local environment, namely surface-confined molecules substrate, solvent, and substrate, influence the LSPR phenomenon (Peixoto de Almeida et al., 2014) (Fig. 2).

Moreover, the enhanced absorption of light by AuNPs has tremendous implications in different medicine sectors such as cancer treatment (Dykman and Khlebtsov, 2011) namely photothermal therapy applications (Bai et al., 2020; De Puig et al., 2015) and NP-based biosensors using colorimetric point-of-care devices (Iarossi et al., 2018). In the latter case, the color of NPs solution changes due to plasmonic coupling between the NPs, as the color shift from red to purple color is due to aggregation in solution (Ghosh and Pal, 2007).

In addition to this, the use of Au in the body is tolerated due to its high reduction potential which makes it less ionizable and then stable in the body (Albanese and Chan, 2011). Several studies performed in living cells and animal



**FIGURE 2.** LSPR of AuNPs (a); Different UV-visible absorption spectra based on AuNPs size (b). Adapted from [Kim and Lee \(2018\)](#).

models demonstrated the biocompatibility of AuNPs ([Zhang \*et al.\*, 2011](#); [de Matteis \*et al.\*, 2020](#)) which therefore is strongly dependent on NPs size and concentrations ([Kang \*et al.\*, 2020](#)) as well as on the cell types. In particular, the small and high doses correspond to the high toxicity ([Vecchio \*et al.\*, 2012](#); [Sani \*et al.\*, 2021](#)).

The surface functionalization of AuNPs with several types of polymers, proteins, peptides, surfactant is a good approach to reduce the impact of NPs in cells ([Mahato \*et al.\*, 2019](#)).

In a typical photothermal treatment, the cancer cells are heated using temperatures between 41°C to 43°C by NIR stimulation after the uptake of Au nanomaterials (such as Au nanorods or Au nanoshells); as consequence, malignant cells can be destroyed ([Zaho \*et al.\*, 2014](#); [de Matteis \*et al.\*, 2021](#)). As well as being a therapeutic agent, AuNPs can be used as diagnostic tools by exploiting other intrinsic characteristics of Au at the nanoscale.

For example, it is well known that the Raman scattering, representing the footprint of a certain molecule, has several applications in different fields ([Cheng \*et al.\*, 2018](#)). However, its use is limited by the small cross section associated to the Raman scattering that is 10–30 to 10–25 cm<sup>2</sup> per molecule

([Nie and Emory, 1997](#)). To improve the scattering, the molecules should be in the proximity of the AuNPs in order to exploit their local optical fields; this triggers a signal enhancement called Surface-Enhanced Raman Scattering (SERS) ([Shuker and Gammon, 1970](#); [Lee \*et al.\*, 2011](#)). The latter is dependent on the AuNPs physicochemical properties such as shape, size, and aggregation. In particular, the best enhancement can be obtained on AuNPs aggregates or cluster having a size ranging from 20 nm to 60 nm ([Kneipp \*et al.\*, 1997](#)).

LSPR can also modulates the quantum yield of fluorescent dyes near their surface, thus inducing fluorescent enhancement or, alternatively, quenching, a process known as Metal Enhanced Fluorescence (MEF) ([Geddes and Lakowicz, 2002](#); [Shankar \*et al.\*, 2009](#)). The two distinct effects (enhancement or quenching) are functions of the distance between the Au surface and the dye. This phenomenon is based on the fluorescence resonance energy transfer (FRET), responsible of the fluorescence quenching. FRET regards the energy transfer between fluorophores. It is widely used to study the potential interaction of biological molecules (such as proteins, lipids, or nucleic acids) located

in proximity. The mechanism exploits the presence of two fluorescent molecules, called donor and acceptor. The donor can be excited at a specific wavelength. This molecule emits energy which, in turn, can be transmitted to the acceptor, that consequently emits fluorescence at a different wavelength of emission compared to the donor. To this respect, AuNPs can improve the sensitivity and efficiency of FRET due to their large molar extinction coefficients (Chen *et al.*, 2012).

The latter is also extremely useful for enhancing the sensitivity of standard colorimetric assays (Brennan *et al.*, 2009). One advantage is the possibility to make a real-time monitoring with a good reproducibility and sensitivity, making AuNPs suitable for point of care (POC) diagnostics (Mariani and Minunni, 2014). For example, the plasmon-induced color switch has been exploited to detect altered levels of cytokines, proteins, DNA in cancer (Reddy *et al.*, 2012), to monitor the bacteria contamination in food (Waswa *et al.*, 2007) and in laboratory medicine (Helmerhorst *et al.*, 2012).

In the context of X-ray-based diagnostic, AuNPs are applied as alternative tools to the traditional contrast agents like iodine or barium sulfate (Künzel *et al.*, 2013). The need to replace the standard contrast agents is attributable to their high kidney toxicity (Kaller and An, 2022). AuNPs are particularly proper for this application due to the high atomic number ( $Z = 79$ ) and density ( $=19.3 \text{ g/cm}^3$ ) (Cutler *et al.*, 2012). Furthermore, AuNPs show high X-ray attenuation coefficients providing sharper images enhancing the time of blood circulation (Hernandez-Rivera *et al.*, 2017; Leung *et al.*, 2011).

For biomedical, forensic, and environmental applications is crucial to detect chemical and biological molecules. Developing affordable, highly sensitive, miniature sensors is the only way to achieve this goal. As a rule, sensors comprise two parts: a recognition element for selectively binding the target analyte, and a transducer able to highlight the binding event (Naresh and Lee, 2021). The performance of a capable sensor is deeply dependent on these two elements based on selectivity, response time, and limits of detection (LOD) (Mathew *et al.*, 2021). It is well known that nanomaterials have remarkable physicochemical properties that can be of great assistance in creating new recognition and transduction processes for chemical and biological sensors miniaturizing sensor elements (Malik *et al.*, 2021). AuNPs are remarkably interesting for their several properties as described above and, in addition, high oxidative stability and low toxicity compared to others types of inorganic materials (Bakand *et al.*, 2012). They can use alone or in combination with other types of nanomaterials such as covalent organic frameworks-AuNPs composite (COFs-AuNPs) to monitor kidney injury (Boyacıoğlu *et al.*, 2022) Tumor Necrosis Factor-alpha (TNF- $\alpha$ ) (Yola and Atar, 2021) or to detect toxins potentially dangerous for living organisms and environment (Karaman *et al.*, 2021).

## Diagnosis of COVID-19 Using AuNPs

### Common standard tests

It is indeed accepted that an early diagnosis represents the best method to prevent the uncontrolled virus transmission. Three

broad groups of diagnostic tests are developed to monitor the spread of COVID-19 (Jarrom *et al.*, 2022):

- (i) Tests able to detect the existence of coronavirus in the respiratory secretion consisting in the amplification of viral nucleic acid by Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) (Kevadiya *et al.*, 2021);
- (ii) Antigen tests (rapid diagnostic tests) to directly detect SARS-CoV-2 proteins produced by virus in respiratory secretions (Interim guidance WHO, 2020). Today, represents the golden standard for a rapid, yet effective way to monitor the positive cases.
- (iii) Serum tests revealing the presence of antibodies against SARS-CoV-2 in blood.

Specialized laboratories process the samples from nasopharyngeal swabs but, as the spread of the virus progresses, self-screening tests have also been developed. Then, the antigenic as well as the serological approaches have been developed as laboratory-based tests. Serological tests can be useful to indirectly identify the virus, that is through the detection of antibodies produced in the serum following the interaction with the pathogen. Rapid *in vitro* diagnostic tests for the qualitative detection of IgG and IgM antibodies against SARS-CoV-2 in human serum, plasma, venous whole blood, and acupuncture were developed by some companies such as Abbott BinaxNow (<https://www.abbott.com/>), bioMérieux SA (<https://www.biomerieux.it/>), AESKU Diagnostics (<https://www.aesku.com/>), Roche (<https://www.roche.com/>) and other ones. However, the limitation about sensitivity and specificity as well as the LOD quantity highlights the need for different and more sensitive approach able to monitor the COVID-19 spread in the population.

In this framework, a lot of nanomaterials, thanks to the aforementioned properties seems to be the best choice (Hasanzadeh *et al.*, 2021).

### AuNPs-based tests

The WHO guidelines reported the methodology of Point-Of-Care (POC) devices based on the detection of viral genes and proteins (Amer *et al.*, 2013) These “pieces” of virus are anchored on the NPs surface and the detection is based on electrical, optical, and electrochemical strategies (Lee *et al.*, 2018). The physicochemical properties of AuNPs have been thus investigated in terms of both detection and treatment of the SARS-CoV-2 (Bidram *et al.*, 2021). In particular, the AuNPs-based colorimetric detection is particularly suitable for the red-to-blue shift as consequence of LSPR coupling among NPs (Li *et al.*, 2015) permitting a rapid screening without the use of expensive instruments. Some works improved AuNPs using anti-spike (Pramanik *et al.*, 2021) or anti-Nucleocapsid (N) antibodies attached on the AuNPs surface by colorimetric reading. SERS was used for the virus identification, using 4-aminothiophenol as reporter molecule linked on AuNPs by Au-S bonds (Pramanik *et al.*, 2021). The addition of the virus with the AuNPs in the reaction mixture induced the aggregation of NPs, which was immediately distinguished even by naked eye thanks to the solution color change from pink to blue. The aggregated AuNPs created “hot-spots” showing a strong SERS signal

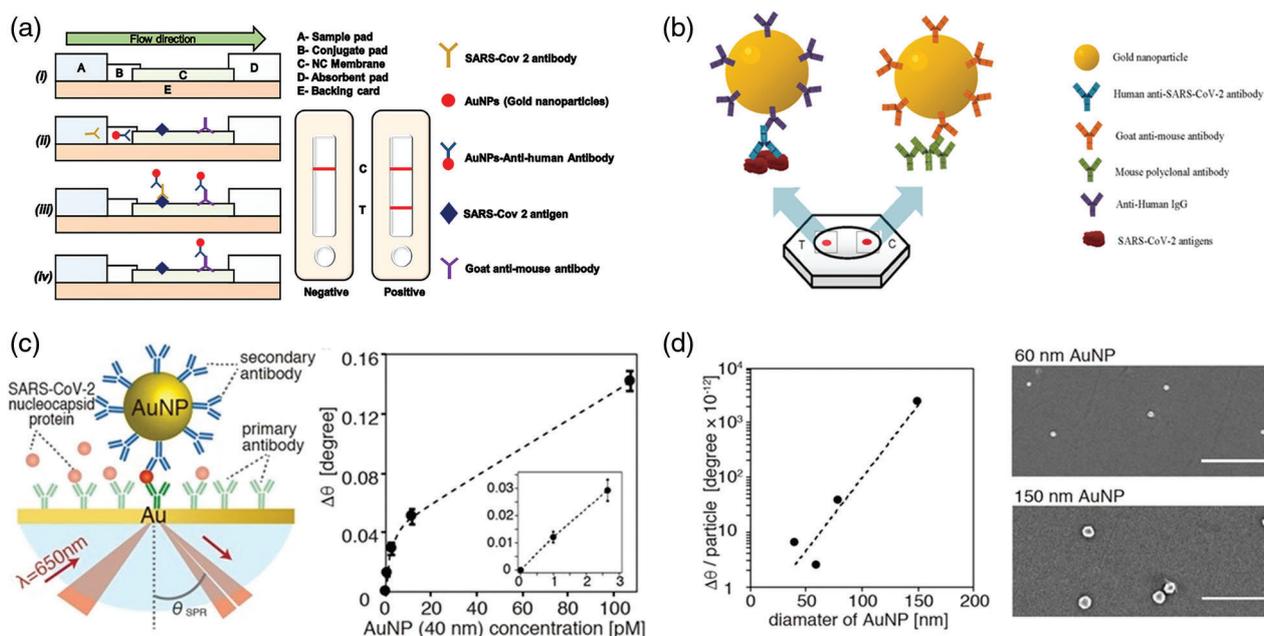
derived from 4-aminothiophenol and antibody attached on the NPs surface. This methodology allowed detecting up to 4 pg/mL of viral proteins in five minutes. Moreover, the effectiveness of the nano system on the virus spread was demonstrated using Cellosaurus HEK293T cells, known to express angiotensin-converting enzyme 2 (ACE2) receptor. Then, anti-spike antibodies functionalized AuNPs effectively blocked the SARS-CoV-2 infection by inhibiting its replication. In a recent strategy (Aithal *et al.*, 2022), AuNPs were decorated by a certain aptamer able to bind the SARS-CoV-2 spike proteins. The spikes were recognized upon addition of a coagulant. According to this approach, the AuNPs that did not recognize the spike underwent an aggregation process, whereas the binding event was detected by SPR. This system allowed detecting a concentration down to 16 nM of free spike proteins.

Femtomolar detection of spike proteins was carried out using plasmonic metasensor technology which can compress electromagnetic fields (Ahmadivand *et al.*, 2021). The advantages of these structures were the low-radiative losses and a strong electromagnetic fields confinement. A plasmonic label-free toroidal metasurfaces at terahertz frequencies were fabricated ( $\sim 0.4$  THz) coupled with colloidal AuNPs functionalized by specific monoclonal antibody to improve the binding features of the molecules. After excitation, the resonance shifts based on the different concentrations of spike proteins binding antibodies. The LOD of this device was about 4.2 fM. Engineered terahertz (THz) plasmonic metamaterials, in particular toroidal materials, are new technologies for diagnosis for their low impact *in vivo* and *in vitro* systems and in addition, they are non-destructive platforms (Ahmadivand *et al.*, 2018). As firstly described, the

coupling with AuNPs is very useful for the detection of proteins due to the opportunity to detect several targets observing distinct resonance peaks (Xu *et al.*, 2016).

#### AuNPs-based serum tests

In this context, smart devices were developed for IgG detection, using AuNPs (Li *et al.*, 2015; Liu *et al.*, 2020a; Pohanka, 2021; Ahmadi *et al.*, 2021). Among these, an interesting platform based on a lateral flow immunoassay strip (LFIA) has been recently created (Wen *et al.*, 2020; Gupta *et al.*, 2020) (Fig. 3a). The nucleocapsid proteins of SARS-CoV-2 were immobilized on the strip to specifically bind IgG-functionalized AuNPs. The goodness and reproducibility of the assay was finally assessed using serum samples from clinically diagnosed cases of COVID-19. The presence of target proteins was evaluated following the color footprint of AuNPs, making this test particularly suitable to equip the conventional test and to use it in low-resource countries. In addition, LFIA can be potentially applied in mass screening or home tests. A colorimetric assay was developed to detect IgGs in plasma using AuNPs functionalized by antigenic epitopes (Lew *et al.*, 2021). These immunodominant linear B-cell epitopes are present on spike and nucleocapsid proteins of SARS-CoV-2 showing great affinity with IgG. For this reason, the AuNPs surface was decorated with epitopes to bind specific antibodies. The binding event induced NPs aggregation triggering the plasmonic absorbance change, thus allowing detection of the virus with a LOD of 3.2 nM. The clinical tests, performed on human plasma medium, showed the identification of coronavirus with 100% of specificity, also in circulating COVID-19 variants (Lew *et al.*, 2021).



**FIGURE 3.** (a) Schematic representation of Lateral flow assay (LFA) using AuNPs. In the figure is reported the conjugation between the antibody and antigen as well as the relative positive results on the test (Gupta *et al.*, 2020); (b) Rapid flow-through dot-blot immunoassay (FT-DBA) using AuNPs functionalized by anti-human IgG. AuNPs with goat anti-mouse can bind to mouse polyclonal antibody (control) (Sil *et al.*, 2021); (c) identification of nucleocapsid protein of SARS-CoV-2 using AuNPs and SPR angular shift ( $\Delta\theta$ ) degree related to 40 nm AuNPs at different concentrations (pM); (d) Angular shift as function of different sizes of AuNPs and SEM acquisitions of AuNPs after SPR measurements (Yano *et al.*, 2022).

Another work focused on the recognition of SARS-CoV-2 using serological test implemented with dot-blot assay (FT-DBA) for the detection of IgG (Sil *et al.*, 2021). In this approach, AuNPs were functionalized with anti-human IgG (hIgG-AuNPs) able to bind the virus antigens immobilized on nitrocellulose membrane.

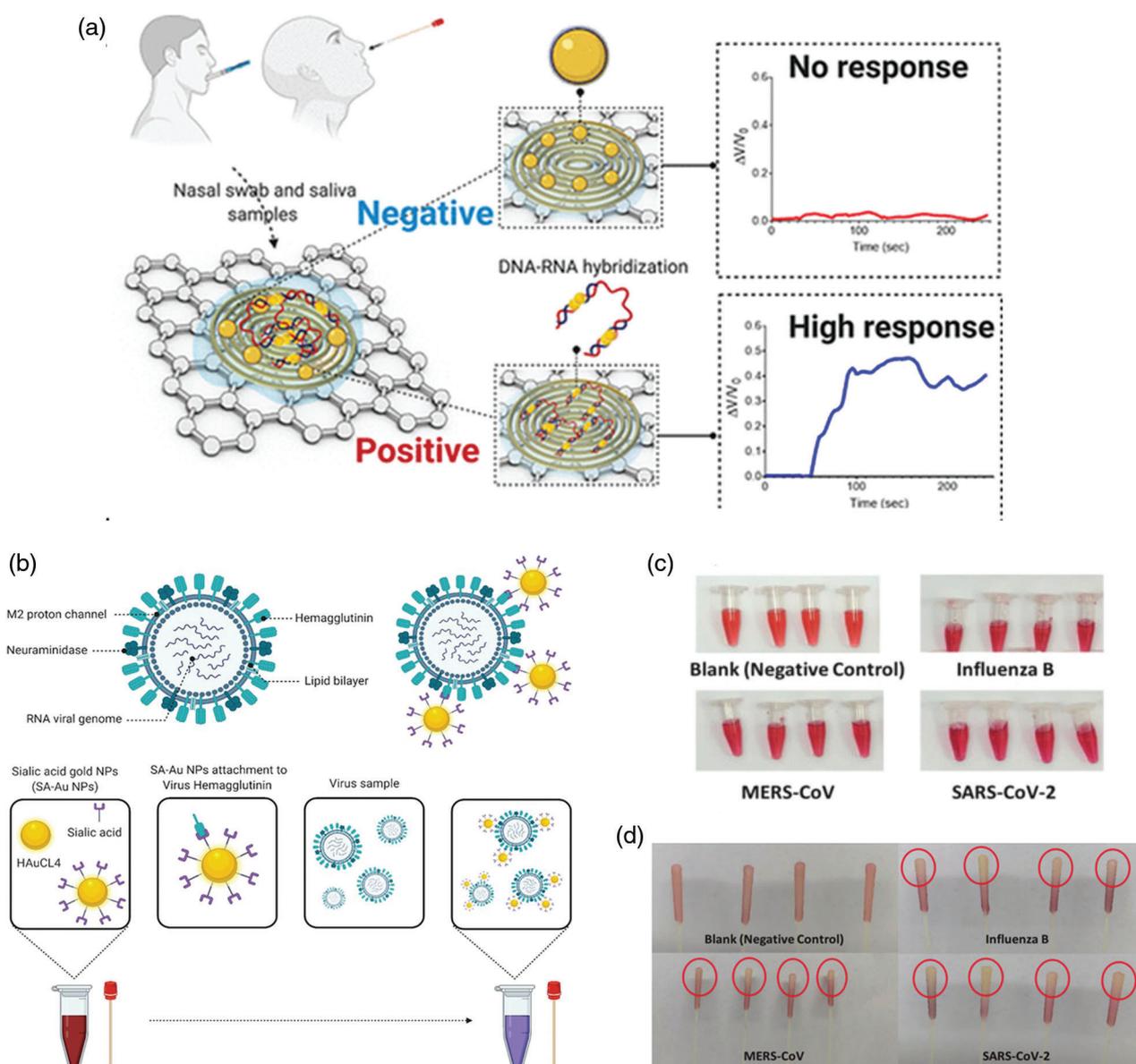
The selectivity and sensibility were higher compared to ELISA standard tests, obtaining results within two minutes, with sensitivity and specificity of about 98% (Fig. 3b).

Recently, Yano *et al.* (2022) created a platform in which the SPR of AuNPs, conveniently functionalized by antibodies, was used to find the nucleocapsid proteins of the SARS-CoV-2. The large size of the AuNPs (150 nm) enhanced the sensitivity detection with respect to the smaller AuNPs allowing to found protein virus at fM levels. The experimental data were both theoretical and experimental

comparing size of 40 nm and 150 nm showing an increase of SPR angle of 0.94 degree for larger NPs; the smaller exhibited only a 0.06 degree (Figs. 3c and 3d).

#### AuNPs-based nasal swab tests

The nasal swab are the most popular tests. In a recent experimental work, an Au antigen-antibody nanoplatform was developed showing a sensitivity and specificity higher than 95% (Della Ventura *et al.*, 2020). The photochemical immobilization technique (PIT), a surface functionalization procedure, was employed to absorb antibodies on the AuNPs surface. Then, the nanoplatform was used to bind three surface viral proteins, namely the spike, the envelope, and the membrane proteins (S, E, and M, respectively). The AuNPs-protein interaction triggered the red shift of the absorption spectra. Alafeef *et al.* (2020) developed a paper-based colorimetric sensors to



**FIGURE 4.** (a) Schematic principle of paper based colorimetric sensors with AuNPs-ssDNA. In the presence of SARS-CoV-2, the AuNPs-ssDNA bind viral RNA triggering a strong electrochemical signal. Reprinted by permission of American Chemical Society (Alafeef *et al.*, 2020); (b) Colorimetric detection of SARS-CoV-2 based on AuNPs conjugated with sialic acid, (c) positive swabs of different kind of viruses included SARS-CoV-2 after immersion in solution containing AuNPs and (d) colored tips of samples compared to the negative control (Alfassam *et al.*, 2021).

digitally detect SARS-CoV-2 by its viral RNA. The sensors were created using AuNPs functionalized by antisense oligonucleotides (ssDNA) able to bind nucleocapsid coronavirus protein, which generated electrochemical response by graphene-ssDNA-AuNP surface (Fig. 4a).

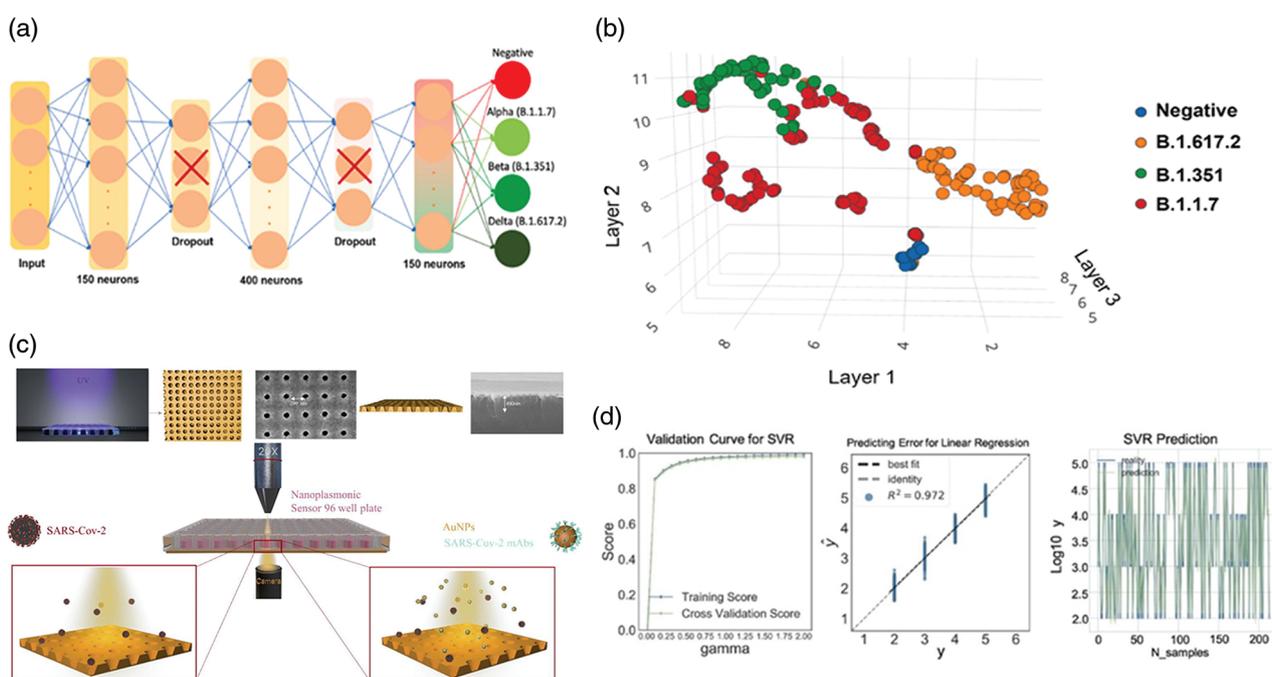
Hyperspectral microscopic data, together with the evidence on clinical samples of positive COVID-19 and healthy asymptomatic subjects, confirmed the effectiveness of this sensors with 100% of accuracy and a sensitivity of 231 copies  $\mu\text{L}^{-1}$ . The LOD was 6.9 copies/ $\mu\text{L}$  without other amplification cycles. A detailed protocol for a nano-amplified colorimetric test to relieve SARS-CoV-2 without the need of RNA extraction was recently reported (Alafeef *et al.*, 2021). This test was very effective due to the nano-amplification of plasmonic AuNPs previously functionalized by antisense oligonucleotides (ASOs), used as a colorimetric reporter to bind and amplify the viral genetic material. The use of ASOs was crucial for their ability to bind nucleocapsid proteins; the NPs aggregation induced a change in plasmonic response of the NPs upon interaction with the virus. The LOD was recorded to 10 copies/ $\mu\text{L}$  showing high specificity and sensitivity without using expensive equipment. In addition, this test can be used for a quantitative response using a handheld optical reader. Rodríguez-Díaz (Rodríguez-Díaz *et al.*, 2022) produced a colorimetric test based on AuNPs (15–38 nm) and PCR. In this specific conformation, the cholesterol is folded inside and the AuNPs solution takes on a red color. On the other hand, when the target is present, the molecules change their conformation anchoring the target, thus determining the aggregation of the nanostructures. The result was spectrophotometrically quantifiable using patient respiratory swab samples. Finally, the authors implemented PCR procedure to complete the detection in 1 h. Recently (Alfassam *et al.*, 2021), AuNPs were conjugated with sialic acid, a glycoprotein binding lung epithelial cell. This molecule

represented a common target of respiratory virus, as it binds the viral surface protein hemagglutinin. Nasopharyngeal swabs containing SARS-CoV-2 were immersed in the solution containing the SA-AuNPs and, after 20 min, the authors observed a change in color detected by the UV-Vis spectrophotometry (Figs. 4b–4d).

### AuNPs Combined Machine Learning to Detect SARS-CoV-2

The validation of the SARS-CoV-2 devices is a critical challenge to verify the optimal performance of diagnostic, especially the one carried out at home for self-monitoring (Vandenberg *et al.*, 2021). In this perspective, a recent work developed a Dense Neural Network (DNN) as a machine learning (ML) approach (Beduk *et al.*, 2022) to validate the accuracy of POC device in which AuNPs were associated with a graphene sensor. Firstly, the device can recognize the ACE2 enzyme (due to its affinity to bind spike proteins of coronavirus) and it was finally integrated in a potentiostat connected with a smartphone by wireless.

The nasopharyngeal swabs were collected by several patients affected by alpha (B.1.1.7), beta (B.1.351), delta (B.1.617.2) virus variants as well as negative patients. The SARS-CoV-2 was found using the machine learning model showing an accuracy of 99.37% (Figs. 5a and 5b). Similar approach was used by Liang *et al.* (2022) to overcome the limitation in the use of SPR based AuNPs for the detection of novel coronavirus. In particular, the authors focused on the disadvantages emerging in the analysis by SPR since the information of spatial and temporal distribution during the analysis were ignored reducing the accuracy and sensitivity. The authors proposed a test based on the analysis of images acquired by microscopy. These images were extracted following interaction between spike proteins adsorbed on



**FIGURE 5.** Graphical representations of The Dense Neural Network (DNN) (a) and records acquired from positive and negative tests performed using nasopharyngeal swabs (b), Adapted from Beduk *et al.* (2022); (c) summary scheme of the plasmonic device for SARS-CoV-2 detection using microscope. Machine learning SVM regression models to predict the virus concentration ( $R^2 > 95\%$ ), (d) Adapted from Liang *et al.* (2022).

TABLE 1

LOD, specificity, Sensitivity, time and sample volume used by different AuNPs-based SARS-CoV-2 tests

Reference	LOD	Specificity	Sensitivity	Time	Sample volume
<a href="#">Pramanik et al., 2021</a>	4 pg/mL <sup>-1</sup>	–	–	5 min	–
<a href="#">Aithal et al., 2022</a>	16 nM	–	–	10 min	20 µL
<a href="#">Ahmadivand et al., 2021</a>	42 fM	–	–	20 min	–
<a href="#">Liu et al., 2020a</a>	–	97.47%	95.85%	15 min	10 µL
<a href="#">Ahmadi et al., 2021</a>	–	96.6%	90%	15–20 min	20 µL
<a href="#">Wen et al., 2020</a>	1.2 mg/mL <sup>-1</sup>	100%	69.1%	15–20 min	10 µL
<a href="#">Lew et al., 2021</a>	3.2 nM	100%	83%	30 min	20 µL
<a href="#">Yano et al., 2022</a>	85 fM	–	–	1 min	70 µL
<a href="#">Della Ventura et al., 2020</a>	–	>95%	>95%	Few min	100 µL
<a href="#">Alafeef et al., 2020</a>	6.9 copies/µL	100%	231 copies/µL <sup>-1</sup>	5 min	–
<a href="#">Alafeef et al., 2021</a>	10 copies/µL	100%	96.6%	<1 h	<10 µL
<a href="#">Rodríguez-Díaz et al., 2022</a>	>103–104 copies/µL	–	–	15 min	10–15 µL
<a href="#">Beduk et al., 2022</a>	5.14 ng/mL for S1 and 2.09 ng/mL for S2	–	–	1 min	–
<a href="#">Liang et al., 2022</a>	125.28–106 vp/mL	–	–	12 min	50 µL
<a href="#">Behrouzi et al., 2021</a>	5 ng/mL	–	–	–	4 µL

the sensors and AuNPs decorated with anti-SARS-CoV-2 antibodies. Binding results in transmission inhibition in the far field. Consequently, a reduction in gray values can be observed that is proportional with virus concentration that was detected in the range of 125.28 to 106 vp/mL with a LOD of 100 vp/mL. At this point, following further extractions of other images with different shades of colors corresponding to certain concentrations of pathogen, these were inserted into the machine learning program useful to detect SARS-CoV-2 quickly and safely (Figs. 5c and 5d).

Behrouzi and Lin (2021) developed a convolutional neural learning able to increase the efficiency of SARS-Cov-2 test by analysis of images. The method is based on the double-coffee ring phenomenon using LSPR by the presence of AuNPs functionalized using specific antibodies against the coronavirus permitting to detect virus with a LOD of 5 ng/mL. With this aim, as sensing system a hydrophilic membrane characterized by a porous pattern and hydrophobic barriers was used. When AuNPs bond to viral units, a specific image characterized by a double coffee ring can be displayed. In this way, the neural network will be able to detect this interaction. Indeed, a small ring was formed in the center due to the interaction between the NPs surrounded by a second ring produced instead by the hydrophobic barrier.

In the Table 1 are reported the most important characteristic of the sensors described in this review.

## Conclusions

In the last year we witnessed a huge and common human effort towards the fight of the life-threatening COVID-19 infections. If on one side mass vaccination surely stopped the uncontrolled circulation of the virus, on the other a huge diagnostic campaign helped tracking positive cases, which can be then

easily quarantined. With respect to the latter approach, we have witnessed a fast development of several different diagnostic kits, used both in the clinic as well as within common pharmacies. In this work, we described the most recent efforts to produce SARS-CoV-2 diagnostic tool based on AuNPs. Thanks to their unique physicochemical properties, AuNPs allowed indeed the detection of viral antigens even at picograms level, without the need of specialized equipment, in a cheap way. Since the pandemic situation is it is constantly evolving, we believe the development of next generation diagnostic kits should take into consideration the use of nanomaterials, to develop a nanoscale personalized platform able to detect viral RNA in specific manner with an optimal limit of detection and high sensitivity.

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