Minute-scale detection of SARS-CoV-2 using a low-cost biosensor composed of pencil graphite electrodes

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COVID-19 has led to over 3.47 million deaths worldwide and continues to devastate primarily middle- and low-income countries. High-frequency testing has been proposed as a potential solution to prevent outbreaks. However, current tests are not sufficiently low-cost, rapid, or scalable to enable broad COVID-19 testing. Here, we describe LEAD (Low-cost Electrochemical Advanced Diagnostic), a diagnostic test that detects severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) within 6.5 min and costs $1.50 per unit to produce using easily accessible and commercially available materials. LEAD is highly sensitive toward SARS-CoV-2 spike protein (limit of detection = 229 fg·mL⁻¹) and displays an excellent performance profile using clinical saliva (100.0% sensitivity, 100.0% specificity, and 100.0% accuracy) and nasopharyngeal/oropharyngeal (88.7% sensitivity, 86.0% specificity, and 87.4% accuracy) samples. No cross-reactivity with other viruses was detected and the test displayed a viable shelf-life of 5 d when stored at 4°C.

Significance

High-frequency testing is urgently needed to help prevent the spread of COVID-19. Here, we introduce Low-cost Electrochemical Advanced Diagnostic (LEAD), a diagnostic test that detects SARS-CoV-2 within 6.5 min, costs $1.50 per unit, and uses easily assembled materials such as human angiotensin-converting enzyme 2 (ACE2) receptor and a plastic vial (Fig. 1 A–C). This testing device enables on-site SARS-CoV-2 detection within 6.5 min, faster than tests currently approved by the Food and Drug Administration (FDA) (Fig. 1D). Each LEAD unit can be manufactured for a total cost of $1.50, including the vial ($0.30), graphite leads ($0.20), and all the modifiers required to ensure high sensitivity ($1.00), i.e., glutaraldehyde (GA), gold nanoparticles (AuNPs), cysteamine (cys), ACE2, and bovine serum albumin (BSA). The graphite working electrode (WE) was modified with AuNPs stabilized with cys to allow anchoring of ACE2 (16). BSA was used to block the remaining active sites on the electrode surface to avoid nonspecific interactions between the clinical sample and the biosensor. In summary, we describe a low-cost and rapid COVID-19 test that is easy to assemble and may enable population-wide high-frequency testing. Due to its reduced cost and DIY design, LEAD provides an opportunity to increase access to testing to underserved populations.
element to ensure sensitive and selective viral detection (19). The WE, where the (electro)chemical reaction/interaction takes place and is subsequently converted to a detectable analytical signal, was functionalized by the drop-casting method. The graphite WE was polished with a 2,000-grit sandpaper to remove impurities from the surface, and a contact area of 1.0 cm length by 0.7 mm diameter was obtained. Next, in order to generate a cross-linked polymer, the graphite pencil electrode (GPE) was immersed in a 25.0% (vol/vol) GA solution for 1 h at 37 °C. GA has been extensively used to modify electronic devices as it introduces aldehyde functional groups that facilitate the covalent attachment of compounds containing amine terminal moieties (20, 21). Here, we leveraged GA to modify the GPE’s surface with AuNPs functionalized with cys (AuNPs-cys). First, we synthesized the AuNPs following protocols similar to those reported by our group previously (22). The AuNPs were functionalized with cys by their thiol groups. Next, the modified graphite substrate was kept under an AuNPs-cys solution at 37 °C for 75 min to allow the immobilization of AuNPs-cys by cross-linking between the aldehyde functional groups of GA and the amine functional groups of cys (20). Subsequently, we added a solution containing the preprepared reactive intermediary N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) with ACE2, EDC-NHS-ACE2, to enable anchoring between the amine groups of AuNPs-cys and the EDC-NHS-ACE2, yielding ACE2-AuNPs-cys after 30 min at 37 °C. The electrodes were then incubated with BSA at 37 °C for 30 min in order to block the electrode’s remaining active sites after immobilization of ACE2 (Fig. 1B). BSA is a functionally inert protein with a high density of superficial lysine residues that is commonly used for biosensor development (23). Next, we exposed the sensor to samples containing SP or SARS-CoV-2 and changes in the peak current (ip) of a redox probe ([Fe(CN)6]3−/4− solution). (24, 25). The AuNPs-cys were then anchored covalently to the surface of the WE by pre-}

Fig. 1. LEAD, a rapid and low-cost electrochemical biosensor. (A) Schematic representation of graphite electrodes used in LEAD. (a) AuNPs-cys functionalization on graphite electrodes after modification with glutaraldehyde. (C) Modification of AuNPs-cys with ACE2 using EDC and NHS to enable amide bond formation and BSA for surface blockage. (B) Molecular Tests. (C) Characterization Assays. Experiments were then performed to characterize the biosensor. Fig. 24 presents an ultraviolet-visible (UV-vis) spectrum obtained from a HAuCl4 solution with a maximum absorption band at 309 nm. After AuNP-cys formation, a wine color was obtained displaying a UV-vis absorption band at 532 nm. Successful formation of the spherical AuNP-cys solution was confirmed by scanning electron microscopy (SEM) images (Fig. 2B), presenting a mean diameter of 14.13 ± 0.18 μm. The bare GPE presented a flat surface containing stacked carbon sheets (Fig. 2C). The AuNP-cys appeared well-distributed within the GPE surface (Fig. 2D) after the optimized functionalization process, facilitating the subsequent ACE2 immobilization onto the surface of the electrode. The WE, where the (electro)chemical reaction/interaction takes place and is subsequently converted to a detectable analytical signal, was functionalized by the drop-casting method. The graphite WE was polished with a 2,000-grit sandpaper to remove impurities from the surface, and a contact area of 1.0 cm length by 0.7 mm diameter was obtained. Next, in order to generate a cross-linked polymer, the graphite pencil electrode (GPE) was immersed in a 25.0% (vol/vol) GA solution for 1 h at 37 °C. GA has been extensively used to modify electronic devices as it introduces aldehyde functional groups that facilitate the covalent attachment of compounds containing amine terminal moieties (20, 21). Here, we leveraged GA to modify the GPE’s surface with AuNPs functionalized with cys (AuNPs-cys). First, we synthesized the AuNPs following protocols similar to those reported by our group previously (22). The AuNPs were functionalized with cys by their thiol groups. Next, the modified graphite substrate was kept under an AuNPs-cys solution at 37 °C for 75 min to allow the immobilization of AuNPs-cys by cross-linking between the aldehyde functional groups of GA and the amine functional groups of cys (20). Subsequently, we added a solution containing the preprepared reactive intermediary N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) with ACE2, EDC-NHS-ACE2, to enable anchoring between the amine groups of AuNPs-cys and the EDC-NHS-ACE2, yielding ACE2-AuNPs-cys after 30 min at 37 °C. The electrodes were then incubated with BSA at 37 °C for 30 min in order to block the electrode’s remaining active sites after immobilization of ACE2 (Fig. 1B). BSA is a functionally inert protein with a high density of superficial lysine residues that is commonly used for biosensor development (23). Next, we exposed the sensor to samples containing SP or SARS-CoV-2 and changes in the peak current (ip) of a redox probe ([Fe(CN)6]3−/4− solution). (24, 25). The AuNPs-cys were then anchored covalently to the surface of the WE by pre-}

**Results**

**Biosensor Design.** The electrochemical device was designed to explore the remarkable binding affinity between SARS-CoV-2 spike protein (SP) and human ACE2, its receptor in the human body (17, 18) (Fig. 1A). Thus, ACE2 was used as our recognition element to ensure sensitive and selective viral detection (19). The WE, where the (electro)chemical reaction/interaction takes place and is subsequently converted to a detectable analytical signal, was functionalized by the drop-casting method. The graphite WE was polished with a 2,000-grit sandpaper to remove impurities from the surface, and a contact area of 1.0 cm length by 0.7 mm diameter was obtained. Next, in order to generate a cross-linked polymer, the graphite pencil electrode (GPE) was immersed in a 25.0% (vol/vol) GA solution for 1 h at 37 °C. GA has been extensively used to modify electronic devices as it introduces aldehyde functional groups that facilitate the covalent attachment of compounds containing amine terminal moieties (20, 21). Here, we leveraged GA to modify the GPE’s surface with AuNPs functionalized with cys (AuNPs-cys). First, we synthesized the AuNPs following protocols similar to those reported by our group previously (22). The AuNPs were functionalized with cys by their thiol groups. Next, the modified graphite substrate was kept under an AuNPs-cys solution at 37 °C for 75 min to allow the immobilization of AuNPs-cys by cross-linking between the aldehyde functional groups of GA and the amine functional groups of cys (20). Subsequently, we added a solution containing the preprepared reactive intermediary N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) with ACE2, EDC-NHS-ACE2, to enable anchoring between the amine groups of AuNPs-cys and the EDC-NHS-ACE2, yielding ACE2-AuNPs-cys after 30 min at 37 °C. The electrodes were then incubated with BSA at 37 °C for 30 min in order to block the electrode’s remaining active sites after immobilization of ACE2 (Fig. 1B). BSA is a functionally inert protein with a high density of superficial lysine residues that is commonly used for biosensor development (23). Next, we exposed the sensor to samples containing SP or SARS-CoV-2 and changes in the peak current (ip) of a redox probe ([Fe(CN)6]3−/4− solution). (24, 25). The AuNPs-cys were then anchored covalently to the surface of the WE by pre-
infrared (FTIR) spectroscopy analysis (SI Appendix, Fig. S1) (26, 27). The functionalization of GPE with AuNPs-cys led to decreased values of $R_{CT}$ (47.6 ± 1.3 $\Omega$) and increased $i_p$ (410.5 ± 13.7 $\mu$A) compared to the previous functionalization step (Fig. 3A). The higher current and lower charge transfer resistance detected resulted from the high electrocatalytic and surface area presented by AuNPs-cys.

![Figure 2](image1.png)

**Fig. 2.** AuNPs-cys and GPE characterization studies. (A) UV-vis spectrum obtained for HAuCl$_4$ solution (yellow color with a maximum absorbance band at 309 nm) and AuNPs-cys formation (wine color and absorbance band at 532 nm). (B) SEM image of AuNPs-cys dispersion with a histogram inset. The spherical AuNPs-cys present a mean diameter of 14.13 ± 0.18 nm. (C) SEM image of the bare GPE electrode polished showing a flat surface containing carbon sheets. (D) SEM image of the GPE electrode modified with GA and AuNPs-cys, which present a high AuNPs-cys distribution throughout the electrode, thus facilitating ACE2 immobilization and SARS-CoV-2 SP detection.

![Figure 3](image2.png)

**Fig. 3.** Functionalization steps and electrochemical characterization of LEAD. (A) Schematic representation of stepwise functionalization steps of LEAD. (B) CVs recorded for each modification step of the GPE surface in a solution of 5.0 mmol L$^{-1}$ [Fe(CN)$_6$]$^{3-}$/$^{4-}$ containing 0.1 mol L$^{-1}$ KCl as the supporting electrolyte at a scan rate of 50 mV s$^{-1}$. (C) Nyquist plots were obtained using the same conditions as in A. (Inset) A zoomed view of the plots in high-frequency regions. The following experimental conditions were used for these experiments: frequency range from $1 \times 10^5$ Hz to 0.1 Hz and 10-mV amplitude. All measurements were performed at room temperature.
by the AuNPs, which contributed to fast electron-transfer kinetics and an active nanostructured electrode surface (28, 29), thus conferring very attractive features for sensor development. In addition, the -NH$_3^+$ functional groups present on the AuNPs-cys-modified GPE led to favorable electrochemical interactions of the anionic probe [Fe(CN)$_6$]$_{3/4}$, providing a preconcentration of the redox probe close to the electrode interface. This led to an improved electrochemical response, i.e., higher current peak (30).

The synthesized AuNP-cys dispersion demonstrated adequate stability for up to 7 d when stored at 4 °C under a light-protected environment (SI Appendix, Fig. S2). This stability allowed AuNP-cys synthesis to be carried out weekly, facilitating the large-scale modification process of GPE.

The electrochemical functionalization with AuNPs-cys was evaluated through the redox behavior of the adsorbed AuNPs by CVs recorded using 0.1 mol·L$^{-1}$ H$_2$SO$_4$ after different periods of immersion in the AuNPs-cys suspension (SI Appendix, Fig. S3). Indeed, an anodic peak was observed at +880 mV and a cathodic peak at +522 mV, which correspond to the redox processes of Au (III) within the electrode surface (SI Appendix, Fig. S3A). We next optimized the period of electrode exposition to AuNPs-cys suspension to be 80 min (SI Appendix, Fig. S3B) based on the peak current of the anodic process derived from oxidation of the gold adsorbed on the GPE/GA surface. Subsequently, we immobilized the recognition element ACE2 onto the surface of the nanoelectrochemically functionalized GPE using EDC·NHS (magenta line). This led to increased RCT values (182.6 ± 2.2 μΩ) and decreased ip values (247.2 ± 4.1 μA) compared to the previous functionalization step (SI Appendix, Fig. S3C), confirming the proper anchoring of our recognizing element (ACE2) to the electrode surface, which leads to the hindrance of the faradic processes of [Fe(CN)$_6$]$_{3/4}$- taking place at the modified GPE surface. As a final functionalization step, we immobilized BSA (green line) to block the remaining unmodified electrode area to avoid nonspecific and undesired adsorption of other molecules (e.g., proteins and lipids). This step resulted in the highest RCT values (985.7 ± 12.7 Ω) and lowest ip (177.4 ± 3.2 μA), suggesting a continued decline in the charge transfer kinetics after anchoring the AuNPs-cys due to the insertion of nonconductive materials (i.e., ACE2 and BSA).

**Analytical Performance of LEAD.** We used square wave voltammetry (SWV) for SARS-CoV-2 detection. This technique is highly sensitive, especially for detecting reversible redox species (31), such as potassium ferri- and ferrocyanide. Our electroanalytical method is based on the signal suppression induced by highly specific interactions between the nanoelectrochemically functionalized GPE using EDC·NHS and NHS (magenta line). This led to increased RCT values (182.6 ± 2.2 Ω) and decreased ip values (247.2 ± 4.1 μA) compared to the previous functionalization step (SI Appendix, Fig. S3C), confirming the proper anchoring of our recognizing element (ACE2) to the electrode surface, which leads to the hindrance of the faradic processes of [Fe(CN)$_6$]$_{3/4}$- taking place at the modified GPE surface. As a final functionalization step, we immobilized BSA (green line) to block the remaining unmodified electrode area to avoid nonspecific and undesired adsorption of other molecules (e.g., proteins and lipids). This step resulted in the highest RCT values (985.7 ± 12.7 Ω) and lowest ip (177.4 ± 3.2 μA), suggesting a continued decline in the charge transfer kinetics after anchoring the AuNPs-cys due to the insertion of nonconductive materials (i.e., ACE2 and BSA).

Next, we evaluated the optimal incubation time for detecting SARS-CoV-2 in clinical samples by evaluating the analytical sensitivity parameter obtained from dose–response curves at very low SP concentrations (Fig. 4A). The experiments were recorded in triplicate using increased concentrations of SP, from 1 × 10$^{-12}$ to 1 × 10$^{-8}$ g·mL$^{-1}$. The results were expressed as $\Delta i = I - I_0$, where $I$ corresponds to the current recorded for the redox probe ([Fe(CN)$_6$]$_{3/4}$) after incubating the sample on the electrode surface and $I_0$ corresponds to the current recorded for the redox probe before exposing the biosensor to the sample (Fig. 4A). Five minutes were determined to be the optimal incubation time due to the highest value of the angular coefficient of the dose–response curves, demonstrating fast binding kinetics between the SARS-CoV-2 SP and the immobilized ACE2 on the electrode surface.

We also obtained an analytical curve for different concentrations of SP in 0.1 mol·L$^{-1}$ phosphate-buffered saline (PBS) (pH = 7.4) under optimized experimental conditions (Fig. 4B). Note that the SWV response for the redox probe [Fe(CN)$_6$]$_{3/4}$ decreased with increased concentration of SP due to suppression of the analytical signal (ip) induced by the highly specific interaction between the SP and the ACE2-modified GPE (17, 33). Binding of SP to the biosensor surface partially blocked the electroactive sites of LEAD, leading to current suppression and yielding a positive result indicative of the presence of SARS-CoV-2 SP.

The SWV signals ($n = 3$ measurements using different biosensors) obtained at each concentration were plotted as a logarithmic function of the SP concentration (Fig. 4C). The analytical curve (Fig. 4C) was calculated at concentrations ranging from 1 × 10$^{-14}$ g·mL$^{-1}$ to 1 × 10$^{-6}$ g·mL$^{-1}$ of SP and displayed a linear behavior at the concentration range between 1 × 10$^{-13}$ g·mL$^{-1}$ and 1 × 10$^{-9}$ g·mL$^{-1}$ SP, resulting in an analytical sensitivity value of 0.0575 ± 0.0020 μA·g$^{-1}$·mL$^{-1}$ and a high linear correlation (R$^2$) of 0.994.

The limit of detection (LOD) and limit of quantification (LOQ) of LEAD were calculated according to the four-parameter logistic (4PL) curve (SI Appendix, Fig. S5), using Eqs. 1 and 2 (34). This method is commonly used for assays that determine biological binding interactions and reflect the underlying binding kinetics (35–37). Thus, the LOD and LOQ values of LEAD were 229 fg·mL$^{-1}$ and 0.91 pg·mL$^{-1}$, respectively. Therefore, our device enabled the rapid detection of SP at very low concentrations (less than picograms per milliliter), providing high sensitivity (Fig. 4C) and a low LOD using highly accessible materials, such as pencil graphite and a plastic vial. In SI Appendix, Table S1 we show a side-by-side comparison of the performance of LEAD and other electrochemical detection methods described for SARS-CoV-2 diagnosis. Note that LEAD enables high detectability (LOD = 229 fg·mL$^{-1}$), rapid testing time (6.5 min), and a very low production cost ($1.50 per test). The testing time was recorded to be 6.5 min considering the sample incubation period (5 min), the time required to record two SWVs (before and after sample incubation, 1 min), and the washing step with PBS after incubating the sample (30 s).

To assess the diagnostic capability of LEAD we calibrated our biosensor using tittered solutions of inactivated SARS-CoV-2 ranging from 10$^2$ to 10$^6$ plaque-forming units (PFU)·mL$^{-1}$ (Fig. 4E and F). LEAD exhibited high sensitivity, presenting an LOD of 2.07 PFU·mL$^{-1}$, corresponding to the order of 10 RNA copies per microliter, which is similar to RT-qPCR sensitivity (38, 39).

$$L_C = i_{\text{blank}} + t(1 - \alpha, n - 1)\sigma_{\text{blank}}, \quad [1]$$

where $L_C$ is a value of blank limit, $i_{\text{blank}}$ is the mean of signal intensities for $n$ blank (negative control) replicates, $\sigma_{\text{blank}}$ is the SD of blank replicates, and $(1 - \alpha, n - 1)$ is the $1 - \alpha$ percentile of the t-distribution given $n - 1$ degrees of freedom, $\alpha = \beta = 0.05$ significance levels.

$$L_d = L_C + t(1 - \beta, m(n - 1))\sigma_{\text{test}}, \quad [2]$$

where $L_d$ is the LOD in the signal domain, $\sigma_{\text{test}}$ is the pooled SD of $n$ test replicates, and $t(1 - \beta, m(n - 1))$ is the $1 - \beta$ percentile of the t-distribution given m(n − 1) degrees of freedom. Again, we set $\alpha = \beta = 0.05$, but these significance levels can be selected depending on the needs of a given study.

Our electrochemical biosensor was applied for SARS-CoV-2 detection in clinical samples containing a wide range of viral loads. The threshold cycle (Ct) of the RT-PCR data for all clinical samples analyzed ranged from 21.5 to 34.3 Ct. It is important to
highlight that our results (current suppression – ΔI) presented a high linear correlation ($R^2 = 0.954$) with Ct values ranging from 22.3 to 34.3 (SI Appendix, Fig. S6).

**Batch-to-Batch Reproducibility and Stability Assays.** We performed reproducibility assays of our device to ensure that different test batches performed similarly. To verify the reproducibility of the manufacturing and functionalization processes of LEAD we recorded SWVs in the presence of 5 mmol·L$^{-1}$ [Fe(CN)$_6$]$^{3-}$/4$^-$ after incubating the sensor with $1 \times 10^{-12}$ g·mL$^{-1}$ of SP prepared in 0.1 mol·L$^{-1}$ PBS (pH = 7.4). A relative SD of 4.31% was obtained for the analytical signal (current suppression of the redox probe) using six sensors ($n = 6$) from different batches, indicating excellent reproducibility (SI Appendix, Fig. S7).

Next, we evaluated the stability of LEAD under different temperature and storage conditions (25 °C, stored dry at 4 °C, stored at 4 °C in PBS, and at −20 °C) over 7 d. Analytical curves were generated in 0.1 mol·L$^{-1}$ PBS (pH = 7.4) at a concentration ranging from $1 \times 10^{-12}$ g·mL$^{-1}$ to $1 \times 10^{-9}$ g·mL$^{-1}$ of SP (Fig. 4D). Sensors stored under dry conditions at 4 °C (without PBS) were stable for 24 h. However, after 72 h the stability decreased to 50% of the initial sensitivity (Fig. 4F). Interestingly, electrodes stored at 4 °C in PBS solution were stable for 120 h, and the mean sensitivity of the device decreased by only 25% after 6 d compared to
the initial performance of LEAD (Fig. 4F). These results demonstrate that the immobilized ACE2 maintained its activity over prolonged periods of time (up to 5 d) when stored in a refrigerated aqueous solution.

**Cross-Reactivity Experiments.** Cross-reactivity studies using other viruses were carried out to investigate the specificity of our biosensor toward SARS-CoV-2 and rule out potential off-target reactivity. Using the same experimental conditions as for SARS-CoV-2 (Fig. 5), we tested four other viral strains: H1N1 (A/California/2009), Influenza-B/Colorado, herpes simplex-virus-2, and murine hepatitis virus (MHV). No cross-reactivity was detected against any of these viruses as indicated by the response to all strains, which presented a current suppression (ΔI) lower than the cutoff value of 10 μA obtained by SWV for the lowest SP concentration detected (Fig. 4C). These results further highlight the translatable nature of our sensor toward COVID-19. Interestingly, LEAD displayed a higher affinity to the highly infectious SARS-CoV-2 UK variant 1.1.7.B (Fig. 5B) than to wild-type SARS-CoV-2 (Fig. 5A), which is in agreement with recent studies demonstrating that mutations in the receptor-binding domain of the spike glycoprotein variant led to enhanced binding affinity toward ACE2 (40, 41).

**Detection of SARS-CoV-2 in Clinical Samples.** To evaluate the diagnostic efficacy of LEAD, we tested 103 NP/OP (Table 1) and 10 saliva samples (*SI Appendix*, Tables S2 and S3) obtained from inpatients from the Hospital of the University of Pennsylvania after heat inactivation. All samples were confirmed as COVID-19 positives and COVID-19 negatives by RT-PCR. Table 1 shows that out of the 103 NP/OP tested (53 COVID-19–positive and 50 COVID-19–negative samples) our device accurately detected 90 (88.7% sensitivity, 86.0% specificity, and 87.4% accuracy). To evaluate the diagnostic efficacy of our device in a more complex biological environment, we tested 10 saliva samples (3 positive and 7 negative samples) as a proof of principle. LEAD presented higher accuracy, sensitivity, and specificity (100.0%) for saliva samples than for NP/OP swabs, although the sample size was substantially different between the two groups.

**Discussion**

We present a simple, inexpensive, and portable electrochemical biosensor that enables diagnosis of COVID-19 within 6.5 min using 50 μL of sample and highly accessible and commercially available materials (i.e., graphite pencil leads and a plastic vial), yielding a test that costs $1.50. The WE can be functionalized in less than 3 h and remains stable for over 5 d when stored in a PBS solution at 4 °C. LEAD displayed excellent sensitivity for detecting SARS-CoV-2 SP (LODs of 229 fg·mL⁻¹ and 2.07...
Characterization Studies. Electrochemical techniques, such as CV, were carried out in a potential window from 0.7 to −0.3 V with a scan rate of 50 mV s⁻¹. EIS was conducted in the frequency range from 1 × 10⁻² to 1 Hz using an amplitude of 10 mV and under open circuit potential. Both electrochemical techniques were used to characterize the electrochemical behavior of electrodes in each modification step. In SWV studies, potentials were scanned from −0.4 to 1.0 V, corresponding to a frequency of 80 Hz, amplitude of 75 mV, and step low of 8 mV. All electrochemical characterizations were performed in 0.1 mol L⁻¹ KCl solution containing 5.0 mmol L⁻¹ of the mixture [Fe(CN)₆]₃⁻/⁴⁻ solution. All electrochemical experiments were carried out at room temperature (25 ± 3 °C).

Morphological characterizations of the synthesized AuNP-cys dispersion and GPE before and after superficial functionalization with AuNP-cys/ACE2/BSA were performed using SEM images acquired using a JEOLE 7500F HRSEM from the Singh Center for Nanotechnology (University of Pennsylvania). SEM images were recorded with 15,000 to 150,000x magnifications, acceleration voltage of 5 kV, and using SE mode. The spectrophotometry analysis was performed using a PerkinElmer Multimode 3, version 7.0.21 Spectrophotometer (model EnVision). The absorbance of AuNP-cys dispersion was measured at 532 nm (at room temperature) over 7 d to evaluate its stability. FTIR spectra were obtained for GPE/AuNP-cys and GPE/AuNP-cys/ACE2 samples in a PerkinElmer Spectrum 2 equipped with a Diamond UATR 2 detector ranging from 500 cm⁻¹ to 4,500 cm⁻¹ and 32 scans were performed for each measurement.

Synthesis of AuNPs. The AuNPs-cys were prepared according to methodologies described in the literature (42, 43). First, 100 µL of cys (213.0 mmol L⁻¹) was dropped into 1.5 mmol L⁻¹ HAuCl₄ in a final volume of 10.0 mL, under vigorous stirring for 20 min at room temperature. Subsequently, 10.0 µL of NaBH₄ (10.0 mmol L⁻¹) was added and kept under stirring for 20 min in a light-protected environment at room temperature. The resulting yellow color changed to wine red as a consequence of the formation of AuNPs. Finally, the solution was stored at 4 °C in a refrigerator for up to 7 d, conditions at which it presents high stability when stored in the absence of light (SI Appendix, Fig. S1) (42, 44).

The cys presents an amine group (−NH₂) and a mercaptan group (−SH) in its extremities, the latter of which favorably binds onto the AuNPs surface through an Au–S bond. Thus, cys serves to provide greater stability to the AuNPs due to the electrostatic repulsion process among the amine groups, which generates free positive charges (26, 43, 45).

Modification of Graphite Lead Electrodes. The WE was polished with 2,000-grit sandpaper, and a contact area of 1.0-cm length by 0.7-mm diameter was obtained. Next, the GPE was immersed in a 25.0% (vol/vol) glutaraldehyde (25%, vol/vol) were purchased from Sigma-Aldrich. Graphite pencils (0.7 mm) were used as the WE. The Ag/AgCl conductive ink was painted over one of the graphites to create the working and counterelectrodes, and graphite pencils (0.7 mm) were used as the graphite electrodes.

Cryogenic vials were used as the electrochemical cell (2.0-mL volume), each containing a graphite electrode. Electrochemical measurements were carried out using a MULTI AUTOLAB PGSTAT 302N potentiostat with six channels, controlled by the NOVA 2.1 software. All reagents used in this work were of analytical grade. The deionized water (resistivity ≥98%, gold(III) chloride trihydrate (HAuCl₄·3H₂O) (99.99%), sodium borohydride (NaBH₄) with ≥98% purity, cysteamine hydrochloride (cy) with ≥98% purity, phosphate buffer saline solution, pH = 7.4, and glutaraldehyde (25%, vol/vol) were purchased from Sigma-Aldrich. Graphite pencils (0.7 mm) were used as the WE. The Ag/AgCl conductive ink was painted over one of the graphites to create the working and counterelectrodes, and graphite pencils (0.7 mm) were used as the graphite electrodes.

Spectrum 2 equipped with a Diamond UATR 2 detector ranging from 500 cm⁻¹ to 4,500 cm⁻¹ and 32 scans were performed for each measurement.

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Modification of Graphite Lead Electrodes. The WE was polished with 2,000-grit sandpaper, and a contact area of 1.0-cm length by 0.7-mm diameter was obtained. Next, the GPE was immersed in a 25.0% (vol/vol) glutaraldehyde solution for 1.0 h as a first modification step. This process allowed the graphite surface to be functionalized with aldehyde groups. Then, the modified graphite substrate was kept in a AuNPs-cys solution with protonated amine groups from cys at pH 7.4, which enabled covalent anchoring of the AuNPs to the substrate by the formation of an amide bond (26). The AuNPs-cys presented a maximum adsorption time of 75 min and, after 100 min, the AuNPs-cys presented low adsorption to the substrate, and its color gradually faded (SI Appendix, Fig. S2).

Subsequently, the solution containing 50 mmol L⁻¹ EDC, 25 mmol L⁻¹ NHS, and 10 µg mL⁻¹ of ACE2 diluted in 0.1 mol L⁻¹ PBS (pH = 7.4) was incubated on the substrate containing GPE/GA/AuNP-cys. After 30 min, the ACE2 immobilized onto the substrate reached the anchoring stability and provided a highly sensitive SWV response. In the presence of EDC and NHS, the carboxyl group of ACE2 bound to the primary amine of cys through a covalent bond. The reaction between carboxyl groups and EDC–NHS resulted in the formation of a stable ester, which undergoes nucleophilic substitution with the amine groups present on the substrate surface (AuNPs-cys), resulting in the formation of an amide bond between the modified GPE/GA/AuNPs-cys surface and ACE2. In the final step, nonspecific sites present within the electrode surface were blocked by incubation in a 1% (mass/vol) BSA solution for 30 min. Fig. 1 illustrates the simplified process for modifying LEAD (32, 46).

Table 1. Detection of SARS-CoV-2 in OP/NP and saliva samples by LEAD

|                  | RT-qPCR |  |  |  |  |
|------------------|---------|  |  |  |  |
|                  | Positive| Negative| Total| Sensitivity| Specificity| Accuracy |
| NP swabs         | 47      | 7       | 54   | 47/53 (88.7%)| 90/103 (87.4%)|  |
| Negative         | 6       | 43      | 49   | 43/50 (86.0%)|  |
| Saliva           | 3       | 7       | 3    | 3/3 (100.0%)| 10/10 (100.0%)|  |

Analytical features of LEAD obtained using clinical samples. The sensitivity, specificity, and accuracy of LEAD for NP/OP and saliva samples were measured. Positive and negative values for the clinical samples were obtained by RT-qPCR.
COVID-19 Sensing Using LEAD. For diagnosing SARS-CoV-2, a volume of 50 μL of VTM or a 0.1 mol L−1 PBS sample containing SP was applied to the WE using a plastic pipette for 5 min. After the incubation period, the WE was gently washed with 0.1 mol L−1 phosphate buffer solution (pH = 7.4) to remove unbound virus or sample. Next, the electrodes were stored in a 2.0-mL cryogenic vial for subsequent electrochemical detection. One milliliter redox probe solution (5.0 mmol L−1 Fe(CN)3−6/4− in 0.1 mol L−1 KCl) was used for the voltammetric measurements and to detect the current suppression due to binding of SARS-CoV-2 SP to the biosensor. Subsequently, the electrochemical response was monitored using the SWV technique. Notably, this procedure was applied to other studies such as cross-reactivity, reproducibility, incubation time, and SARS-CoV-2 detection in clinical samples. The total diagnostic time was calculated to be 6.5 min considering the sample incubation period (5 min), the time required to record two SWVs (before and after sample incubation, 1 min), and the washing step after sample incubation (30 s).

Reproducibility, Stability, and Cross-Reactivity Studies. The reproducibility study was performed by recording the analytical signal (current suppression of the redox probe) obtained for six different sensors (from different batches) after incubation with 1 μg mL−1 SP solution. The reproducibility of LEAD was evaluated by the analytical sensitivity value extracted from analytical curves in a concentration range from 1 × 10−6 to 1 × 10−2 g mL−1 of SP in different conditions (25 °C, 20 °C dry at 4 °C, and stored at 4 °C in PBS medium (pH = 7.4) over 7 d. All modified electrodes were stored inside capped plastic vials during the stability study.

The specificity studies were carried out using the following different viruses: MHV at 105 PFU mL−1 (coronavirus); H7N9, A/California/2009; Influenza B, B/Colorado; and HSV2, herpes simplex virus-2 (all at 105 PFU mL−1). The highly contagious SARS-CoV-2 B.1.1.7 UK variant was used to assess the capability of LEAD to detect SARS-CoV-2 variants.

Clinical Sample Analyses. The clinical performance of LEAD was assessed using clinical samples acquired from the Hospital of the University of Pennsylvania and deidentified prior to use. We set a current suppression (ΔI) cutoff value higher than 10 μA for diagnostic purposes in accordance with the analytical response obtained for the lowest concentration of SP detected (10−14 g mL−1) in the dose–response curve (Fig. 4C), i.e., samples that exhibited ΔI > 10 μA were deemed positive for SARS-CoV-2 infection. One hundred and three NIP0P swab samples in VTM were obtained from patients and heat-inactivated (50 negatives and 3 positives). We also used 10 saliva samples (3 positives and 7 negatives). All samples were analyzed and the results obtained were compared to those from RT-PCR (SI Appendix, Tables S2 and S3). The Ct values obtained by RT-PCR for the clinical samples ranged from 21.5 to 34.3.

Data Availability. All study data are included in the article and/or SI Appendix.

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