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Development of gold nanoparticles-based lateral flow sandwich assay for colorimetric and electrochemical detection of bacterial infection biomarker

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Abstract

In this study, two distinct technologies were used for quantitative and qualitative analysis of bacterial infection (BI) specific procalcitonin (PCT) biomarker. lateral flow assay (LFA) strips polyclonal antibody-gold nanoparticles ((pAb-AuNP)/ monoclonal antibody (mAb)/PCT) with the same PCT concentration are used as novel analytical performance on both platforms, and their results are investigated based on the existence of the test line bands. The authors present modified LFA strips (pAb-AuNP/mAb/PCT) for electrochemical studies using electrochemical impedance spectroscopy (EIS). A red band appeared on the test line, implying excellent outcomes. The limit of detection (LOD) for qualitative PCT detection using this LFA has been observed to be 1 ng/mL. The results of LFA- based test lines are validated further using quantitative colorimetric techniques. It demonstrates that the concentration used in qualitative LFA yields positive results and demonstrates a good linear correlation between visually detected ranges of 2 to 60 ng/mL. On the other hand, the quantitative electrochemical method, has a much higher sensitivity for the detection of PCT, with a linear range of 1 to 60 ng/mL, and 1 ng/mL LOD is observed. Furthermore, an LFA-based strip electrode was used in quantitative electrochemical measurements without any electrochemically active surface modifications, ensuring a simple and fast procedure. The quantitative platforms for PCT detection presented not only provide simple, rapid, and impacted methods, but they often can enhance sensing technology in other areas of health diagnostic research.

Keywords: Procalcitonin, Gold nanoparticles, Lateral flow assay, Electrochemical impedance spectroscopy

1. Introduction

On a global scale, Bacterial Infection (BI) is one of the presumed principal reasons for death, and it has become a more serious problem as a result of persistent bacterial growth. As per a World Health Organization (WHO) study, antibiotic-resistant bacteria represent a public health risk [1,2]. As a result, an on-site detection technique is required for BI prognosis and immediate response. Innumerable immunoassays, such as immunochromatographic tests (ICT), enzyme-linked immunosorbent assays (ELISA), time-resolved fluoro immunoassays (TRFIA), and chemiluminescence immunoassays (CLIA) [3,4], have already been proposed in a wide range of research applications for screening and treatment of BI specific biomarkers. procalcitonin (PCT) continues to act as a prelude of the calcitonin hormone and can recognize the difference between microbial infections in the prognosis of BI or septic shock [5,6]. The accumulation of PCT in serum is 0.1 ng/mL, and it rises with the severity of the inflammation. Throughout BI, the PCT ranges in healthy individuals are 0.05 g/mL; if the systemic infection is extremely improbable but regionalized infection is possible, the PCT ranges are 0.05-0.5 g/mL; and in circumstances such as major surgery, major trauma, cardiogenic shock, and severe sepsis, the PCT ranges are 0.5-2 g/mL [7-9]. Even so, lateral flow assay (LFA) persists as an effective method for on-site screening of BI and other clinical manifestations [10]. To make complex tests more visible to consumers, this method allows even inexperienced users to run them at the right time in an economical way and without equipment. For qualitative and semiquantitative antigen detection, this technology primarily requires the use of immunogenic complexes and a labeling agent. Due to their increased intensity, photonic consistency, biological

activity, and ease of surface functionalization, gold nanoparticles (AuNP) remain particles of interest for LFA advancement [11-13]. Numerous studies have demonstrated the use of LFA techniques for detecting BI-specific biomarker PCT using AuNP as a label [5,14]. Some of the existing research has resulted in the development of an LFA strip that detects vastly different PCT concentration levels on different test lines by using popcorn-like and spherical-shaped AuNP [15-17]. High specificity, detection limit, minimal test and measurement cost, and intrinsic microfabrication potential with advanced analysis performance are all appealing characteristics of electrochemical detection strategies. Until now, significant advancement in LFA-based electrochemical analysis has relied on transducers viz., glassy carbon electrodes (GCE), indium tin oxide (ITO), and gold electrodes that are very well appropriate for standard use in an LFA [18]. Furthermore, the advancement of nanomaterials provides an incentive to optimize transducer efficiency. For example, by increasing transmittance, enhancing electrocatalyst effect, and continuing to increase electrochemical performance and electrode surface area [19,20]. 3D-architecture electrocatalytic transmitters seem to be more appealing used in LFAs than some other measurements even though sample liquid can pass through the transmitter, helping to promote fostering growth between analyte and transmitter surface [21]. Furthermore, the enhanced electrochemical surface area may be able to increase the sensor's contrast ratio.

Previous studies used multiple test lines for LFA test results and quantitative detection techniques used are expensive [13,22]. Multiple test lines may cause the failure of antibody-antigen immunogenic complex formation at the test line. So, the accuracy of results may not occur every time. So, to overcome the limitation, the presented article is based on only single test line results of the LFA strip which is further used for colorimetric quantitative and electrochemical quantitative detection of PCT. Both quantitative and qualitative test results are compared in this report. Out of which, electrochemical quantitative detection is better as it gives results in a short period of time.

As a model antigen, PCT was selected from the literature for the detection of BI in the current study. Electrochemical impedance spectroscopy (EIS) and colorimetric quantitative detection were used to examine the results of the test line of LFA strips with the new approach for PCT detection. LFA test strips were prepared, followed by the sandwich assay, to detect PCT qualitatively. AuNP are used as a labeling agent for PCT-specific pAb tagging. This colloid AuNP and pAb complex (pAb-AuNP) is applied to the LFA strip's conjugate pad. The establishment of a sandwich complex between pAb-AuNP and mAb results in the development of a red band in the strip's test line, designed to allow the PCT concentration to be optically focused. These qualitative test results were additionally quantified using an LFA reader. The portion of the nitrocellulose membrane (NM) on which the immunogenic complex was previously detected at the test line affirms the electrochemical quantification. EIS analysis was done to investigate the reliability of the testing results and the signal strength. The identification of low redox signals in the modified LFA band reveals the potential of a one-phase analysis pattern with electrochemical measurements. These findings enhanced the integrity of the EIS analysis.

2. Materials and methods

2.1 Reagents and Instrument

For the quantitative detection of analyte quantitative immunoassay reader (FIA8000) was purchased from SOWAR Private Limited. Advanced electrochemical potentiostats 204 three-electrode system was used for electrochemical signal detection. Bovine albumin serum (BSA), sodium azide, sucrose, sodium citrate dihydrate, and auric chloride were purchased from CDH, tween-20 from Thomas baker. Monoclonal and polyclonal antibodies (Mab and pAb) were acquired from micro mybiosource.com. Polyester conjugation pad, sample pad, NM, and absorption pad were acquired from microdevices methylene diphenyl diisocyanate (MDI) membrane.

2.2 AuNP-antibody conjugate preparation

The citrate method was selected to obtain ruby red color AuNP (Figure 1) [23-26]. To prepare the conjugated complex, pre-prepared AuNP and 0.01 mg/mL concentration of pAb was used. For pAb antibody dilution, 100mM PBS buffer (pH, 7.4) was used. With a solution of colloidal gold and diluted antibodies, sodium hydroxide was used to retain the pH at 6.5. The diluted solution was kept for 60 min at 30°C before adding 0.5 percent obstructing agent BSA and stirring for another 30 minutes. The pAb-AuNP was then ready after specifying the consecrated concentration of binding pAb. Ultraviolet-visible (UV-Vis) spectroscopy was used to affirm the conjugation of pAb-AuNP by evaluating the absorption peak at approximately 527 nm. The whole suspension was sonicated at 6000 rpm for about 30 min. The uninflected antibody filtrates were eliminated after centrifugation. The pallets were mixed thoroughly in buffer solutions having 10 M mol PBS, 0.25 % sucrose, 0.5 BSA, and freshly prepared 0.5 % sodium azide solution, and stored at 40°C for further examination.

2.3 Arrangements of LFA strip components

Figure 1 depicts the whole assemblage of the LFA test strip constituents. The adhesive was used to adhere the NM to a plastic backing plate. The conjugate pad was applied in a 0.5 cm overlap with the NM (2.5 x 0.5 cm^2). On either side of the membrane, an absorbent pad was 0.5 cm superimposed. After that, the sample application pad was pasted over the conjugate pad with a 0.5 cm overlap. To ensure that the constituents were properly placed and implemented, the PCT sample was poured from the sample pad. The concentration of 0.06 mg/mL Mab antibody was prepared in PBS buffer solution (pH 7.4) and used to make the test line of the LFA strip. 10 µ l of the Mab antibody solution was dropped onto the NM and drying it at room temperature. The LFA strips were placed in a dry place after getting these fully assembled. (2.5 x 0.5 cm^2).

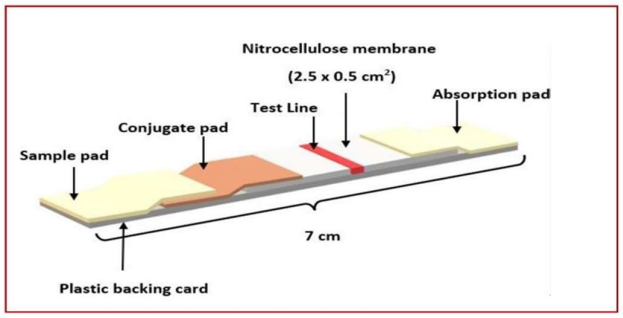

Figure 1 Schematic illustration of LFA Strip.

3. Results and discussion

In this investigation, we discussed the qualitative, colorimetric quantitative, and electrochemical quantitative detection of PCT biomarkers. PCT levels in sepsis range from 2 to 10 ng/mL, while levels above 10 ng/mL imply a condition of septicemia. Nevertheless, PCT concentrations ranging from 2- 0.15 ng/mL reveal unpredictability in the existence of BI. PCT is selected as the model biomarker for establishing the LFA sandwich format for BI diagnosis. A micropipette was used to dispense the PCT-specific Mab to the test line. When the conjugated AuNP with sample antigen flows through the NM and reacts with the Mab (that has already been dropped on the test line area), an immunogenic complex with a red band is formed, indicating the existence of an analyte in the sample. The inclusion of AuNP in the sandwich complex causes a red band to appear on the test line, revealed the existence of this complex. The sandwich complex LFA-based strip was quantified using colorimetric and electrochemical techniques. The LFA technique was used first in this procedure (Figure 2). Sandwich analysis of LFA was performed using different concentrations (1 to 60 ng/mL). Following the flow of each concentration of PCT through the sample pad. At the test line, the observation of positive and negative tests was monitored. When the sample antigen came into contact with the pAb-AuNP intricate as it flowed through the sample pad, a labelled immunogenic complex was formed. This complex begins to move along a permeable membrane in the test line, where anti-PCT Mab were previously dropped, resulting in the formation of a sandwich complex. On the test line, the sandwich complex has been shown with a red color band. Visually, a very weak band was observed at 1 ng/mL, and an absence of analyte was noticed at <1 ng/mL due to the absence of a red band at the test line. When the concentration of PCT continued to increase from 2 to 60 ng/mL, the density of the positive color band increased. To validate the test, we had prepared the test strips with test line and control line and performed LFA with 1 ng/mL and 60 ng/mL PCT concentrations that correspond to minimum and maximum detection limits of our assay, respectively Figure 2 inset. At minimum detection limit (1 ng/mL), the test line showed a faint red colour, whereas the colour intensity of control line was fairly good. This could be co-related to proper functioning of strip for LFA, wherein, the sample had flowed through the test line but due to low concentration of PCT, it could not be captured at the test line itself. However, the free antibody conjugated quantum dot that had passed through the test line were captured at the control line presenting red line at this site and thus verified that the antibody conjugated AuNP were active. Also, we had performed the assay at 60 ng/mL that corresponded to maximum detection limit of our Assay. However, at this concentration the test line presented a strong red band corresponding to positive test result, whereas the control line was faintly coloured. This can be attributed to the fact that the high complex density at the test line, that had been formed due to high PCT concentration, left a very less number of pAb-AuNP to be captured at the control line. The strength of the red color band at the test line affects the quantity of PCT, which has been quantified further using colorimetric and electrochemical techniques. The read time for colorimetric and qualitative determination was 15 min and 5 min respectively, while the read time for electrochemical quantification was 120 sec, which was significantly

less than the colorimetric and qualitative determination of PCT. It has been predicted that the detection time could be reduced by electrochemical quantification of analytes.

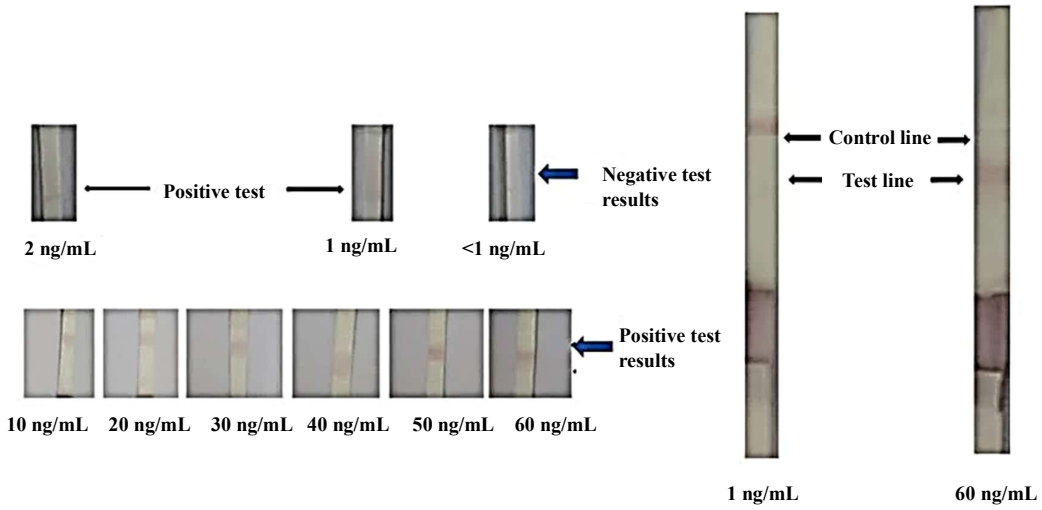

Figure 2 LFA- based test line positive and negative results and the validity of LFA test results.

3.1 Quantitative colorimetric detection

The part of test results of the LFA strip (NM, 2.5×0.5cm^2) was further confirmed with a quantitative immunoassay reader (FIA8000) that is specific for PCT samples. A strip of each concentration was placed in the reader. The results were obtained in the order of decreasing concentration (60 to 1 ng/mL) which was consistent with a detection pattern similar to that of the PCT analyte with the naked eye (Figure 3). The results observed by the reader after 15 min. The intensity of each signal was quantified by the band attributes on the test line. The output of the immunoassay reader indicates the presence or absence of a specific antigen at the test line. To generate quantitative data, a profile of the intensities of these bands was used. A substantial amount of data has been accumulated using varying concentrations of PCT. Signal attributes describe metrics of pixel intensities in the corresponding bands and were based on the local intensity and/or color of the pixels. Alternatively, the test line signal intensity could be attributed to the antigen-antibody interaction. With the colorimetric detection, LOD was found at 2 ng/mL Through the calibration curve, it was found that the response by the LFA reader was not noticed at 1 ng/mL which was visually detected as a very weak red band at the test zone by using qualitative and electrochemical detection. Figure.3 Inset shows the linear relationship from 2 to 50 ng/mL and 60 ng/mL is not considered because sepsis conditions are analyzed clinically, using PCT ranges, which means that higher ranges demonstrate the presence of PCT in the sample. The regression coefficient for this quantitative colorimetric detection is 0.98. Evaluation with the naked eye showed visual detection limits of assay requirements from 1 to100 ng/mL.

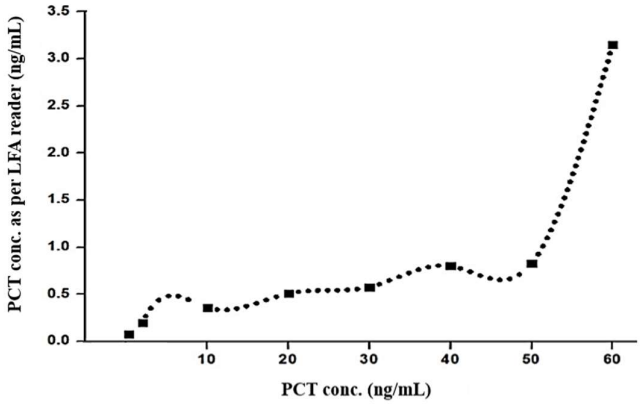

Figure 3 Colorimetric test results of 1 to 60 ng/mL concentration of PCT and inset indication linear relationship in between 2 to 50 ng/mL.

3.2. Quantitative electrochemical detection

To investigate the electrochemical response studies of the NM part of the LFA test strip, EIS was performed to investigate the electrochemical response studies of the NM part of the LFA test strip, EIS was performed. EIS is among the utmost broadly used investigational techniques for determining the behavior of electrochemical systems, permitting the assessment of various physical phenomena within a cell. The EIS is a measuring device of the resistance of a circuit to a current once power is applied. The EIS strategy provokes a reaction based on a phase transition or changes in temperature within solvent or implemented current, which produces the electrode to relocate out of equilibrium. An alternating potential is being used to measure the electrical resistance in an electrochemical cell. EIS represents a set of electrochemical methods which can be used as faradaic or quasi mechanisms to study physicochemical characteristics and test procedures [27,28]. The NM part's testing area was used to investigate the EIS signal and was used as a working lectrode, reference and counter electrode, and Ag/AgCl and platinum foil for signal processing. Each electrod was estimated and conducted as a function of antigen PCT concentration 1 to 60 ng/mL in 0.1 M KCl with 10nM of $[Fe(CN)_6]3-/4-$ redox probe using an EIS study presented in Figure 4A. With the addition of each PCTconcentration, the current value was examined. Electrically insulated antibody-antigen complexes are formed due to the specific interaction between PCT and anti-PCT, which shows a decrease in current value, and the reduction in the amperometric current at the surface of the electrode was predicted [28]. Regular decreases in the kinetic barrier for the electron transfer were noticed in the ranges of 1 to 60 ng/mL of PCT concentration which shows the decrease in Rct values. Figure 4B shows the calibration plot obtained in the range of 1 to 60 ng/mL of the PCT concentration The response current and impedance biosensor calibration line was covering the required detection range. It was found that the logarithmic value of PCT concentrations and Rct variation have a linear correlation within a range of 1 to 60 ng/mL which is appropriate for PCT concentrations. The linear correlation resulted in a regression coefficient of 0.99 was observed. Linearity correlation can be followed by Equation (1).

$$I\,(\mu A) = 4315.92\,(\mu A\ ng/mL)\,[PCT(ng/mL)] + 6818.08\,\mu A \quad (1)$$

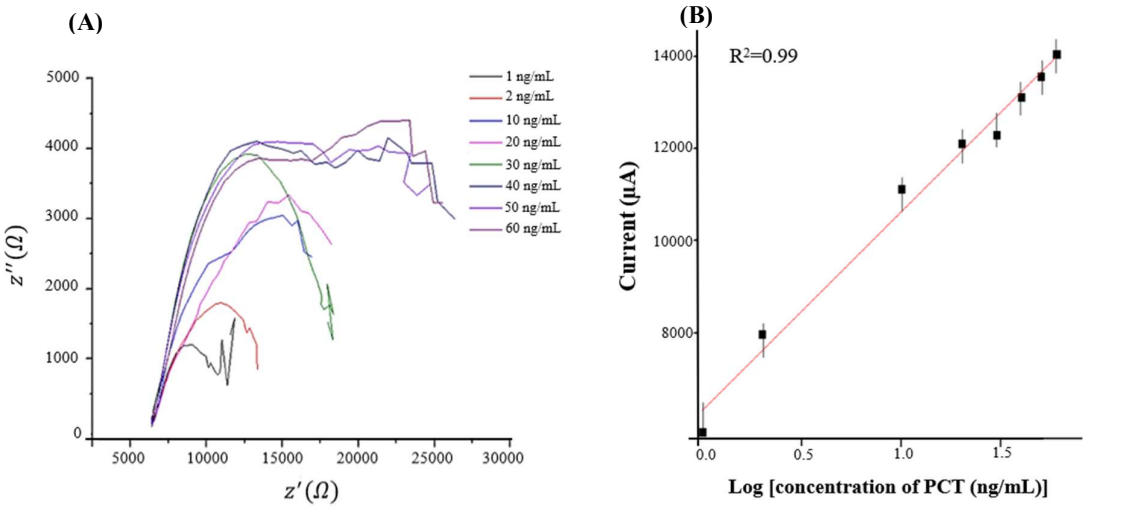

Figure 4 (A) Presenting decreasing order of electrochemical impedance peak in different concentrations and, (B) Calibration curve as a function of PCT concentrations.

3.3 Spectroscopic characterization

The absorption spectrum can be used to ascertain whether or not a nanoparticle solution has deteriorated over time. AuNP solution appears ruby red before conjugation and purple after conjugation because particles are conjugated. Figure 5A depicts the UV-visible AuNP and its conjugate absorption peak. The red curve in this graph indicates pAb-AuNP conjugation, while the black curve signifies AuNP peak. The identification of a bare AuNP peak at 522 nm confirmed the presence of spherical shell AuNP with notable flocculation steadiness and uniformity in terms of volume and proportions. the pAb-AuNP peak was observed at 529 nm, indicating that pAb-AuNP conjugation takes place.

Copper grids coated with carbon were used for Transition Electron Microscopic (TEM)analysis. The AuNP solution (10 µl) was sprayed onto the grid and allowed to dry for 48 h. The TEM images show that the AuNP

that was synthesized are well separated and spherical (Figure 5B), indicating that it was stable in solution. Furthermore, the synthesized particles were monodispersed.

The scanning electron microscope (SEM) samples were prepared by using the drop-casting method. This method was used to produce an AuNP-film on the ITO surface. ITO (1.0×1.0 cm^2) surface was washed with ethyl alcohol and ammonia before preparing the AuNP film. The AuNP solution was then evenly applied to the ITO surface and allowed to dry for two days. After that, the film was dried for 1.5 hours at 120°C. Thereafter thin film was used for SEM analysis (Figure 5C).

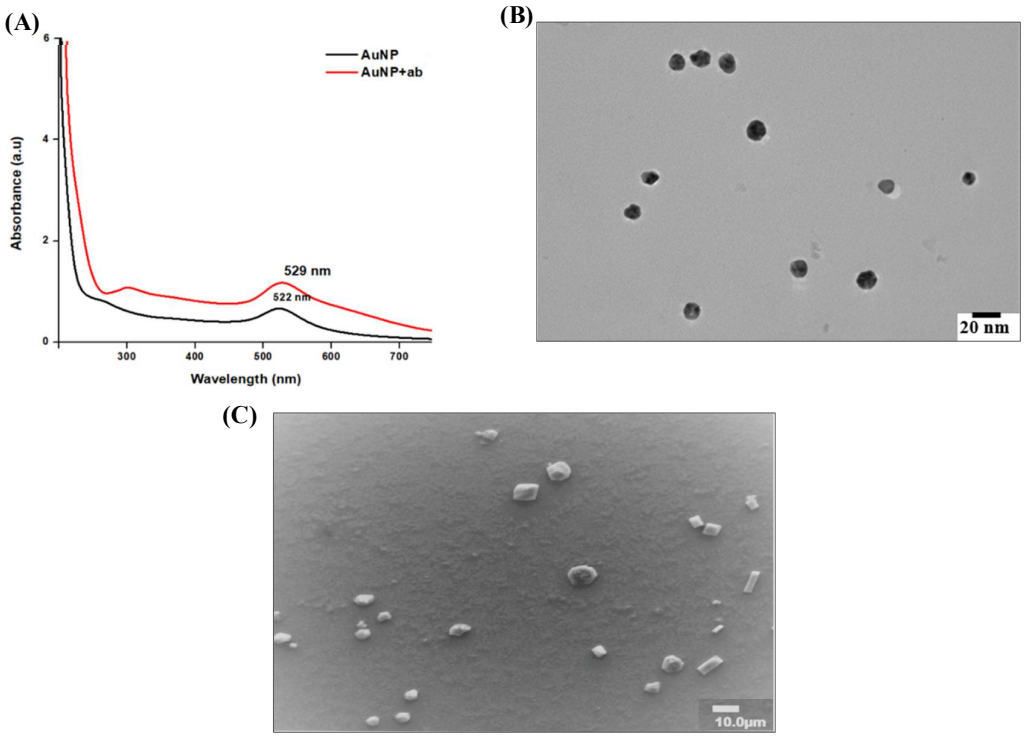

Figure 5 (A) UV-vis spectroscopy of AuNP and its conjugate, (B) TEM image of AuNP, and (C) SEM images of AuNP.

4. Conclusion

Rapid detection of BI-specific biomarker PCT accurate identification is crucial in disease prevention, treatment, and monitoring. In this study, we validated the PCT test results of LFA-based strips using colorimetric and electrochemical quantitation methods. These detection methods are capable of producing accurate results in a short period of time. For colorimetric quantification, a different portable reader will be used to improve optical density. Future instrument enhancements may include further technological advances and digitization, as well as software for the detection of other analytes.

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