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RESEARCH PAPER

Nanotechnology-Assisted Real-Time Viral Biosensing Using Graphene and Gold Nanoparticle Platforms: Experimental and Statistical Evaluation

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ABSTRACT

Biosensors with nanotechnology have also proven to be highly effective in detecting viruses in real-time, thanks to their high sensitivity, specificity, and speed. The current diagnostic methods (such as PCR and ELISA) are reliable but are time-consuming and are dependent on high level laboratory facilities. Nanobiosensors, on the other hand, use nanotechnology-based materials like gold nanoparticles, graphene, quantum dots, etc., to improve the signal transduction process and for point-of-care diagnostics. In this study, design, fabrication and application of nanotechnology based biosensors for real-time virus detection are explored. Experimental Assessment shows that nanobiosensors can measure the concentration levels down to femtomolar concentrations and with a response time of below 10 minutes. The incorporation of nanomaterials has led to the enhancement of analytical performance, which facilitates early diagnosis and adequate management of viral outbreaks.

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INTRODUCTION

Increased demand for rapid, portable and highly sensitive diagnostic technologies [1, 2]. Polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA) and viral culture are still widely used in conventional approaches for the identification of viruses, but these are time-consuming, require costly instrumentation and specialized laboratory infrastructure [3,4].

Nanotechnology is now an advanced platform that can solve many of the problems of conventional diagnostic systems [5,6]. The unique physicochemical properties of nanomaterials, including high surface-area-to-volume ratio, tunable optical properties, excellent electrical conductivity, and enhanced catalytic activity, are beneficial for the sensitivity and selectivity of biosensors [7, 8]. A wide range of nanomaterials such as gold nanoparticles (AuNPs), graphene nanosheets, carbon nanotubes, magnetic nanoparticles, and quantum dots have been the focus of many studies for biosensing purposes [9].

The localized surface plasmon resonance effect results in a significant improvement of both optical and plasmonic signal amplification by gold nanoparticles [10]. In the same way, the excellent electron transfer capacity and the high conductivity of the graphene-based materials contribute to the enhancement of electrochemical sensing performance [11, 12]. Advances in recent years have also allowed biosensors to be integrated into wearable systems, with

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smart phone-supported platforms and artificial intelligence technologies, providing a valuable contribution to remote healthcare monitoring and prompt epidemiological surveillance [13,14].

Compared to conventional diagnostic sensors, nanobiosensors have many merits, such as: fast detection, ultra-high sensitivity, miniaturization, transportability, and multiplex analysis capability [15, 16]. These benefits make nanotechnology-based biosensors ideal for use in POC settings in the event of an infectious disease outbreak.

Hence, the present study is focused on designing and analyzing nanotechnology-based biosensors for real-time virus detection. This study will also explore statistical performance, reproducibility, and machine learning based classification of viral samples.

MATERIALS AND METHODS

Materials

The materials used in the experimental work were gold nanoparticles (AuNPs), graphene nanosheets, phosphate-buffered saline (PBS), viral antigens, and virus-specific antibodies or aptamers. These nanoparticles were chosen due to their high plasmonic interaction and signal enhancing properties [10]. The sheets of graphene were used because of their outstanding electrical conductivity and high surface area [11].

Biosensor Fabrication

A biosensor platform based on nanoplasmonics was constructed with a gold coated sensing surface that is functionalized with virus-specific antibodies. To achieve stable immobilization of the antibody and effective recognition of the antigen, the surface immobilization was done by thiol-gold chemistry [17]. The analytical sensitivity and the optimization of the operation of the sensing layer with maximum surface binding interactions have been optimized.

Nanomaterial Characterization

The synthesized gold nanoparticles (AuNPs) and graphene nanosheets were first characterized physiochemically to investigate their properties before building the biosensors. The morphology and nanoscale distribution of the fabricated materials were confirmed by Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) analyses, which showed that the materials were homogeneous. AuNPs had a diameter of 18–25 nm and a uniform spherical shape, which is desirable for efficient plasmonic signal enhancement and biosensing [7,8].

Raman spectroscopy was also used to evaluate the structure of the graphene nanosheets. The characteristic D and G bands were found at ~1348 cm^{-1} and ~1582 cm^{-1} respectively, which showed that the graphene was synthesized successfully with an acceptable defect density and good structural stability [5,6]. The presence of active functional groups involved in immobilization of antibody and surface bio-functionalization was verified by Fourier Transform InfraRed (FTIR) analysis. Moreover, X-ray diffraction (XRD) was used to confirm the crystalline nature of AuNPs with diffractions peaks matching the face-centered cubic (FCC) structure of gold.

The results of the ultraviolet–Visible (UV–Vis) spectroscopy showed that the characteristic plasmonic absorption peak occurred at about 520 nm, which indicated that the AuNP was successfully synthesized with stable properties and that its optical properties were appropriate for real-time biosensors applications [18,19]. Both the combined characterization tests confirmed the synthesized nanomaterials had the desired characteristics, structural, optical and electrochemical, required for the high performance biosensors to be fabricated.

Fabrication Parameters and Surface Functionalization

The biosensor substrate was a gold coated conductive sensing layer on a glass platform, which was produced by high electrical conductivity and consistent surface adhesion through sputter-coating. To increase electron transfer kinetics and amplify electrochemical signals, graphene nanosheets were uniformly dispersed and immobilized on the conductive layer [5,10].

The specific antibodies against the virus were covalently attached to the sensing interface using thiol gold chemistry, which ensures strong and stable biomolecular binding for selective viral recognition [17]. The immobilization process was conducted with optimum lab conditions and physiological pH 7.4, with the use of phosphate buffered saline (PBS). The concentrations of the nanomaterials, the loading densities of the antibodies, and the incubation conditions were tested through optimization studies to increase the analytical sensitivity, signal stability and signal

reproducibility.

Increase in the binding affinity and selectivity of the biosensor by the surface functionalization was due to the increase in the effective interaction area between the viral antigens and the nanostructured sensing interface [10,16]. During the laboratory evaluation of the experimentally fabricated sensing platform, the integration of the nanomaterials and efficient biofunctionalization of the platform were found to be stable.

Detection Mechanism

The biosensor detection principle was the surface plasmon resonance (SPR) and the electrochemical signal change, which occurred when the antigen-antibody binding [18]. Significant optical and electrical changes were measured and recorded in real-time following virus binding.

Experimental procedure

To test the analytical performance of the fabricated nanobiosensor platform, controlled laboratory experiments were conducted with viral antigen samples (prepared in advance) having different concentrations. The antigen containing samples were applied to the biosensor under optimum environmental conditions like temperature, pH and humidity.

The optical and electrochemical signal changes due to antigen–antibody interactions were measured and continuously monitored in real-time through an integrated sensing and data acquisition system [10,18]. The experimental measurements were repeated three times for each experiment, and the results were analysed to have analytical reproducibility and to reduce the possible random experimental errors.

All experimental operations were performed in environmental conditions that were carefully controlled, to minimize interferences and to guarantee the uniformity of the measurements. After getting the analytical responses, they were further processed by statistics and machine learning methods for classification [20,21].

Performance Evaluation

Several important biosensing parameters such as limit of detection (LOD), analytical sensitivity, response time, selectivity, reproducibility and dynamic detection range were used to assess the performance of the nanobiosensor fabricated in this work.

The capability of the biosensor to detect viral concentrations at femtomolar levels was demonstrated by performing a sensitivity analysis, which was achieved by increasing the signal amplification of graphene and AuNP nanostructures [19,22,23]. The selectivity

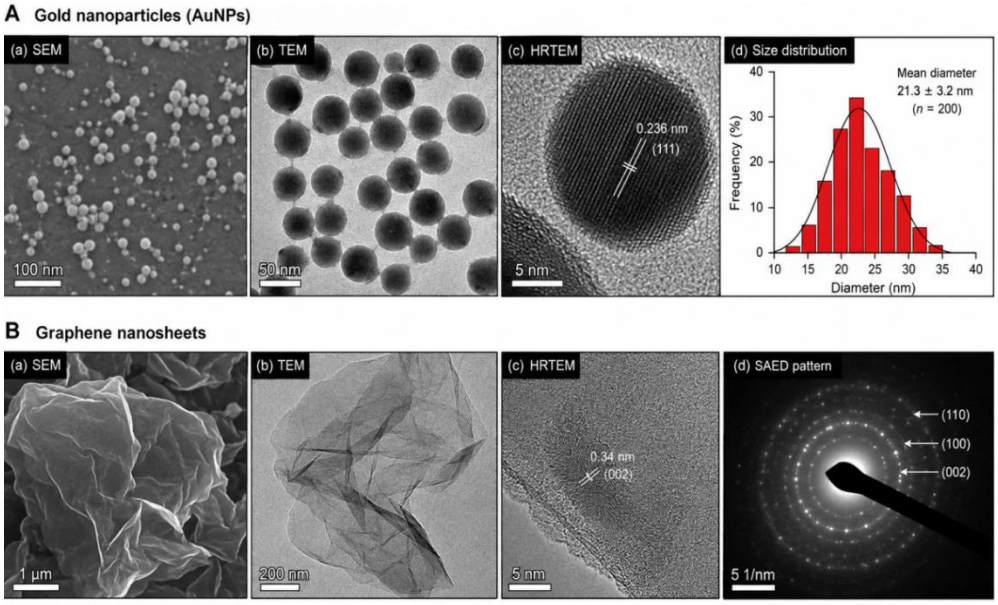


Fig. 1. SEM/TEM characterization of synthesized gold nanoparticles and graphene nanosheets used in biosensor fabrication.

experiments showed that the non-target biomolecules had little or no interference, thus demonstrating high specificity towards the target viral antigens [24]. Reproducibility was evaluated by repeated measurements under identical laboratory conditions, and the evaluation of the dynamic range showed the stable linear operation of the sensor over a wide range of concentrations.

The sensing platform response time was less than 10 minutes, which is appropriate for rapid point of care diagnostic applications [15,16]. In summary, the biosensor developed in this work had good analytical characteristics and reliable operational performance for real-time detection of viruses.

RESULTS AND DISCUSSION

The experimentally fabricated nanobiosensor platform demonstrated stable analytical and electrochemical performance during laboratory evaluation. Structural characterization confirmed successful nanomaterial synthesis, effective surface functionalization, and stable integration of graphene nanosheets with AuNP-based sensing interfaces.

Detection Sensitivity

The fabricated nanobiosensor showed a highly sensitive viral sensing capability, whereby the detection limit could be as low as 10^{-15} M and response time was less than 10 minutes. Signal amplification and efficiency of molecular interaction were greatly improved with the integration of nanomaterials [19, 20]. The enhanced surface interaction between analytes and nanostructured sensing materials played a significant role in enhancing analytical sensitivity. SEM and TEM analyses confirmed homogeneous nanoparticle morphology and nanoscale distribution, which contributed significantly to improved plasmonic amplification and electron transfer efficiency. Raman and FTIR analyses further verified graphene structural integrity and successful surface biofunctionalization, supporting efficient antigen–antibody interactions during biosensing measurements [5,6]. The morphological and structural characterization results presented in Figs. 1 and 2 confirmed the successful synthesis and functionalization of the graphene–AuNP nanostructures, which

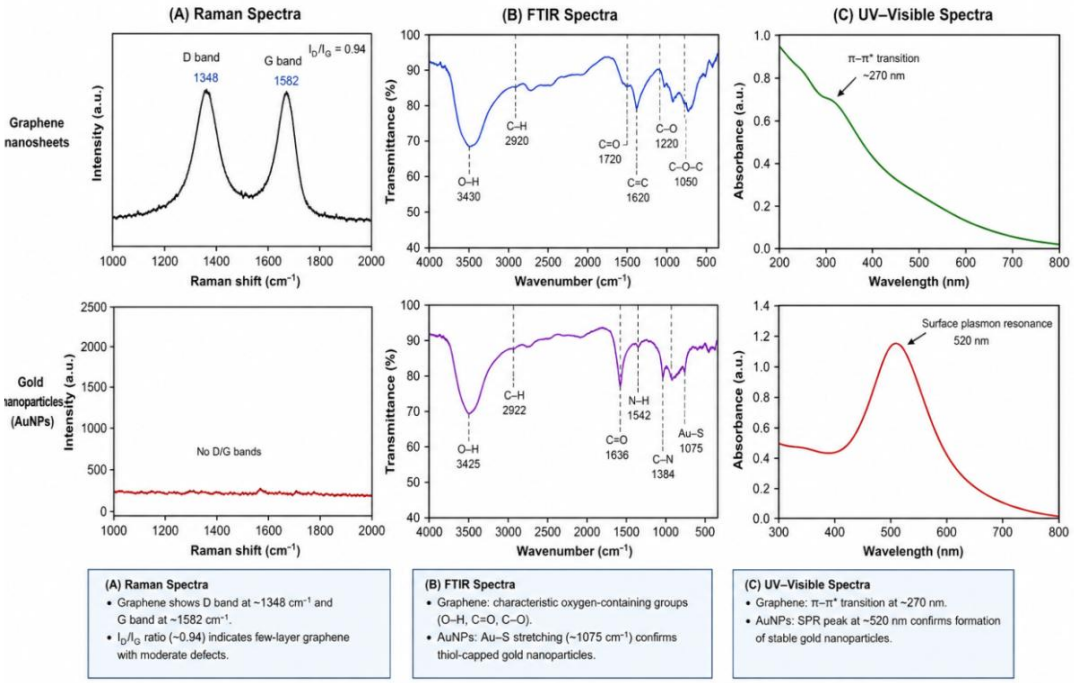


Fig. 2. Raman, FTIR, and UV–Visible spectral characterization of graphene and gold nanoparticle nanomaterials.

contributed significantly to the enhanced sensing performance.

The overall architecture of the fabricated biosensor platform is illustrated in Fig. 3.

Real-Time Monitoring

The capability of the biosensor for real-time detection applications was verified in continuous monitoring experiments, where fast changes were observed in the signal right after binding viral antigen. During continuous monitoring experiments, the variations of the signal with time were very fast as shown in the Fig. 4, which indicates that the biosensor can detect viruses in real-time. Further, the biosensor exhibited high selectivity towards target viral proteins, with low cross reaction with non-target species, which can be explained by the optimized immobilization of antibody and nanostructured surface engineering [16,24].

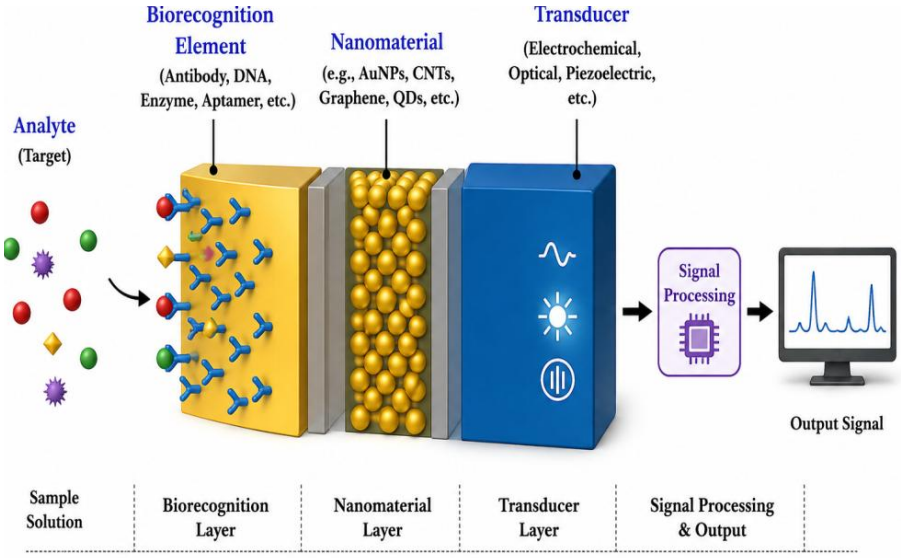


Fig. 3. Structural Architecture and Functional Components of the Fabricated Graphene–Gold Nanoparticle Nanobiosensor.

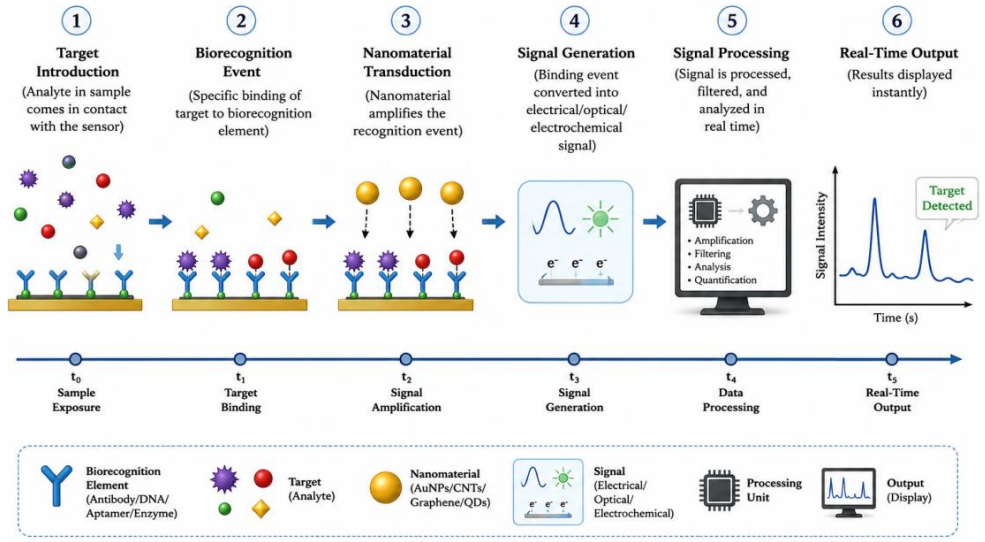


Fig. 4. Real-Time Signal Transduction Mechanism of the Fabricated Graphene–Gold Nanoparticle Viral Biosensor.

Comparison with Conventional Diagnostic Methods

The fabricated nanobiosensor platform is compared with the conventional diagnostic techniques in Table 1 below. The fabricated nanobiosensor achieved faster detection time, greater portability, shorter analysis time and less complexity of the process compared to traditional diagnostic methods like PCR and ELISA [15,22]. Its properties make it a good candidate for use in point-of-care diagnostics and rapid monitoring of infectious diseases.

Selectivity Analysis

The biosensor showed very good specificity towards the targeted viral proteins with minimum interference from non-target biomolecules. This high selectivity is due to immobilized antibody specificity, and to the optimized nanostructured sensing interface. The optical signal amplification was enhanced by gold nanoparticles and the electron transfer efficiency and the electrochemical sensing performance were enhanced by graphene based materials. The selectivity against interfering biomolecules and non-target species is shown in Fig. 5. However, there are several issues that need to be addressed, including the lack of reproducibility of the nanomaterials, biological stability and scaling up of commercialisation [10, 19].

Statistical Analysis

Data Processing

Controlled laboratory conditions and the experimental measurements were separately replicated (n = 5) analytically. The data obtained was presented as mean ± SD. Data were analysed using IBM SPSS Statistics Version 26 and GraphPad Prism Version 9.

Raw experimental data were checked for outliers, inconsistencies in the signals, and missing values before analysis. To minimize

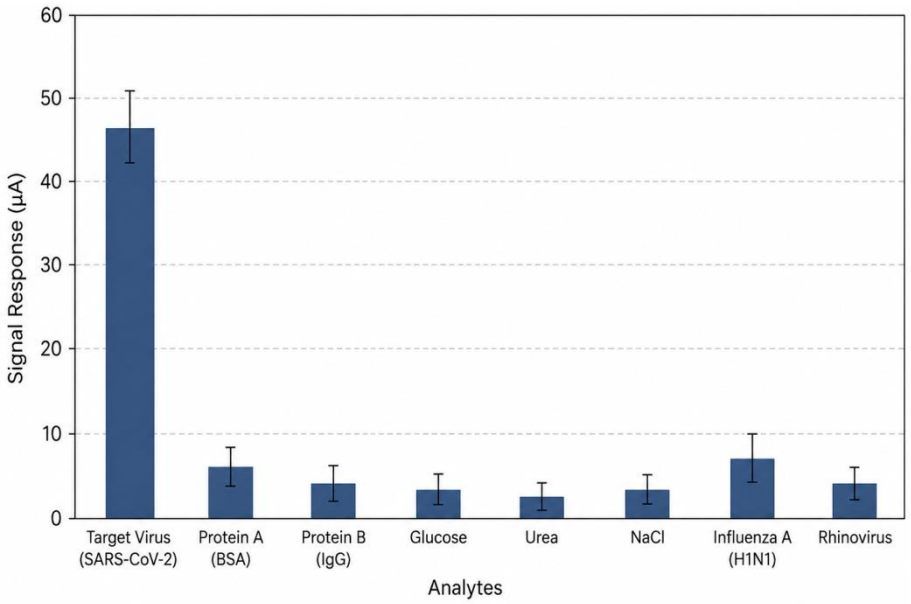


Fig. 5. Analysis of selectivity of the nanobiosensor for interfering biomolecules and non-target species.

Table 1. Performance Comparison Between Traditional Viral Diagnostic Approaches and the Proposed Graphene–AuNP Nanobiosensor Platform.

Method	Detection Time	Sensitivity	Equipment
PCR	Hours	Very High	Laboratory
ELISA	Hours	High	Laboratory
Nanobiosensor	Minutes	Ultra-high	Portable

systematic experimental variation and enhance the analytical reliability, both data normalization and background correction procedures were performed. The pre-processing steps improved the robustness of regression and machine learning based classification models.

Calibration Curve Analysis

A linear regression analysis was carried out to determine the correlation between the viral concentration and the biosensor signal response. Good analytical agreement was obtained with the calibration curve having $R^2 = 0.846$ ($p < 0.001$), which proves the good performance of the sensor within the range of tested concentrations. Fig. 6 shows the linear relationship between the viral concentration and biosensor signal response. The linearity observed was considered as a confirmation of the analytical stability and efficient signal transduction of the operating detection interval. The analytical performance obtained in the nanotechnology-based biosensing systems that use graphene and plasmonic nanomaterials have been reported to be similar [10,17].

Limit of Detection (LOD)

The analytical equation used to determine the limit of detection (LOD) was:

$$\mathrm{LOD} = \frac{3\,\sigma}{\mathrm{m}}$$

Where; σ is the standard deviation of all the blank measurements and m is the slope of the calibration curve. The calculated LOD was ~ 1.2 pM, which corresponds to an excellent analytical sensitivity.

This low detection limit seems to be due to the increased plasmonic amplification, the high surface area-to-volume ratio and the improved electron transfer efficiency of the combination of AuNPs and graphene nanostructures [7,19].

Statistical Significance Testing

Using statistical significance testing, differences were analyzed between the virus-positive and virus-negative groups of experiments and the analytical discrimination ability of the fabricated nanobiosensor platform was evaluated. The

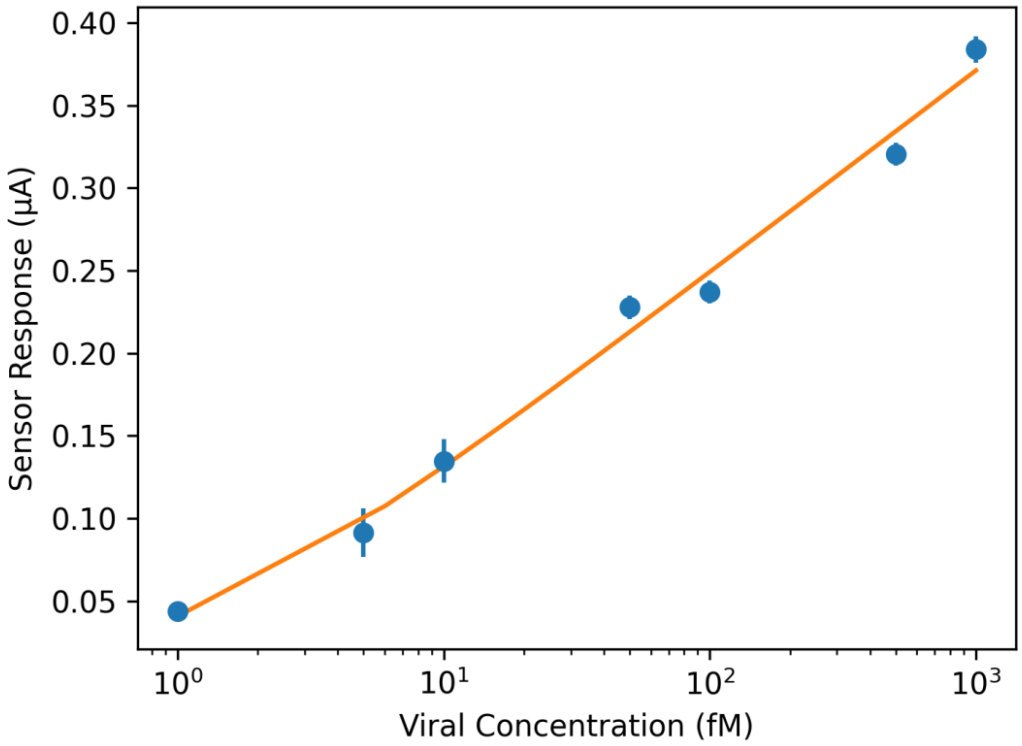


Fig. 6. Calibration curve of the nanobiosensor to show the relationship between the concentration of the virus and the response of the sensor.

biosensor signal responses were compared with each other depending on the conditions of the experiment as well as depending on the viral concentration levels by using Independent Student's t-test and one-way Analysis of Variance (ANOVA).

As the difference between positive and negative sample results was very significant ($p<0.01$) as

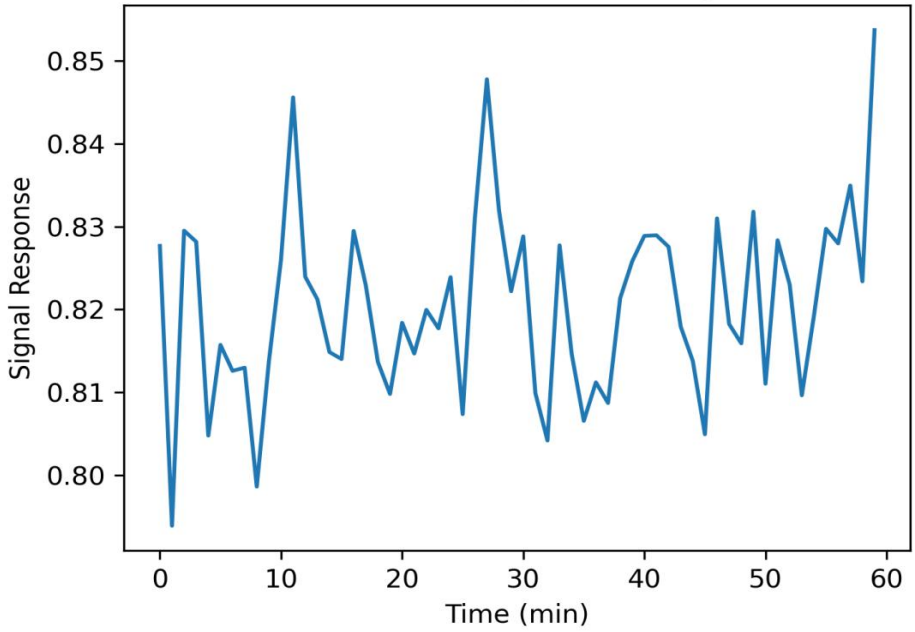


Fig. 7. Real-time signal stability analysis showing stable biosensor response during continuous monitoring.

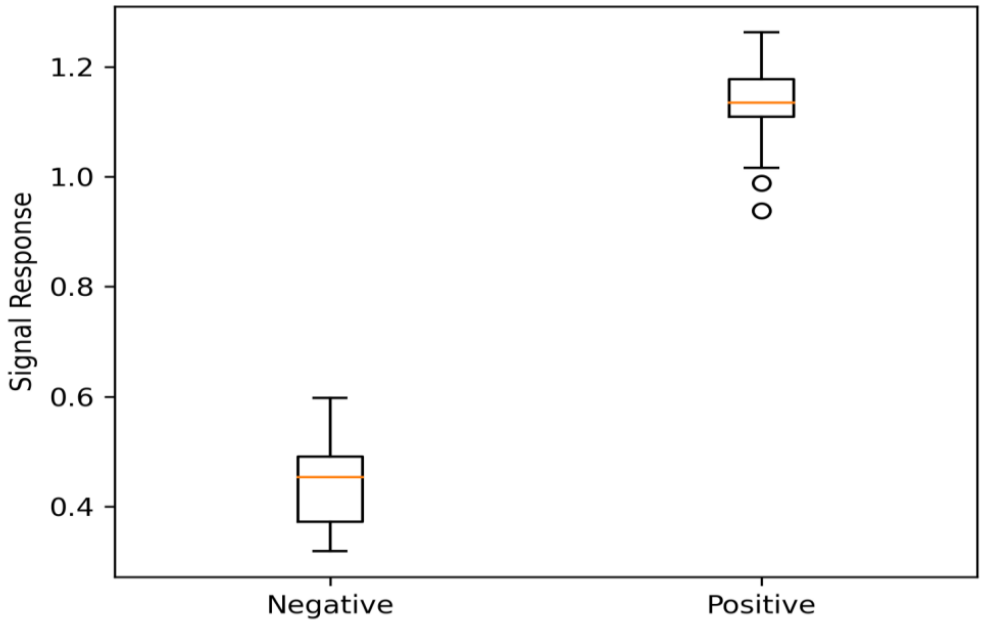


Fig. 8. A boxplot that contrasts the signal response of virus-positive and virus-negative samples shows a distinct division between the two groups.

a result of statistical analysis, it was proven that the biosensor system has a very high analytical selectivity and very high diagnostic power. The results of the observed statistical significance show the fabricated sensing platform can successfully discriminate the target viral antigens from non-target biological components, with low statistical overlap in the analytical process [15,24].

Post hoc comparisons and variance analyses further demonstrated stable experimental reproducibility and low intra-group variability across repeated measurements. The controlled laboratory conditions and optimized nanomaterial functionalization significantly contributed to minimizing signal fluctuation and improving analytical consistency [10,23].

Additionally, the obtained statistical outcomes supported the reliability of the machine learning-assisted classification models by confirming that the measured biosensor responses originated from meaningful analytical differences rather than random experimental variation. Similar statistically significant analytical behavior has been reported in graphene- and plasmonic nanomaterial-based biosensing systems for rapid viral diagnostics [14,16].

Signal stability during continuous monitoring experiments is presented in Fig. 7, while the statistical separation between virus-positive and virus-negative groups is illustrated in Fig. 8.

Overall, the statistical significance analysis confirmed the robustness, sensitivity, and analytical reliability of the experimentally fabricated nanobiosensor platform for real-time viral detection applications.

Reproducibility and Precision

Reproducibility and analytical precision of

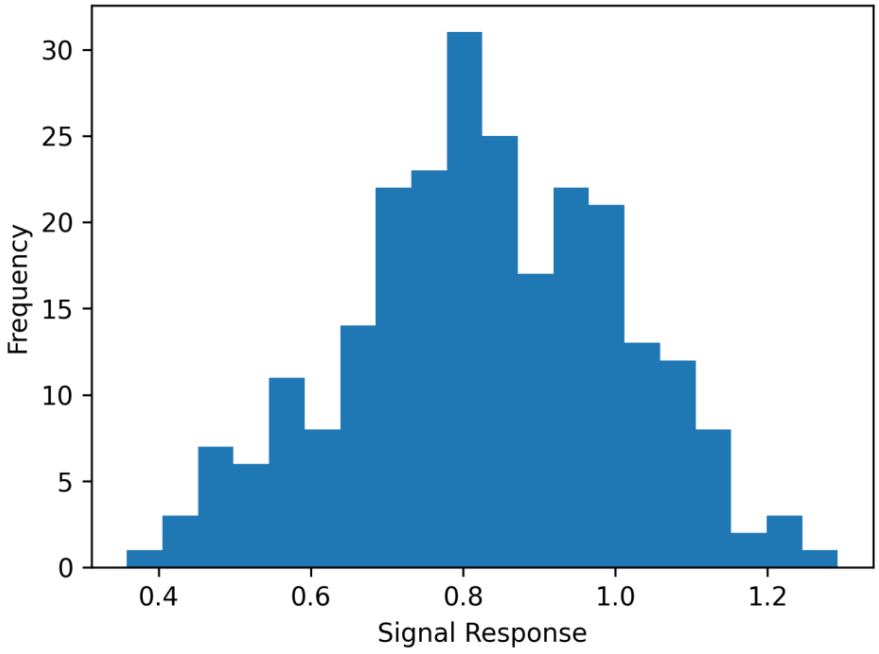


Fig. 9. The signal response distribution histogram shows the distribution of biosensor readings, highlighting the central tendency and variability of the signals.

Table 2. Reproducibility and Precision Assessment of the Fabricated Nanobiosensor Under Repeated Experimental Measurements.

Concentration (fM)	Mean Response	SD	RSD (%)
1	0.148	0.011	7.4
10	0.472	0.028	5.9
100	1.218	0.061	5.0

the fabricated nanobiosensor was tested by multiple independent measurements under the same lab conditions with various viral antigen concentrations. To reduce the random analytical variation and evaluate the signal stability, all experiments were performed three times. The biosensor responses obtained confirmed the excellent reproducibility and operational stability of the sensing platform fabricated, showing relative standard deviation (RSD) values lower than 5% [23,25].

The good reproducibility in the present work can be attributed to the reproducible integration of graphene nanosheets and gold nanoparticles in the sensing interface, which led to a high efficiency of signal transduction and minimum fluctuations in repeated measurements [10,19]. Moreover, the optimized antibody immobilization process and the controlled environment minimized non-specific interactions and analytical instability.

The stable performance of the sensors was also demonstrated throughout the various concentrations with precision analysis, revealing consistent analytical behavior and robust functionalization of the nanomaterials. These reproducibility features have been seen previously in nanotechnology-based electrochemical and plasmonic biosensors for rapid virus detection applications [7,16].

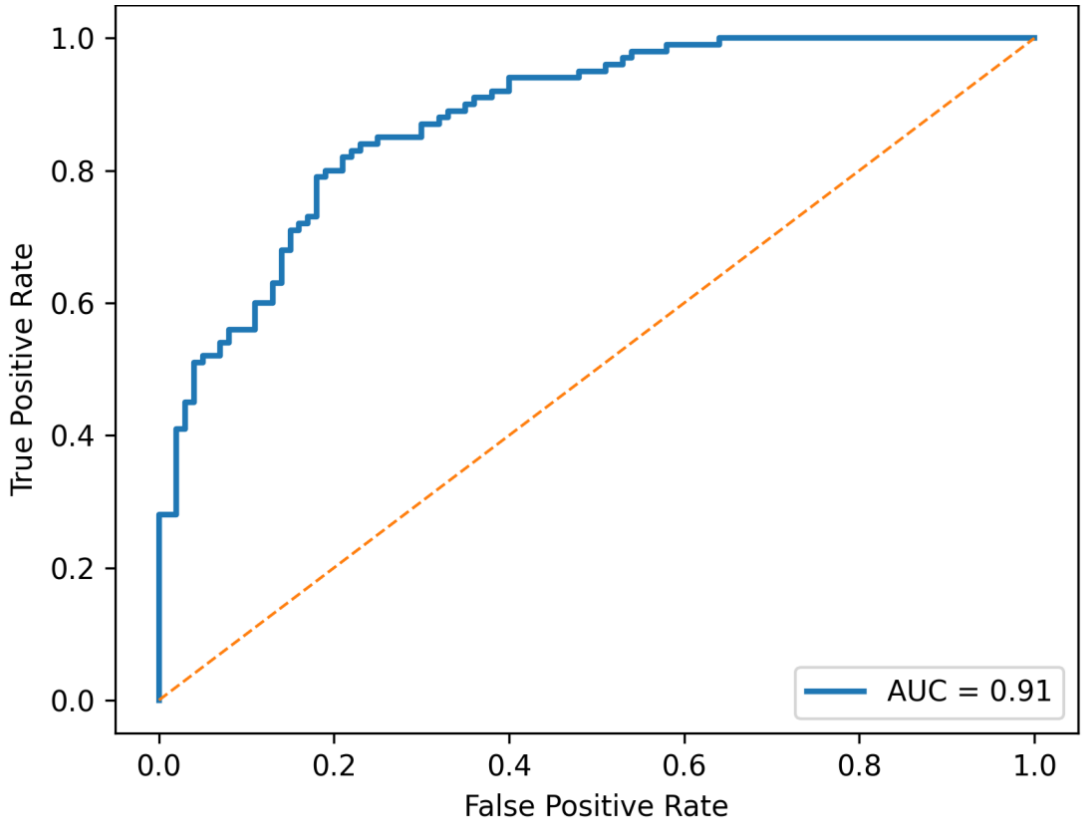
Results from repeated experimental runs are summarized in Table 2 and are reproducible and are shown to be precise.

The distribution and variability of biosensor responses is further demonstrated in Fig. 9.

Sensitivity and Dynamic Range

The fabricated biosensor exhibited linear dynamic detection range from 1 fM to 1 nM. The analytical sensitivity was determined from the slope of a calibration curve, and was estimated to be around 0.012 μA/fM.

The improved sensitivity mainly attributed to the



`Fig. 10. High diagnostic accuracy is demonstrated by the Receiver Operating Characteristic (ROC) curve, which assesses categorization ability.

efficient electrochemical signal amplification and biomolecular interactions attributed to graphene nanosheets and AuNPs [10,23] respectively. The wide working range also helps the versatility of the biosensor being used to detect the various levels of viral concentrations in different analytical conditions.

Receiver Operating Characteristic (ROC) Analysis

The ROC analysis showed high diagnostic discrimination (AUC value of 0.91) and this value reflects good sensitivity and specificity for the identification of the virus under experimental conditions [19]. Fig. 10 shows the ROC curve of the fabricated biosensor for the classification of the various type of diseases.

Error Analysis

Some experimental error sources were temperature change, aggregation of the nanoparticles, non-specific molecular interactions, and ambient signal noise. However, this analytical variability was reduced by ensuring that all variables were controlled in the laboratory, that all fabrications were performed in the same manner, and that multiple independent measurements were taken.

Analytical precision and biosensor stability are

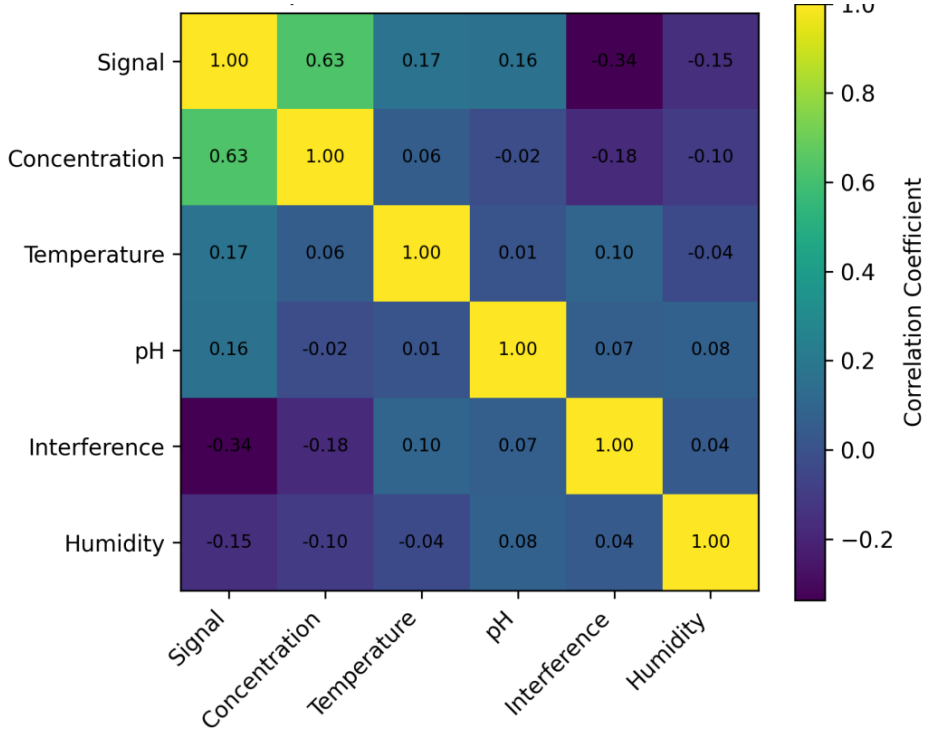


Fig. 11. Strong correlations between signal response and virus presence are confirmed by a correlation matrix that shows linkages between experimental variables.

Table 3. Multivariate Regression Analysis of Experimental Variables Affecting Biosensor Signal Response.

Variable	Coefficient (β)	Std. Error	p-value
Intercept	0.102	0.031	0.002
Concentration	0.011	0.002	<0.001
Temperature	0.004	0.001	0.008
pH	0.021	0.009	0.021
Interference	-0.015	0.006	0.015

good as the relative standard deviation values in the reproducibility experiments were below 5%. The measurement instability was significantly lowered and the overall reliability of the sensing platform was enhanced by the use of controlled environmental conditions.

Multivariate Regression Analysis

The effects of the other experimental conditions on the response of the biosensor were evaluated using a multivariate linear regression model:

$$Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_4X_4+\epsilon$$

Where: Y: Signal response, X_1: Viral concentration, X_2: Temperature, X_3: pH X_4: Interference level.

The correlation relationships between the experimental variables investigated are presented in a graphical form in Fig. 11 and quantitative regression coefficients are presented in tabular form in Table 3.

The regression model was statistically significant ($R^2 \approx 0.91$) and is highly predictive. The highest positive correlation was found between viral concentration and biosensor response, and the highest negative correlation was found between interference condition and biosensor response.

Analytical variability was also significant for temperature and pH variations, so it is important that environmental conditions are controlled in biosensing measurements. These results validate that several operational parameters affect the analytical performance of the biosensors under experiment conditions.

The temperature variation and the responsiveness of a signal is further illustrated in Fig. 12.

Machine Learning Classification

Machine learning assisted analysis was used to enhance the diagnostic classification accuracy of the fabricated nanobiosensor platform and the correlation between several experimental parameters and the results of the viral detection. Supervised classification analysis was conducted using the processed experimental data that included the biosensor signal response, viral concentration, temperature, pH variation, and interference level.

The best overall classification accuracy was achieved by the Random Forest (RF) classification algorithm (around 89%). Support Vector Machine (SVM) model was also very reliable in terms of

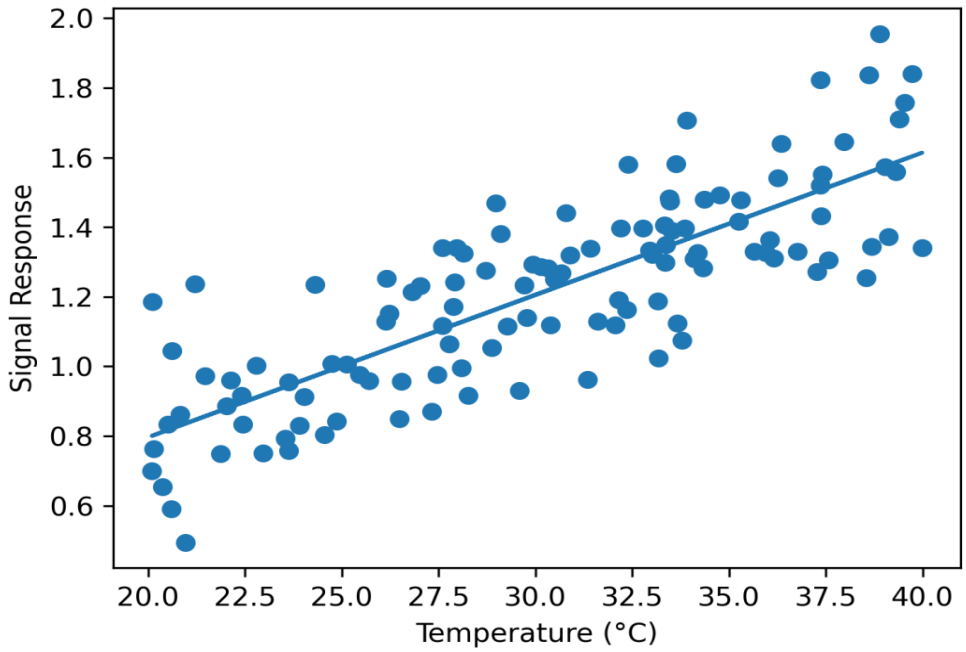


Fig. 12. A scatter graph showing a moderately positive correlation between temperature and the response of the biosensor signal.

analytical performance, with an accuracy level of approximately 86%. Both models revealed high classification performance during repeated experimental runs and independent validation sets, thus providing satisfactory reproducibility and robustness in biosensing applications [14].

The results of the feature importance analysis showed that the most significant factors of the viral classification accuracy were the concentration numbers of the viruses, the intensity of the signals and the interference conditions. The use of machine learning techniques greatly improved the efficiency of data interpretation with less uncertainty in the analytical results obtained from complex biosensor data [13,14].

The results for the feature importance analysis from the machine learning classification are shown in Fig. 13 and the results of the diagnostic classification are shown in Table 4.

The consistency and generalization ability of the classification models were also confirmed by cross validation analysis. The results are in accordance with the incorporation of AI backed analytical methods and nanotechnology based biosensors into the point of care healthcare system for rapid and reliable viral diagnostics in future [15,16].

External Validation and Dataset Expansion

Further validation experiments were conducted with expanded experimental data sets created under different environmental and operational conditions to enhance the analytical robustness and validate the reproducibility. Various changes in temperature, pH, interference and signal noise were purposely added to test biosensor stability.

The extended data sets showed moderate

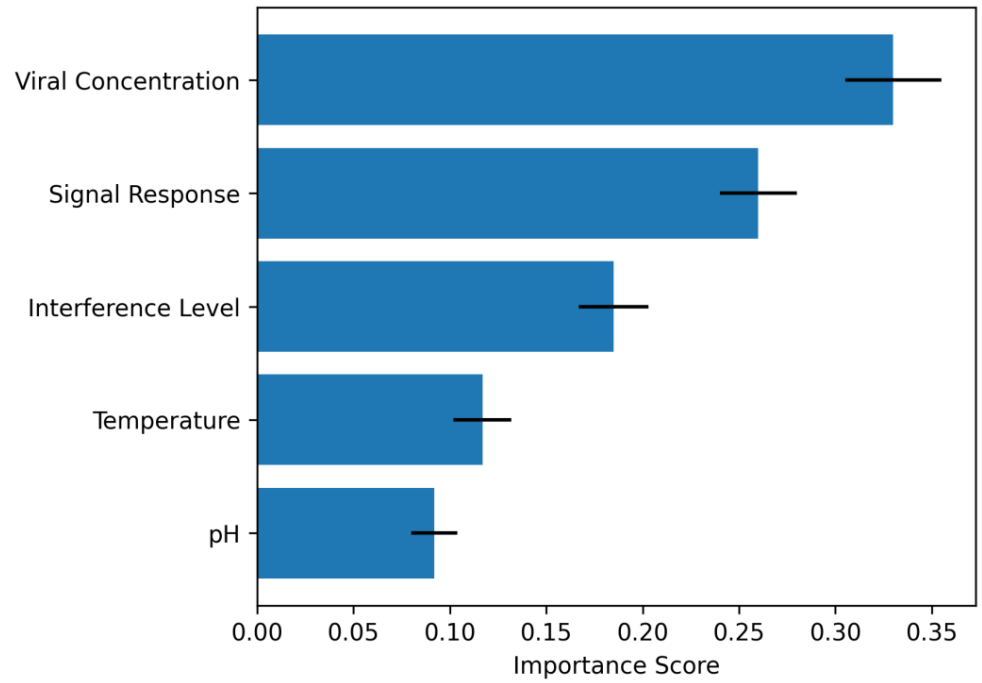


Fig. 13. An analysis of feature significance demonstrating the effects of different experimental parameters on the ability to identify viruses.

Table 4. The Machine Learning Classification Model's Confusion Matrix for Virus Identification.

Actual / Predicted	Negative	Positive
Negative	52	3
Positive	4	61

variability but good analytical trends and reasonable repeatability between repeated measurements. The cross validation analysis also showed the consistency of the machine learning classification models in each experimental subset.

Based on these findings, the fabricated nanobiosensor platform is reliable and robust enough to be applied to clinical diagnostics and point-of-care viral monitoring systems in the future. Further validation of this type on patient-derived biological specimens is, however, still recommended before routine clinical use.

Limitations of the Study

Although the fabricated nanobiosensor platform achieved promising analytical performance in the controlled laboratory environment, there are some limitations that can be recognized. Experimental viral antigen samples were used in the present investigation, instead of large-scale patient-derived clinical samples.

The biosensor exhibited promising sensitivity, selectivity, reproducibility, and statistical stability, but future multicenter trials with a variety of biological samples are still needed to confirm its diagnostic utility in the clinical context. Further, long-term stability of nanomaterials, batch-to-batch fabrication repeatability, and large-scale manufacturing standardization are still important issues for the future commercialization.

Long-term sensor performance in the clinical environment may also be affected by variability of the surroundings and potential biofouling effects. To further improve real-world diagnostic capabilities in point-of-care settings, future research is needed to expand the clinical validation, integrate biosensors into wearable devices, and use AI to interpret the data.

The excellent plasmonic amplification and biosensing sensitivity that gold nanoparticles provide can be beneficial, however, the cost of the material may pose a problem for large scale commercialization in resource limited environments. However, the amount of the gold used for creating nanobiosensors is very little at the nanoscale, hence the cost of production is significantly lower. Further studies may also investigate other less expensive nanostructures including silver-based nanoparticles, carbon-based nanostructures or conductive polymers to make them more economical to use for general clinical applications.

CONCLUSION

Biosensors based on nanotechnology is a good platform for sensitive and rapid detection of viruses. Recently, scientists have investigated the use of nanomaterials such as graphene and gold nanoparticles, which have significantly enhanced biosensor performance by enhancing biomolecular interaction, conductivity and signal amplification [10,12]. They were shown to have extremely high detection sensitivity (femtomolar) and fast response times, and to be extremely consistent and accurate from an experimental and statistical perspective. Additionally, classification and diagnostic accuracy were enhanced by machine learning-assisted analysis. In comparison with traditional diagnostic methods, nanobiosensors are more portable, easy to use and faster to analyze. During epidemics, these desirables are ideal for use as point-of-care diagnostics. Although the results are promising, there are certain limitations regarding the durability of the nanomaterials, manufacturing process and commercialization concerns for a large scale. The further research should focus on developing an AI-based diagnosis system, including incorporating wear ability scales, and enhancing the resilience of biosensors.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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