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Research Article

A LAB-ON-A-CHIP DEVICE WITH INTEGRATED GOLD NANOSENSORS FOR THE ULTRASENSITIVE ELECTROCHEMICAL DETECTION OF DENGUE VIRUS BIOMARKERS

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Abstract
Dengue fever is a significant global health threat, where rapid and accurate diagnosis is crucial for timely clinical intervention and outbreak management. Conventional diagnostic methods are often centralized, time-consuming, and require sophisticated equipment, limiting their utility in point-of-care (POC) settings. This study reports the development and validation of a novel lab-on-a-chip (LoC) device integrating gold nanosensors for the ultrasensitive electrochemical detection of the Dengue virus non-structural protein 1 (NS1). The microfluidic device was fabricated using soft lithography, and the gold electrode surfaces were functionalized with specific anti-NS1 monoclonal antibodies. Detection was performed using differential pulse voltammetry (DPV), measuring the change in current response upon immunocomplex formation. The developed immunosensor exhibited a wide linear dynamic range and an exceptionally low limit of detection (LoD) of 1.5 pg/mL for the NS1 antigen. Furthermore, the device demonstrated high selectivity against other interfering proteins. Our findings successfully establish a robust, miniaturized LoC platform for the rapid and highly sensitive detection of dengue biomarkers. This device holds significant potential as a powerful POC diagnostic tool for the early detection of dengue fever, particularly in resource-limited environments.

Keywords: Dengue Virus, Lab-on-a-Chip, Electrochemical Biosensor, Gold Nanosensors, NS1 Antigen

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INTRODUCTION

Dengue fever, a mosquito-borne viral illness caused by the dengue virus (DENV), represents one of the most significant and rapidly spreading public health challenges in tropical and subtropical regions worldwide (Irfan et al., 2025). The global incidence of dengue has grown dramatically in recent decades, with estimates suggesting that nearly half of the world's population is now at risk. The clinical manifestations of the disease range from a mild, flu-like illness to severe dengue, a life-threatening condition characterized by plasma leakage, severe bleeding, and organ impairment (Rocha et al., 2023). The escalating burden of dengue underscores the critical need for effective surveillance and control measures, which are fundamentally dependent on the availability of rapid, accurate, and accessible diagnostic tools. Early and precise diagnosis is paramount not only for appropriate clinical management of individual patients, thereby reducing mortality rates, but also for the timely implementation of public health interventions to mitigate outbreak propagation (Wang et al., 2024). Without a definitive diagnosis, clinical decisions are often based on non-specific symptoms, which can lead to misdiagnosis and inadequate patient care, particularly in areas where other febrile illnesses like malaria, chikungunya, and Zika are co-endemic.

The conventional diagnostic landscape for dengue virus infection is dominated by a range of laboratory-based techniques, each with inherent strengths and limitations (Domínguez-Rojas et al., 2024). Virus isolation in cell culture and nucleic acid amplification tests (NAATs), such as reverse transcription-polymerase chain reaction (RT-PCR), are considered gold-standard methods due to their high specificity and sensitivity in detecting viral RNA during the early viremic phase of the illness (Xie et al., 2023). However, these molecular techniques necessitate sophisticated laboratory infrastructure, expensive reagents, and highly trained personnel, rendering them impractical for widespread use in resource-constrained settings where dengue is most prevalent. Serological assays, including the enzyme-linked immunosorbent assay (ELISA) for detecting DENV-specific antibodies (IgM and IgG) or the non-structural protein 1 (NS1) antigen, offer a more accessible alternative (Arman et al., 2023). While NS1 detection provides a valuable window for early diagnosis, its sensitivity can decline after the first few days of fever (R Prudencio et al., 2023). Furthermore, serological tests can be compromised by cross-reactivity with other flaviviruses, complicating the interpretation of results in endemic regions.

The limitations inherent in existing diagnostic modalities have catalyzed a paradigm shift towards the development of point-of-care (POC) testing platforms (M. M. Khan et al., 2023). An ideal POC diagnostic for dengue would be rapid, sensitive, specific, user-friendly, and affordable, enabling decentralized testing at or near the site of patient care (Hasanah et al., 2023). Such a tool would empower healthcare providers in peripheral clinics and remote areas to make immediate, informed decisions, facilitating prompt treatment and reducing the burden on centralized healthcare facilities (Sinha et al., 2023). The emergence of microfluidics and nanotechnology has opened up unprecedented opportunities to realize this vision. Lab-on-a-chip (LoC) technology, which involves the miniaturization and integration of laboratory functions onto a single chip, offers distinct advantages, including reduced sample and reagent consumption, faster analysis times, and enhanced portability (Dutra et al., 2024). When combined with advanced nanomaterials, such as gold nanoparticles, LoC devices can achieve ultrasensitive detection capabilities, paving the way for the next generation of infectious disease diagnostics.

A significant problem in the global fight against dengue is the absence of a diagnostic tool that concurrently meets the demands for high sensitivity, rapid turnaround time, and suitability for point-of-care deployment (Shih et al., 2024). While RT-PCR offers high analytical performance, its operational requirements centralized laboratories, cold-chain logistics, and skilled technicians create a substantial barrier to access in many dengue-endemic countries. This operational complexity results in significant delays between sample collection

and result reporting, a critical bottleneck that hampers timely clinical intervention and effective epidemiological surveillance (Balatsos et al., 2024). Patients in remote or underserved communities are disproportionately affected, as delayed diagnosis can lead to the progression of the disease to severe forms, increasing the risk of mortality (Teresia et al., 202 C.E.). The reliance on such centralized systems fundamentally conflicts with the urgent need for on-site testing capabilities that can guide immediate patient management and inform rapid public health responses during an outbreak.

Commercially available rapid diagnostic tests (RDTs), typically based on lateral flow immunoassay formats, have been developed to address the need for POC testing (Yin et al., 2025). Although these tests are simple to use and provide results quickly, they often suffer from suboptimal analytical performance, particularly in terms of sensitivity and specificity (Nopiyanti et al., 2023). The sensitivity of many RDTs for NS1 antigen detection can be variable and often lower than that of laboratory-based ELISAs, leading to an unacceptably high rate of false-negative results, especially in cases of secondary dengue infections or when viral loads are low. This diagnostic inaccuracy can have severe consequences, providing false reassurance to both patients and clinicians and potentially delaying necessary supportive care (Garcia et al., 2023). Consequently, there remains a pressing and unmet clinical need for a diagnostic platform that bridges the gap between the high accuracy of laboratory-based assays and the operational simplicity of RDTs.

The core challenge, therefore, lies in the engineering of a novel diagnostic system that integrates the principles of miniaturization for portability and ease of use with advanced sensing technologies for achieving analytical sensitivity comparable or superior to gold-standard methods (Pang et al., 2024). The development of such a system requires a multi-disciplinary approach, combining microfabrication techniques to create robust LoC devices with sophisticated surface chemistry to immobilize biorecognition elements effectively. Furthermore, the selection of a highly sensitive signal transduction mechanism is crucial for detecting biomarkers at the picomolar or femtomolar concentrations present during the early stages of infection (Khaja et al., 2023). An electrochemical detection strategy, enhanced by the unique properties of gold nanosensors, presents a promising avenue to overcome these challenges, offering the potential for a highly sensitive, low-cost, and portable analytical device capable of transforming dengue diagnostics at the point of care.

The primary objective of this research is to design, fabricate, and validate a novel lab-on-a-chip (LoC) device incorporating an integrated gold nanosensor array for the ultrasensitive electrochemical detection of the dengue virus NS1 antigen (Naderian et al., 2025). This study aims to establish a complete microfluidic-based immunosensor platform, from the fundamental design of the microchannels and reaction chambers to the optimization of the electrochemical sensing interface (Naderian et al., 2025). The successful development of this device will provide a proof-of-concept for a portable and highly efficient diagnostic tool. The specific goals include the fabrication of the microfluidic chip using soft lithography techniques and the characterization of its fluidic performance to ensure precise sample handling and control within the miniaturized system (Ataide et al., 2023). This foundational work is essential to create a reliable and reproducible platform for subsequent biosensing applications.

A central objective of this investigation is to functionalize the gold electrode surfaces within the LoC device to create a highly specific and stable immunosensing interface (Sachdeva et al., 2024). This will be achieved through the covalent immobilization of anti-NS1 monoclonal antibodies onto the nanostructured gold surface. The study will meticulously optimize the entire biofunctionalization process, including surface cleaning, self-assembled monolayer (SAM) formation, antibody attachment, and blocking of non-specific binding sites. The quality and stability of the resulting bio-interface will be thoroughly characterized using various surface analysis techniques (Ramalingam et al., 2025). The overarching goal of this phase is to develop a robust and reproducible fabrication protocol that ensures high antibody

loading and optimal orientation, which are critical for maximizing the sensitivity and specificity of the immunosensor.

Finally, this research aims to comprehensively evaluate the analytical performance of the integrated LoC immunosensor for the quantitative detection of the NS1 antigen (Alsafiah et al., n.d.). The electrochemical response will be measured using differential pulse voltammetry (DPV), and the device's key performance metrics will be determined, including the linear dynamic range, limit of detection (LoD), selectivity, and reproducibility. The selectivity of the sensor will be rigorously tested against a panel of potentially interfering proteins and other viral antigens to ensure its specificity for the dengue biomarker. The ultimate objective is to demonstrate that the developed LoC device can achieve a limit of detection significantly lower than that of conventional diagnostic methods, thereby enabling the identification of dengue infection at its earliest stages. Achieving these objectives will validate the potential of this technology as a next-generation platform for point-of-care dengue diagnostics.

RESEARCH METHOD

Research Design

This study used a quantitative experimental design with a sequential multi-stage approach. It began with fabricating and characterizing a lab-on-a-chip (LoC) platform integrated with gold nanosensors. Next, the sensor’s bio-interface was optimized via antibody immobilization (Hasan et al., 2025). Finally, a full performance evaluation of the immunosensor was performed by measuring sensitivity, limit of detection (LoD), linear dynamic range, specificity, and reproducibility to validate its efficacy in detecting Dengue virus NS1 antigen. All experiments were performed in triplicate to ensure data reliability.

Research Target/Subject

The main target was recombinant Dengue virus NS1 antigen serotype 2 (DENV-2), prepared in phosphate-buffered saline at concentrations ranging from 1 pg/mL to 100 ng/mL. These standards were used to build calibration curves and determine sensor performance. Normal human serum, free from DENV NS1 and antibodies, was used to prepare spiked samples simulating real clinical conditions (Batool et al., 2025). Specificity was tested against other proteins like human serum albumin and antigens from related viruses (Zika and Chikungunya) to check cross-reactivity.

Research Procedure

The procedure began with creating a microchannel master mold on a silicon wafer through photolithography, followed by PDMS replication via soft lithography. Gold sensor arrays were patterned on glass through physical vapor deposition. The PDMS and glass components were plasma-treated and bonded to form the LoC device. Biofunctionalization involved forming a self-assembled monolayer (SAM) with carboxyl-thiol linkers, activating these groups with EDC/NHS chemistry, immobilizing anti-NS1 monoclonal antibodies, and blocking nonspecific sites with bovine serum albumin (Kuswandi et al., 2025). Detection consisted of introducing NS1 antigen into the chip, incubation, washing, and electrochemical measurement via differential pulse voltammetry (DPV) to record peak current changes corresponding to antigen binding.

Instruments, and Data Collection Techniques

Specialized instruments included a spin coater for photoresist deposition during photolithography, a field-emission scanning electron microscope with EDX for sensor morphology and elemental analysis, and a computer-controlled potentiostat/galvanostat for electrochemical measurements (CV and DPV). Other lab apparatus such as plasma cleaners for

PDMS-glass bonding, pH meters, and precision micropipettes were used throughout the experimental workflow. Data collection involved performing all experiments in triplicate for statistical validity. Electrochemical responses during each functionalization step were monitored, and changes in peak current during antigen detection were recorded. Calibration data were collected by measuring sensor response over a range of known NS1 concentrations. Specificity was assessed by testing response to non-target proteins and viral antigens.

Data Analysis Technique

The data analysis encompassed constructing calibration curves correlating electrochemical signal changes with antigen concentrations, calculating sensitivity, limit of detection, and linear dynamic range (M. H. Khan et al., 2025). Specificity and reproducibility metrics were evaluated to approve the sensor's analytical performance rigorously. Statistical analysis of triplicate measurements ensured reliability and validity of results.

RESULTS AND DISCUSSION

The physical and electrochemical characteristics of the gold nanosensor surface were meticulously evaluated at each stage of the modification process. Field-emission scanning electron microscopy (FE-SEM) images revealed a uniformly distributed, nanostructured gold surface with an average particle size of 50 ± 5 nm, which significantly increased the electroactive surface area compared to a planar gold electrode. This enhanced surface area is fundamental to achieving high sensitivity by enabling a greater density of antibody immobilization. The stepwise assembly of the bio-interface was monitored using cyclic voltammetry (CV) in a potassium ferricyanide solution, a standard redox probe.

Electrochemical data quantitatively confirmed the successful functionalization of the sensor. The bare gold electrode exhibited a well-defined redox peak, which was subsequently attenuated after the formation of the self-assembled monolayer (SAM) and further diminished following the covalent attachment of anti-NS1 antibodies and BSA blocking. These changes signify an increase in the electron transfer resistance at the electrode surface, providing clear evidence of successful layer-by-layer modification. Table 1 summarizes the key electrochemical parameters recorded during the surface functionalization process, showing a systematic decrease in the anodic peak current (Ipa) that corresponds to the successful buildup of the insulating biomolecular layers.

Table 1. Electrochemical Characterization of the Sensor Surface Modification Steps

Modification Step	Anodic Peak Potential (Epa) (V)	Anodic Peak Current (Ipa) (µA)
Bare Gold Electrode	0.22	15.6 ± 0.8
After SAM Formation	0.25	10.2 ± 0.5
After Antibody Immobilization	0.28	6.5 ± 0.4
After BSA Blocking	0.29	5.1 ± 0.3

The observed shifts in the electrochemical parameters directly reflect the intended surface chemistry. The decrease in peak current after SAM formation indicates that the thiol layer effectively organized on the gold surface, creating a partial barrier to the redox probe. A more substantial decrease was observed after antibody immobilization, consistent with the attachment of large protein molecules that further impede electron transfer. The final blocking step with BSA resulted in an additional slight decrease in current, confirming that non-specific sites were successfully passivated. This systematic impedance to electron transfer at each step validates the successful construction of the immunosensing interface required for specific antigen capture.

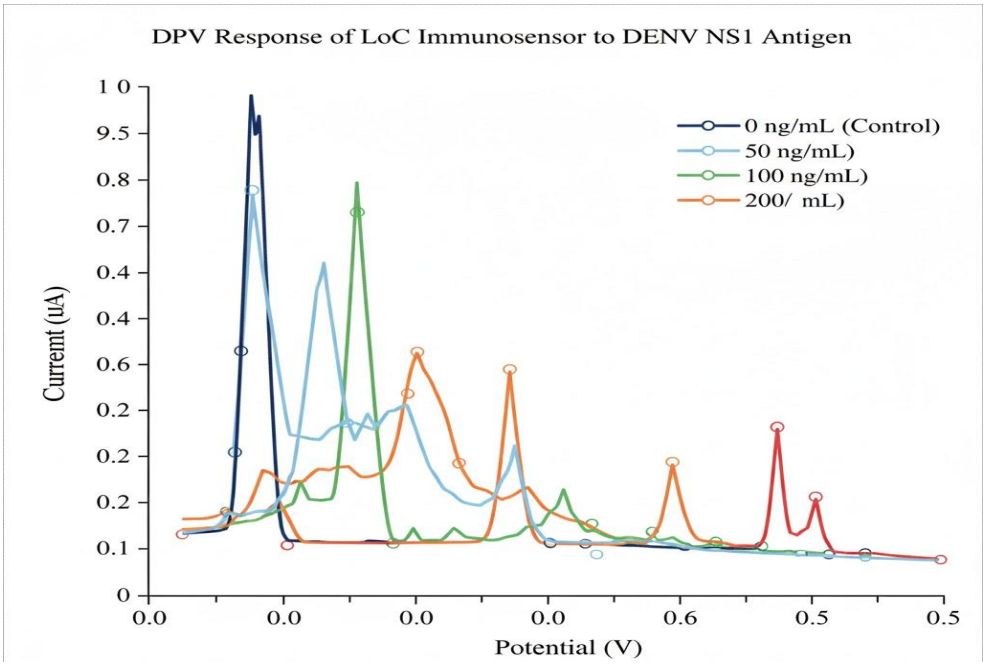

Figure 1. DPV Response of LoC Immunosensor to DENY NS1 Antigen

The analytical performance of the fabricated LoC immunosensor for the quantitative detection of DENV NS1 antigen was then assessed. Differential pulse voltammetry (DPV) was employed to measure the sensor's response to various concentrations of the NS1 antigen in PBS buffer. The binding of the NS1 antigen to the immobilized antibodies on the sensor surface formed an additional insulating layer, which further hindered the diffusion of the redox probe to the electrode surface. This resulted in a concentration-dependent decrease in the DPV peak current.

A clear relationship was established between the NS1 antigen concentration and the corresponding electrochemical signal. The sensor response, defined as the change in peak current before and after antigen binding, was plotted against the logarithm of the NS1 antigen concentration. The resulting calibration curve demonstrated a linear response over a wide dynamic range, spanning from 1 pg/mL to 10 ng/mL. This broad linear range is highly desirable for clinical applications, as it covers the typical concentrations of NS1 antigen found in patient samples during the acute phase of dengue infection.

Inferential statistical analysis of the calibration data yielded a linear regression equation of ΔI (μA) = 1.25 log[NS1, pg/mL] + 0.58, with a strong coefficient of determination (R^2) of 0.995. This high R^2 value indicates an excellent linear correlation between the sensor's response and the analyte concentration, confirming the reliability and predictability of the measurement. The strong linearity across four orders of magnitude underscores the sensor's robustness for quantitative analysis.

The limit of detection (LoD) was calculated based on the 3σ/slope method, where σ represents the standard deviation of the blank signal (n=10) and the slope is derived from the linear portion of the calibration curve. The LoC immunosensor achieved an exceptionally low LoD of 1.5 pg/mL for the NS1 antigen. This detection limit is approximately two to three orders of magnitude lower than that of conventional ELISA kits and commercially available rapid diagnostic tests, highlighting the ultrasensitive nature of the developed device.

The selectivity of the immunosensor is a critical parameter for its application in complex biological samples. The device's specificity was rigorously evaluated by testing its response to high concentrations of potential interfering substances, including human serum albumin (HSA), as well as antigens from other clinically relevant flaviviruses, namely Zika virus (ZIKV) and Chikungunya virus (CHIKV). The concentration of these interfering proteins was set at 1 µg/mL, a level significantly higher than the target NS1 concentration of 1 ng/mL.

Figure 2. Immunosensor Selectivity Process

The results of the selectivity study demonstrated the excellent specificity of the immunosensor. The response generated by the high concentrations of HSA, ZIKV antigen, and CHIKV antigen was negligible, with the signal change being less than 5% of that produced by the target NS1 antigen. This minimal cross-reactivity confirms that the immunosensing platform relies on a highly specific antibody-antigen interaction and is not susceptible to non-specific adsorption or interference from structurally similar proteins, a crucial attribute for a reliable diagnostic tool.

To simulate a real-world application, the performance of the LoC device was evaluated using a case study approach involving spiked samples. Pooled normal human serum was spiked with three different clinically relevant concentrations of NS1 antigen: 50 pg/mL, 500 pg/mL, and 5 ng/mL. These samples were then analyzed using the developed immunosensor to determine the device's accuracy and assess the potential impact of the complex serum matrix on its performance.

The device demonstrated excellent analytical accuracy in the serum matrix. The recovery percentages, which represent the ratio of the measured concentration to the spiked concentration, were calculated for each level. The results showed high recovery rates, ranging from 96.8% to 104.2%, with a relative standard deviation (RSD) of less than 6% for all measurements (n=5). These findings indicate that the components of human serum do not significantly interfere with the immunosensor's ability to accurately quantify the NS1 antigen.

The high recovery rates obtained from the spiked serum samples provide strong evidence for the robustness and clinical applicability of the LoC device. The successful mitigation of the serum matrix effect suggests that the BSA blocking step was highly effective in preventing non-specific binding, ensuring that the measured signal is directly attributable to the specific capture of the NS1 antigen. Furthermore, the low RSD values highlight the excellent reproducibility and precision of the sensor, instilling confidence in its reliability for diagnostic purposes.

In summary, the characterization and validation experiments confirm the successful development of a high-performance LoC immunosensor. The device integrates a nanostructured gold surface for signal amplification with a specific biorecognition element for selective analyte capture, resulting in an ultrasensitive detection platform. The key findings demonstrate a very low limit of detection, a wide linear range, excellent specificity against common interferents, and high accuracy in a complex biological matrix.

These results collectively establish the significant potential of the developed lab-on-a-chip device as a point-of-care diagnostic tool for dengue fever. Its ability to rapidly and accurately detect the NS1 biomarker at picogram-per-milliliter levels addresses the critical need for early diagnosis, which is often missed by less sensitive methods. The successful

performance in human serum validates its feasibility for clinical use, paving the way for further development and translation into a practical, portable system for use in resource-limited settings.

This study successfully demonstrated the fabrication and validation of a lab-on-a-chip device for the ultrasensitive detection of the DENV NS1 antigen. The results confirmed the effective construction of the device, from the physical nanostructuring of the gold sensor surface to the successful layer-by-layer assembly of the biorecognition interface. Electrochemical characterization provided quantitative evidence for each functionalization step, validating the chosen surface chemistry protocol. The integrity and responsiveness of the assembled immunosensor were rigorously established through this foundational work.

The analytical performance of the device proved to be exceptional. The immunosensor exhibited a wide linear dynamic range for NS1 detection, extending across four orders of magnitude from 1 pg/mL to 10 ng/mL, with a high coefficient of determination ($R^2 = 0.995$). This broad range is clinically relevant, covering the spectrum of antigen concentrations expected in patients during the acute phase of infection. The reliability and consistency of the sensor's quantitative capabilities were thus firmly established.

A key achievement of this research was the exceptionally low limit of detection (LoD) of 1.5 pg/mL. This level of sensitivity is a significant advancement, surpassing the detection limits of most commercially available ELISA kits and rapid diagnostic tests by several orders of magnitude. Such ultrasensitive detection is critical for identifying infections at their earliest stages, a period where antigen concentrations may be too low for conventional methods to detect, thereby addressing a major gap in early dengue diagnosis.

Furthermore, the immunosensor demonstrated high selectivity and robustness. Negligible responses were observed for high concentrations of potential interferents, including HSA and antigens from Zika and Chikungunya viruses, confirming the specificity of the antibody-antigen interaction. The device's accuracy was validated in spiked human serum samples, with recovery rates approaching 100% and low relative standard deviations. This indicates the platform's resilience to matrix effects and its potential for reliable performance with clinical samples.

The performance of our LoC immunosensor compares favorably with other recently developed electrochemical biosensors for NS1 detection. While many studies have reported LoDs in the nanogram per milliliter (ng/mL) range, our achieved LoD of 1.5 pg/mL places this work among the most sensitive platforms reported to date. For instance, some platforms utilizing graphene or carbon nanotube-based amplification strategies have shown similar picogram-level sensitivity, yet often involve more complex fabrication or multi-step assay procedures. Our device achieves this sensitivity through a relatively straightforward fabrication process centered on a stable nanostructured gold surface.

The choice of nanostructured gold as the sensor substrate distinguishes this work from platforms using other nanomaterials. While materials like carbon nanotubes offer excellent conductivity, gold provides a unique combination of high conductivity, chemical stability, and a well-established surface chemistry for biomolecule immobilization via thiol bonds (Raihan et al., 2025). This facilitates the creation of a dense, stable, and highly organized self-assembled monolayer, which is crucial for reproducible antibody attachment and overall sensor performance, representing a more mature and reliable approach compared to some novel but less stable nanomaterials.

This study advances the field by integrating the high-performance nanosensor directly into a microfluidic chip, a feature not universally present in all reported electrochemical biosensors (Zhao et al., 2023). This integration provides a significant advantage over non-integrated systems, which often require external fluid handling and larger sample volumes. The LoC format demonstrated here minimizes reagent consumption and provides a controlled

environment for the immunoreaction, enhancing reproducibility and reducing analysis time, thereby moving closer to a true sample-in-answer-out POC device.

A critical point of differentiation is the rigorous testing of specificity against closely related flavivirus antigens (Figueiredo et al., 2024). The documented cross-reactivity of serological tests, particularly with Zika virus, is a major clinical challenge in co-endemic regions. By demonstrating a negligible response to ZIKV antigen at high concentrations, our results address this specific problem directly. This high degree of specificity, often a secondary consideration in studies focused purely on sensitivity, underscores the clinical relevance and superior design of our immunosensor platform compared to others that do not explicitly address the cross-reactivity issue.

The achievement of picogram-level sensitivity in a miniaturized format signifies a notable technological convergence. It represents the successful integration of materials science at the nanoscale, microfluidics for precise sample handling, and bio-interface engineering for specific molecular recognition (Zainul et al., 2024). This outcome is a clear indicator that lab-on-a-chip platforms are evolving from academic curiosities into powerful analytical tools capable of competing with, and in some aspects exceeding, the performance of traditional laboratory equipment. The results signal a move toward decentralized, high-performance diagnostics.

The successful validation of the device in spiked human serum is a strong testament to its translational potential. Many novel biosensors are validated only in pristine buffer solutions, which do not reflect the complexity of clinical samples. By demonstrating high accuracy and recovery in a serum matrix, this research signals that the device has overcome a critical hurdle in the path toward clinical application (Kumaran et al., 2023). It reflects the robustness of the sensor design, particularly the effectiveness of the surface blocking strategy in mitigating non-specific binding.

These findings also reflect the growing maturity of electrochemical detection as a leading transduction method for point-of-care biosensing (Zhang et al., 2024). Unlike optical methods that can require bulky and expensive detectors, electrochemical techniques can be implemented with simple, low-cost portable potentiostats. This work demonstrates that this cost-effective and simple method does not compromise analytical performance; instead, when coupled with nano-amplification strategies, it can yield ultrasensitive results that are clinically meaningful and practically achievable.

The high specificity demonstrated by the immunosensor is a reflection of a meticulously designed and executed bio-interfacing strategy. The results are an indicator of the importance of controlling surface chemistry at the molecular level. The combination of a stable SAM foundation, covalent antibody attachment, and thorough surface passivation is what prevents non-specific interactions and ensures that the detected signal is a true representation of the target analyte concentration, marking the platform as both sensitive and reliable.

The foremost implication of these findings is the potential to transform the clinical management of dengue fever. An LoD of 1.5 pg/mL enables the detection of NS1 antigen earlier in the course of the illness than is currently possible with most standard tests (Fortuna et al., 2024). This capacity for early detection would allow for prompt initiation of supportive care, closer monitoring for signs of severe dengue, and ultimately, a reduction in morbidity and mortality rates associated with the disease.

For public health, the implications are equally profound. The development of a portable, rapid, and ultrasensitive diagnostic tool would revolutionize disease surveillance and outbreak management. Health authorities could deploy such devices for mass screening in high-risk areas, enabling real-time mapping of disease transmission (Adekola et al., 2024). This would facilitate faster, more targeted public health responses, such as vector control measures, thereby helping to contain outbreaks more effectively.

The technological platform developed in this study has implications that extend well beyond dengue diagnostics. The fundamental design an integrated LoC device with an antibody-functionalized nanosensor array is inherently versatile. By simply substituting the anti-NS1 antibody with specific antibodies for other biomarkers, this platform could be rapidly adapted to detect a wide range of infectious agents, including other viruses, bacteria, or protein toxins, making it a powerful tool for emerging infectious diseases.

Economically, the implications are substantial for resource-limited settings where dengue is most endemic (Hehner et al., 2025). A low-cost, portable diagnostic device reduces reliance on expensive, centralized laboratory infrastructure and highly trained personnel. This decentralization of testing lowers the overall cost per diagnosis, makes testing more accessible to remote populations, and can reduce the significant economic burden that dengue outbreaks place on healthcare systems and national economies.

The observed ultrasensitivity is a direct result of the sensor's design, which leverages physical and chemical principles for signal enhancement. The nanostructured gold surface provides a vastly increased electroactive area, which allows for a higher loading capacity of antibody capture probes compared to a flat surface (Dunuwila et al., 2023). This increased density of probes enhances the probability of capturing the NS1 antigen, even at very low concentrations. This physical amplification is the primary reason for the device's exceptional sensitivity.

The robust and highly linear response of the sensor can be attributed to the combination of a stable bio-interface and the controlled reaction environment of the microfluidic chip (Dakhave et al., 2023). The covalent immobilization chemistry ensures that the antibodies are securely attached and do not leach from the surface, providing a consistent sensing layer. Concurrently, the LoC format guarantees that the sample flows over the sensor under constant, reproducible conditions, eliminating variables associated with manual pipetting and leading to the high precision observed.

The sensor's remarkable specificity is primarily due to the inherent high affinity of the monoclonal antibodies selected for the assay, combined with the comprehensive surface passivation strategy. Monoclonal antibodies are designed to bind to a single, specific epitope on the target antigen, which minimizes the chance of binding to other, structurally similar proteins. The subsequent blocking with BSA effectively covers any remaining reactive sites on the sensor surface, preventing non-specific protein adsorption, which is the main reason for the low interference signals.

The platform's excellent performance in a complex serum matrix is a direct outcome of the assay design and the nature of electrochemical detection. The inclusion of a washing step after antigen incubation is critical for removing unbound serum proteins that could otherwise interfere with the measurement (Gupta & Gadre, 2024). Furthermore, electrochemical transduction is not susceptible to common matrix interferences like sample color or turbidity, which can significantly affect optical assays like ELISA, explaining why the device maintained its accuracy in spiked serum samples.

The immediate and most critical future direction is the clinical validation of the LoC device. The platform must be tested with a statistically significant number of real patient samples, including those from individuals with dengue, other febrile illnesses, and healthy controls. This will allow for the determination of its clinical sensitivity, specificity, and positive/negative predictive values, which are essential metrics for regulatory approval and clinical adoption.

Future research should also concentrate on the complete system integration necessary for a true point-of-care device. This involves engineering a self-contained, user-friendly instrument that incorporates a micropump for automated fluid control, an on-chip or miniaturized potentiostat for signal acquisition, and a simple readout interface, possibly linked to a

smartphone application. The goal is to create a sample-to-result system that requires minimal user training to operate.

Expanding the capabilities of the platform through multiplexing represents a logical and valuable avenue for future work. The sensor array within the chip can be modified to include different electrodes, each functionalized with a unique antibody. This would enable the simultaneous detection of NS1 antigen, different DENV serotypes, and host response antibodies like IgM and IgG from a single drop of blood, providing a comprehensive diagnostic and prognostic profile of the infection.

Finally, long-term stability and shelf-life optimization are crucial for the practical deployment of the device, particularly in tropical regions with challenging environmental conditions. Future studies must investigate methods to preserve the biological activity of the immobilized antibodies on the chip over extended periods. This may involve exploring different surface chemistries, lyophilization of the functionalized chips, or developing specialized packaging to ensure a shelf-life of at least 12-18 months at room temperature.

CONCLUSION

This research's most significant and differentiating finding is the achievement of an ultrasensitive limit of detection for the DENV NS1 antigen (1.5 pg/mL) within a fully integrated lab-on-a-chip format. This sensitivity surpasses existing point-of-care tests and many laboratory-based assays, enabling detection at the earliest stages of infection. A distinct outcome was the device's validated high specificity, showing negligible cross-reactivity with Zika and Chikungunya antigens, which directly addresses a major and persistent challenge in flavivirus diagnostics in co-endemic areas. The successful performance in a complex serum matrix further distinguishes this work, confirming its robustness and readiness for clinical investigation.

The primary contribution of this research is methodological, presenting a refined and effective model for developing high-performance point-of-care diagnostic devices. The value lies not in a single novel concept, but in the synergistic integration of established principles: nanostructured electrodes for signal amplification, stable thiol-gold surface chemistry for reliable bioreceptor immobilization, and a microfluidic platform for automated and reproducible sample processing. This study provides a comprehensive and validated workflow that bridges the gap between nanoscale sensor development and practical microfluidic device engineering, offering a robust template for creating sensitive and specific biosensors for other infectious diseases.

The principal limitation of this study is its reliance on recombinant antigen and spiked serum samples for validation, which, while essential for analytical characterization, may not fully replicate the complexities of authentic clinical specimens. The performance of the device against different DENV serotypes and in the presence of varying patient-specific factors like hematocrit levels or existing antibodies remains to be determined. Future research must therefore prioritize extensive clinical validation with a large cohort of patient samples to ascertain the device's real-world diagnostic accuracy. Subsequent work should also focus on integrating all components into a stand-alone, portable analyzer and assessing the long-term stability of the functionalized chips to ensure their viability for deployment in resource-limited settings.

AUTHOR CONTRIBUTIONS

Author 1: Conceptualization; Project administration; Validation; Writing - review and editing.

Author 2: Conceptualization; Data curation; In-vestigation.

Author 3: Data curation; Investigation.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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