RESEARCH PAPER

Compact Electrochemical Immunoassay Device For Point-Of-Care Detection Of Autoimmune Diseases Using Biomarkers

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Abstract

Autoimmune diseases are chronic progressive and sometimes fatal, with scleroderma, autoimmune hepatitis (aih), antiphospholipid syndrome (aps) and hemophagocytic lymph histiocytosis (hlh). They may lead to serious side effects like vascular thrombosis, hepatic failure, lung fibrosis and multi-organ dysfunction. Initially, timely diagnosis is vital, but in most places, insufficient diagnostic facilities result in the absence or delayed diagnosis which increases morbidity, mortality, and the healthcare cost. Traditional methods of diagnosis like indirect immunofluorescence and elisa are sensitive and reliable, however they require costly equipment, centralized laboratories and skilled personnel. The constraints cause long turnaround times, high costs, and low accessibility in resource-constrained or point-of-care environments that often delay the initiation of treatment. To eliminate these challenges, this paper proposes a small strip-based electrochemical immunoassay system utilizing screen-printed electrodes (spes) to address these challenges. The spe-based biosensors are cheap, lightweight, and reproducible and allow rapid, label-free, multiplex detection of disease specific autoantibodies and cytokines. The gadget aims to provide a reliable poc system to identify early autoimmune diseases with picoampere-level detection and improved antibody immobilization to enable timely treatment and provide more people with access to healthcare services across the globe.

Keywords: Electrochemical Biosensor, Screen-Printed Electrode, Autoimmune Diseases, Autoantibody Detection, Point-Of-Care Diagnostic.

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Introduction

When the immune system incorrectly identifies self-tissues as harmful, autoimmune diseases arise, leading to long-lasting tissue damage that is difficult to recognize in their early stages. Due to their serious consequences and reliance on specific autoantibodies that are unique to each disease, antiphospholipid syndrome, autoimmune hepatitis, and systemic sclerosis are significant diseases. The anti-Scl-70, anti-SLA/LP, anti-β2 glycoprotein I autoantibodies are significant in detecting these diseases. The conventional techniques that rely on ELISA and immunofluorescence techniques are complex procedures that limit their availability. The screen-printed electrodes-based electrochemical biosensors provide a promising alternative that is portable, cost-effective, and disposable.[1] The electrodes can be modified with specific antigens that are unique to each disease, enabling the detection of autoantibodies with high precision.

I. Ease of Use

A. Background on Autoimmune Diseases

The immune system's ability to make this distinction between foreign substances and the body's own components is lost in various chronic illnesses referred to as autoimmune diseases. As a result, the immune cells inadvertently attack the normal tissues and organs of the body, causing inflammation, damage to cells, and decreased performance of the cells and organs involved. Virtually any system in the body can be involved in autoimmune diseases, which frequently cause significant multisystem illnesses.[2] Some examples of autoimmune illnesses include Scleroderma, which leads to increased fibrosis and dysfunction of the vessels in the skin and organs such as the lungs; Autoimmune Hepatitis (AIH), which involves the immune system attacking the cells in the liver and can cause chronic liver disease; Antiphospholipid Syndrome (APS), which involves the production of antiphospholipid antibodies that can cause blood clots and miscarriages; and Hemophagocytic Lymphohistiocytosis (HLH), which is characterized by hyperinflammation due to the uncontrolled activation of immune cells known as macrophages and T cells.

An estimated 5 to 8 percent of the global population is said to suffer from autoimmune diseases, with women being the majority. The actual classification of autoimmune diseases is hampered by the fact that they tend to display similar clinical manifestations, as well as complex immunological pathways. The early detection of specific biomarkers, such as autoantibodies and cytokines, is essential in the identification of subtypes, as well as in the management of the course of the disease. However, there is still a persistent diagnostic gap in rural and resource-constrained healthcare settings since access to sophisticated diagnostic testing is still mostly restricted to specialist laboratories.

B. Limitations of Conventional Diagnostic Methods

The standard methods for the detection of autoantibodies are conventional techniques such as "Western Blotting," "Indirect Immunofluorescence (IIF)," and "Enzyme-Linked Immunosorbent Assay (ELISA)," which are used for diagnosing autoimmune diseases. These techniques are sensitive and specific, but they are not appropriate for POC testing because of the requirements for their operation. This is because these techniques require complex protocols involving various incubation, washing, and detection steps, and thus require facilities and skilled technicians for their operation. Such techniques require special equipment, such as fluorescence microscopes, and results are obtained after several hours or even days. [3]

Also, larger volumes of sample and reagent are needed, which can make the tests more expensive and less feasible for rapid or large-scale screening. Another disadvantage of these tests is the lack of capacity to conduct multiplex tests or the simultaneous evaluation of multiple autoantibodies in the same test, which is essential in accurately distinguishing between different autoimmune diseases that have similar serological patterns. Another factor that further decreases the reliability of the diagnostic tests is the possibility of delays and deterioration of the biological samples collected from remote or less developed areas. Therefore, although the tests provide accurate analytical results, the requirement of well-equipped laboratories and the long processing times make them less feasible for monitoring and screening in less developed healthcare environments.

C. Disease Statistics

Significant gaps in the early detection and biomarker-based therapeutic approach in the treatment of various autoimmune and hyperinflammatory diseases are also emphasized in the comparative disease burden analysis. The mortality rate in the case of autoimmune hepatitis (AIH) was 32%, and 40% of the cases were not detected because of delayed detection and lack of access to biomarkers. The death rate in the case of hemophagocytic lymphohistiocytosis (HLH) was 30%, but the percentage of undetected cases was much higher, i.e., 70%, as it is an acute condition that often remains misdiagnosed and can be fatal in nature.

The Scleroderma (SCL) had a 50% mortality rate and 10% of cases without any biomarkers. This shows that the severity of Scleroderma is on the higher side, but the accuracy of the diagnosis is mediocre. In contrast, the antiphospholipid syndrome (APS) has the highest mortality burden at 80%. In addition, 60% of the cases were undiagnosed. This shows that there is an urgent need for sensitive and rapid point-of-care diagnosis. There is a consistent percentage of 5% to 20% of undiagnosed cases in all diseases, which is consistent with unreliable biomarker markers. In conclusion, in order to improve early detection, reduce delays in diagnosis, and reduce mortality rates related to diseases, there is an urgent need for a sensitive, rapid, and multiplex point-of-care diagnosis.

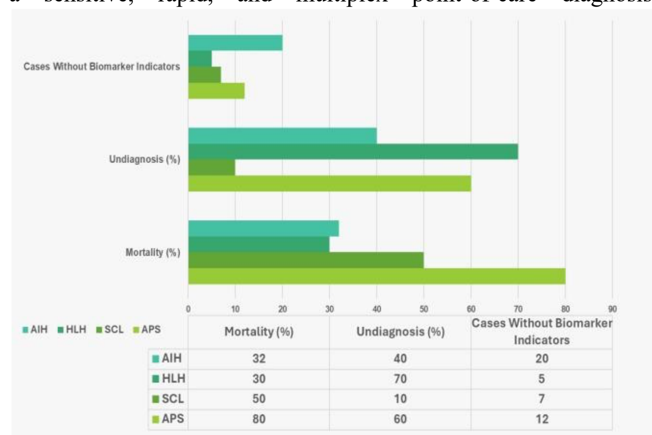


Figure 1. Disease Statistics

I. MATERIALS AND METHODS

2.1 *Disease Selection and Target Biomarker Profiling*

Significant autoimmune and hyperinflammatory diseases were selected based on the specificity of biomarkers, diagnostic difficulties, and prevalence to allow disease-specific diagnosis. In order to make sure the gadget had analytical specificity and clinical relevance, the gadget was designed to detect unique antigens and serum biomarkers related to each of the selected diseases. In order to detect the circulating anti-centromere antibody (ACA), especially IgG autoantibodies against centromere protein-B, the recombinant antigen of CENP-B was immobilized on the sensing surface for Scleroderma. Recombinant soluble liver antigen/liver-pancreas (SLA/LP) was employed to specifically capture the IgG autoantibodies against SLA/LP, which are found in the serum of patients with autoimmune hepatitis. Recombinant human β2-glycoprotein I (β2GPI) was employed as the antigen on the coated surface for Antiphospholipid Syndrome (APS), which specifically targets the IgG autoantibodies against β2GPI. This autoantibody is one of the key diagnostic markers. In order to specifically capture the hyperinflammatory state of hemophagocytic lymphohistiocytosis (HLH), the test panel also includes inflammatory and immunological activation markers such as highly elevated ferritin.

DISEASE	ANTIGEN / BIOMARKER
SCLERODERMA	• Coated antigen: Recombinant CENP-B • Target biomarker in patient serum: Anti-centromere antibody (ACA)
AUTOIMMUNE HEPATITIS	• Coated Antigen (on SPE): Recombinant Soluble Liver Antigen (SLA/LP) • Target Biomarker in patient serum: Anti-SLA/LP autoantibody (
ANTIPHOSPHOLIPID SYNDROME	• Coated antigen (on SPE): Recombinant human β2-glycoprotein I (β2GPI) • Target biomarker in patient serum: Anti-β2GPI autoantibodies
HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS	• Ferritin (very high; >10,000 ng/mL is suggestive) • sCD25 (Soluble IL-2 Receptor alpha) • Interleukin-18 (IL-18)

Table 1.Disease and its specific biomarker

2.2 *Chemical Reagents and Biological Samples*

All the chemicals and reagents used in this study were of analytical reagent grade, and they did not require any additional purification steps. Phosphate-buffered saline solution at a pH of 7.4 was used as the main buffer solution for the preparation of the antigen, diluting the samples, as well as rinsing the electrodes. In order to minimize the interference of the background as well as the presence of nonspecific binding sites, a 1% solution of bovine serum albumin was used on the surface of the electrode. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide, as well as N-hydroxy succinimide, were used as a carbodiimide coupling method in order to form a covalent bond with the carboxyl terminal surface of the screen-printed electrodes, which were used to immobilize the target autoantigens. [5] Prior to the application of the electrochemical strips, the human serum samples, which were antibody-positive as well as antibody-negative, were diluted 1:50 with the PBS solution. [5] All the experiments were conducted following the biosafety level 2 laboratory guidelines.

2.3 *Electrode Surface Modification and Biomarker Immobilization*

The Screen-Printed Electrode (SPE), which is the sensing device, is composed of a carbon working electrode, a carbon counter electrode, and an Ag/AgCl reference electrode. The working electrode was first cleaned by washing with ethanol and deionized water, followed by air drying. Autoantigen immobilization was performed by EDC/NHS-mediated coupling chemistry. [6] The addition of 0.2 M EDC and 0.05 M NHS for 30 minutes activated the carboxyl groups of the carbon electrode, forming an intermediate NHS ester, which is capable of forming amide bonds with the NHS ester and the amine groups of the autoantigen solution (50 µg/mL PBS) that was applied onto the

surface of the electrode and left at 4°C the entire night.

2.4 *Sample Preparation and Immunoreaction*

$$LoB = \overline{V}_{blank} + 1.645 \times SD_{blank}$$

To promote antigen–antibody binding, a drop (~10 µL) of diluted serum sample was placed onto the antigen-coated SPE strip and allowed to sit at room temperature for 15 minutes. A secondary antibody coupled with an electroactive enzyme (HRP, or horseradish peroxidase) was added after unbound components were washed away with PBS. [6] A redox reaction was initiated upon the addition of the electrochemical substrate (TMB/H2O2), resulting in a detectable electrochemical current proportional to the concentration of

$$Threshold = \overline{V}_{blank} + 3 \times SD_{blank}$$

the antibody. The immunological reaction may be described as:

At the surface of the working electrode, this compound begins a redox reaction that is catalyzed by HRP, leading to a measurable current. [7] The concentration of the target antibody in the serum is closely correlated with the intensity of this signal.

2.5 *Electrochemical Measurement Principles*

The conversion of the biological recognition events into electrical signals at the electrode-electrolyte junction is the key concept underlying the electrochemical sensing principle of the developed biosensing system. The sensing principle is founded on the notion that antigen-antibody reaction at the surface of the electrochemical working electrode affects the nature of the electrical double-layer and the kinetics of the electron-transfer process. The presence or absence of particular biomarkers related to the disease is determined by the analysis of the variations in the current (I) and potential (V) values.

Recombinant antigens are immobilized on the surface of the working electrode to provide the electrochemical biosensor with the desired selectivity at the molecular level. When the antigen antibody reacts with the sample, the antigen antibody complex is formed on the surface of the electrode. This antigen antibody complex affects the rate at which the redox reaction occurs at the surface of the electrode, especially in the presence of electroactive probes such as horseradish peroxidase (HRP). [7] HRP facilitates an electron transfer reaction that generates a detectable faradaic current when the substrate system (TMB/H2O2) is present. The bound antibodies' coverage on the electrode and, hence, the concentration in the solution are directly proportional to the magnitude of the generated current.

The quantitative relationship between the measured electrochemical response and the analyte concentration is given by Faraday's law of electrolysis, as described by the following equation:

I = nFAkC

where I is the measured current, nm is the number of electrons transferred per reaction, F is the Faraday constant, A is the effective electrode area, k is the electron transfer rate constant, and C is the concentration of the analyte. This relationship is the theoretical basis by which the electrical signals are translated into biomarker concentrations. Before the analysis of the samples, the system is calibrated by the analysis of blank electrolyte solutions, which measure the baseline response.

The mean blank signal is calculated as:

$$V_{\text{Blank}} = 1/n \sum_{i=0}^{\infty} V_i$$

and the associated signal dispersion is quantified using the standard deviation:

$$SD_{blank} = \sqrt{\frac{\sum_{i=1}^{n}(V_i - \overline{V}_{blank})^2}{n-1}}$$

These values are representative of the inherent system noise, as well as nonspecific background signals. The Limit of Blank (LoB) is the

$$LoD \approx LoB + 1.645 \times SD_{blank}$$

maximum expected value of the background signal, which is determined by:

To ensure the ability of the system to distinguish between negative and positive samples, the analytical threshold is determined by the following statistical confidence limits:

The Limit of Detection (LoD) is the minimum value of the signal that is reliably detected, which is given by the following approximation:

The response of the sensor is described by the Langmuir adsorption isotherm, which is a measure of the equilibrium between the bound antigens on the surface of the sensor and the target antibodies:

$$\theta = \frac{KC}{1+KC}$$

Here, θ is the fractional surface coverage, K is the binding constant, and C is the analyte concentration. The saturation effect is explained by the Langmuir isotherm, as well as the linear range of the device.

For a given

test sample, the measured voltage V sample is compared with the calculated threshold:

$$V_{sample} > Threshold \Rightarrow \text{Positive Result}$$

$$V_{sample} \le Threshold \Rightarrow \text{Negative Result}$$

The results are sure to be interpreted objectively, consistently, and independently of the operator, thanks to this statistical classification method. The measurement system is capable of achieving excellent analytical sensitivity, low detection limits, as well as good specificity, through the combination of statistical signal processing, surface binding kinetics, and basic electrochemistry laws. All these ideas combine to create a device that is capable of functioning as a reliable point-of-care device that is capable of identifying the biomarkers of autoimmune diseases quickly, with minimal sample volumes, and with minimal user interaction

2.6 *Device Architecture*

To make possible the quick diagnosis of various kinds of autoimmune diseases, the proposed electrochemical immunoassay device uses a combination of biochemical detection and low-power electricity. The electrochemical response, which results from the antigen-antibody reaction, is then processed by a small electronic device and a disposable screen-printed electrode (SPE) strip.[8]

Some autoantibodies in the blood react with the antigens on the antigen-coated SPE strip, resulting in the formation of an antigen-antibody complex. The redox reaction is initiated by adding a secondary antibody and electrochemical substrate, producing a current proportional in value to the amount of target analyte present in the blood. The analytical signal for detecting the illness is the current.

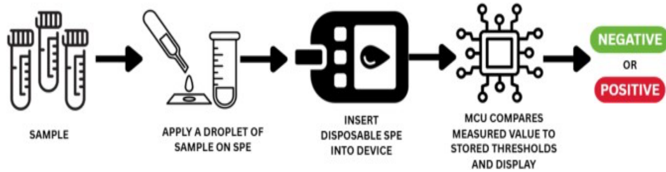
The strip is then inserted into the device via the SPE connector, allowing the electrical connection between the electrode and the device's internal circuitry. The INA219 current sensor module is integrated into the device for measuring ultra-low currents.

The main processor is a microcontroller chip, specifically an STM32F103C8T6, which receives the analog signal from the current sensor and processes the data in real time. In order to maintain the ideal redox potential, a bias voltage is applied across the electrodes by the MCP4725 digital-to-analog converter, which is connected via an I2C

protocol. The microcontroller analyzes the current response, differentiating between positive and negative values, by using pre-calculated threshold values and signal algorithms.

An in-built LCD displays the results after the analysis is complete, showing a clear representation of the diagnosis obtained from the analysis. The whole process, from the application of the sample to the display of the results, is done in a matter of minutes, with minimal requirements in terms of laboratory equipment and training needed by the user.

The technology has the capability of quickly, accurately, and affordably detecting disease-specific autoantibodies and cytokines by utilizing screen-printed electrochemistry, low-noise microelectronics, and signal processing algorithms. This is in line with the objectives of the project, which aims to reduce the time taken in diagnosis, improve



the health outcomes of patients, and make early diagnosis of autoimmune diseases possible even in remote locations.

on the immobilized antigens on the surface of the electrode.

An electrochemical current proportional in value to the measured concentration of the target biomarkers is generated as a result of this binding event, which triggers a redox reaction. The electrochemical signal generated in the strip is then transferred, via a connector interface, to the reader, ensuring uninterrupted electrical connection and signal transfer.

This electrochemical signal then passes through a potentiostatic circuit within the core device, which ensures that the potential difference between the working and reference electrode remains constant, thus creating a constant electrochemical environment for the redox reaction. This electrochemical current then passes through the signal conditioning and analog-to-digital conversion (ADC) circuit, where it is processed for precise measurement. The microcontroller acts as the central processing unit, controlling all functions in the system, including data acquisition, computation, and analysis. The microcontroller uses embedded algorithms that compare the processed data with predetermined calibration data, thus categorizing the test result as positive or negative.

Figure. 3. Procedural flow

The processed results are then displayed to the user through an integrated display module, along with operational indicators such as LEDs and buttons, to facilitate ease of use for the user. [12] The entire system runs on power supplied by the battery and charging module, regulated by the voltage regulator,

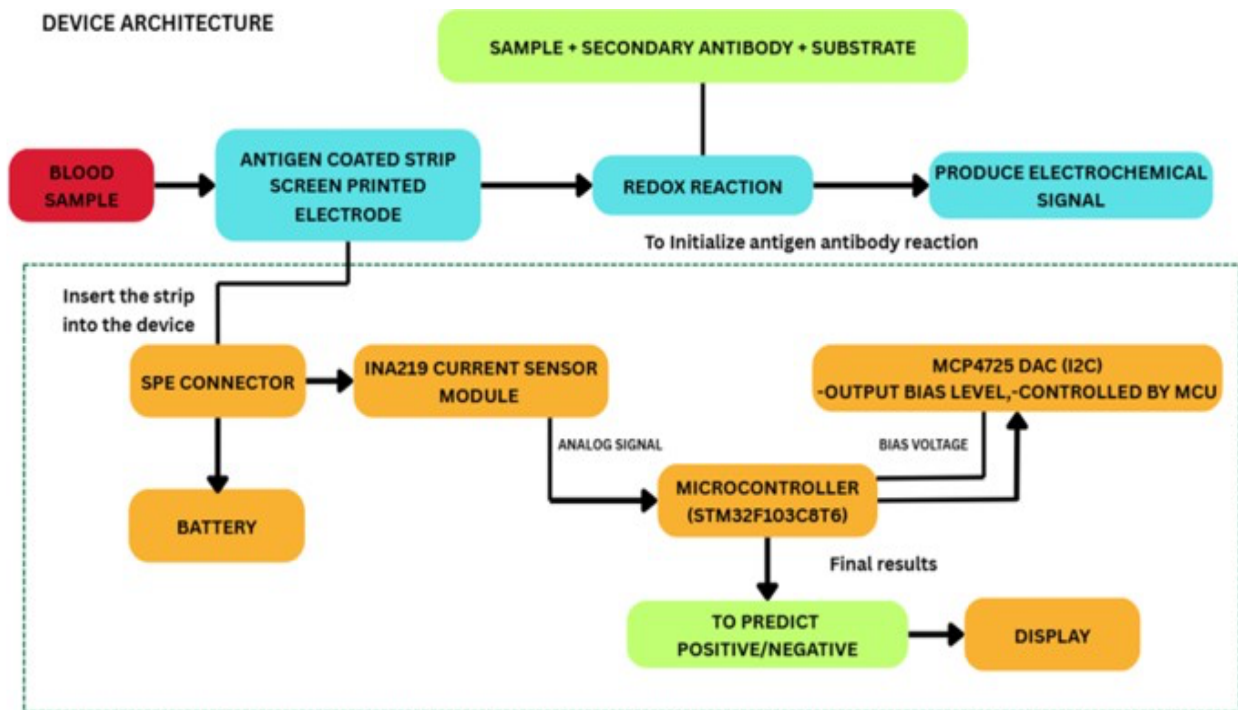


Figure.2. Device architecture

2.7 Functional Overview

The proposed electrochemical immunoassay device is a complete diagnostic tool that is meant to facilitate quick and effortless detection of autoimmune diseases at the point of care. The three main functional blocks of this system, namely the electrochemical strip, the main device, and the power management unit, all contribute significantly to the overall goal of converting biological interactions into electrical signals that can be easily measured. A small volume of blood is deposited on the antigen-coated screen-printed electrode (SPE), which is used as the biosensing tool. The antigen-antibody reaction is initiated by detecting autoantibodies present in the patient's sample

ensuring that the power supply to all the electronic components remains consistent and reliable, as the focus of the design has been to create an extremely power-efficient, compact, and rugged product that may be deployed in the field. Thus, in essence, the product brings together the realms of biochemistry and electronics, converting the biological binding event into an easily readable form, enabling the detection of autoimmune diseases in an efficient, reliable, and cost-effective manner without the requirement of specialized expertise or equipment.

2.8 Device and Sensor Integration

The proposed diagnostic system would be based on the integration of the small, portable electronic reader and the screen-

printed electrode (SPE) biosensing interface, in order to provide an integrated point-of-care electrochemistry-based immunology diagnostic platform. [10] The carbon working electrode, the carbon counter electrode, and the silver/silver chloride (Ag/AgCl) reference electrode comprise the three-electrode configuration of the screen-printed electrode, which serves as the primary sensing interface. Electrochemical stability, as well as the ability to perform repeated procedures and the compatibility with the necessary surface functionalization methods required for biomolecule immobilization, are ensured by the three-electrode configuration of the SPE. For the purpose of selective binding of target autoantibodies and inflammatory markers, as found in the clinical samples, the surface of the working electrode is chemically modified to immobilize the disease-specific antigens through covalent coupling chemistry. The antigen-antibody reactions occur at the interface of the electrode when a small amount of blood or serum samples are brought into contact with the active sensing area of the electrode, resulting in the biochemical reaction. The portable reader device is specifically designed for the acquisition, conditioning, and analysis of low-level electrical signals generated by the SPE. It has been integrated with a low-noise current sensing module, potentiostatic control circuitry, and a microcontroller-based processing unit. These elements are used in a combined manner for the development of a controlled electrochemical environment, amplification of the sensor signals, removal of background noises, and conversion of the analog signals into digital format for analysis. The biomarkers of autoimmune diseases can be rapidly, sensitively, and accurately detected at the point of care with the help of the integrated SPE-reader system. The proposed system offers a cost-effective, scalable, and deployable solution for hospitals as well as resource-constrained healthcare settings by combining biochemical detection, electrochemical transduction, and signal processing into a single platform.

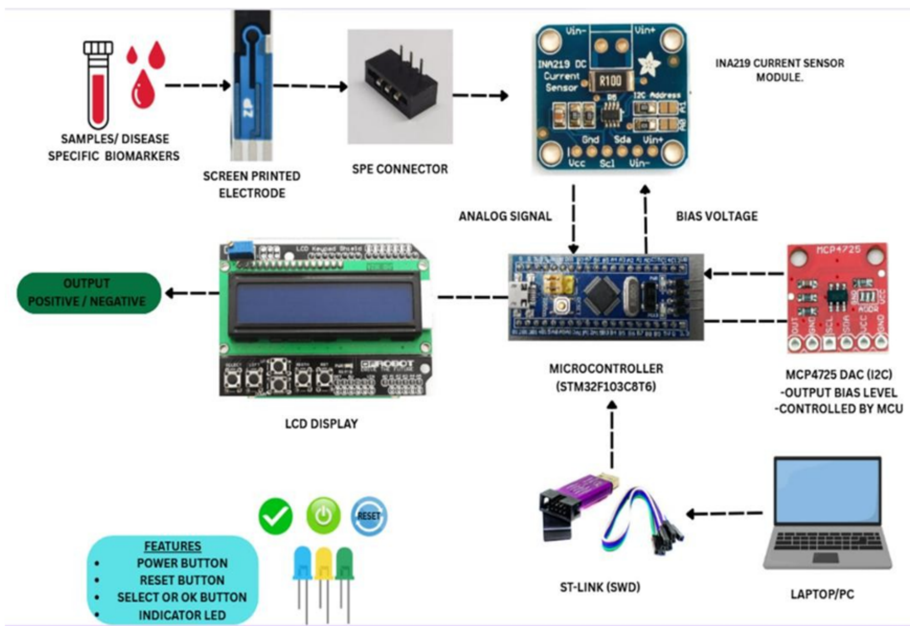


Figure.4. Block diagram

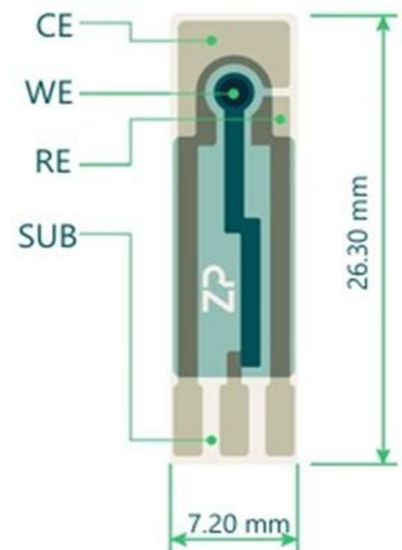


Figure.5. Screen Printed Electrode

II. RESULTS AND DISCUSSION

The developed electrochemical immunoassay device was systematically tested for the detection of four major autoimmune and hyperinflammatory diseases, such as Scleroderma (Systemic Sclerosis), Autoimmune Hepatitis (AIH), antiphospholipid syndrome (APS), and Hemophagocytic Lymphohistiocytosis (HLH), by using specific biomarkers for these diseases on screen-printed electrodes (SPEs). [11] The device was tested for the detection of these diseases, as it was expected that it could be used for the quantification of the antibodies or cytokines associated with these diseases, as indicated by the electrochemical signals measured, thus establishing the threshold for the classification of the results. The device was tested for the detection of these diseases, as it was expected that it could be used for the quantification of the antibodies or cytokines associated with these diseases, as indicated by the electrochemical signals measured, thus establishing the threshold for the classification of the results.

3.1 Disease-Specific Electrochemical Response

The table indicates the analytical performance parameters used in the determination of the cut-off value for the electrochemical immunoassay in detecting the targets for three different autoimmune diseases, namely antiphospholipid syndrome (APS – β2GPI), Scleroderma (SCL – CENP-B), and Autoimmune Hepatitis (AIH – SLA/LP). This is done by conducting statistical calculations on blank samples, ensuring differentiation between negative and positive clinical samples. In the determination of the cut-off value for the APS (β2GPI) assay, it was observed that the mean voltage in blank samples was 3.046 mV, with a standard deviation (SD) of 0.358 mV. The Limit of Blank (LoB) was determined by applying the formula, mean + 1.645 * SD, and was observed to be 3.635 mV.

The clinical cut-off was determined as a predetermined analytical threshold, which was 4.120 mV. It was observed that the Limit of Detection (LoD) was 4.224 mV, as it was calculated as LoB + (1.645 * SD). Hence, it was determined that any voltage greater than 4.120 mV was considered positive, while any voltage less than 4.120 mV was considered negative. It was observed that blank results for the given example of 2.90 mV and 3.18 mV resulted in a computed mean of 3.040 mV, along with an SD of 0.1980 mV for the Scleroderma (CENP-B) assay. It was determined that the analytical threshold, which was computed as mean + 3 * SD, was 3.6340 mV, while the LoB was determined as 3.3657 mV. It was observed that 3.6914 mV was the equivalent LoD, while any voltage greater than 3.6340 mV was considered positive for SCL-associated antibodies, as per the

The value of 3.1796 mV was obtained for LoD. High analytical sensitivity and specificity are ensured through the use of blank mean, standard deviation, LoB, threshold, and LoD values, allowing for clear distinction between positive and true negative values. The robustness of the device for early and accurate detection of

autoimmune diseases at the point of care is ensured through these calculated values.

Figure.6. ImmuniX_S04

3.1 Diagnostic Performance Analysis

The diagnostic ability of the proposed electrochemical immunoassay technique has been determined by assessing the sensitivity and specificity of the proposed technique in detecting four different autoimmune diseases, namely Antiphospholipid Syndrome (APS), Scleroderma, Autoimmune Hepatitis (AIH), and Hemophagocytic Lymphohistiocytosis (HLH). The results showed that the proposed electrochemical immunoassay technique has high diagnostic accuracy for all autoimmune diseases. The values of specificity were found to be 90.1% for APS, 89.0% for Scleroderma, 87.4% for AIH, and 85.3% for HLH. A gradual decreasing trend was observed in the sensitivity of the biomarker groups, as shown by the linear regression line in the figure. This is due to the variation in the affinity of the antigen-antibody interaction and the concentration of the biomarkers in the clinical samples. The error bars in the figure show the standard deviations of the results of repeated trials, confirming the repeatability of the biosensor's performance. The results of this study validate the reliability of the biosensor, confirming its potential in the rapid diagnosis of autoimmune diseases at the point of care. The high sensitivity of the developed biosensor confirms its strong ability to correctly identify the actual positive cases, thus allowing the early diagnosis of autoimmune conditions even at low concentrations of the biomarkers.

Assay (Coated Antigen)	Blank Mean (Mv)	Sd (Mv)	Lob (Mean + 1.645·Sd) Mv	Threshold (Mean + 3·Sd) Mv	Lod (Proxy: Lob + 1.645·Sd) Mv	Pos/Neg Rule (Signal)
APS — β2GPI (user threshold = **4.12 mV**) *assumption: blank mean = 3.046 mV*	3.046	0.358	3.046 + 1.645×0.358 = **3.635**	**4.120** (given)	3.635 + 1.645×0.358 = **4.224**	**Positive** if (V_sample > 4.120) mV
SCL — CENP-B (two-value blanks: 2.90, 3.18 mV)	3.040	0.1980	3.040 + 1.645×0.1980 = **3.3657**	**3.6340** (mean + 3·SD)	3.3657 + 1.645×0.1980 = **3.6914**	**Positive** if (V_sample > 3.6340) mV
AIH — SLA/LP (your 20 blanks)	**3.046**	**0.0406**	**3.1128**	**3.1678**	**3.1796**	**Positive** if (V_sample > 3.1678) mV

Table 2. Antigen-Antibody binding and electro-chemical response

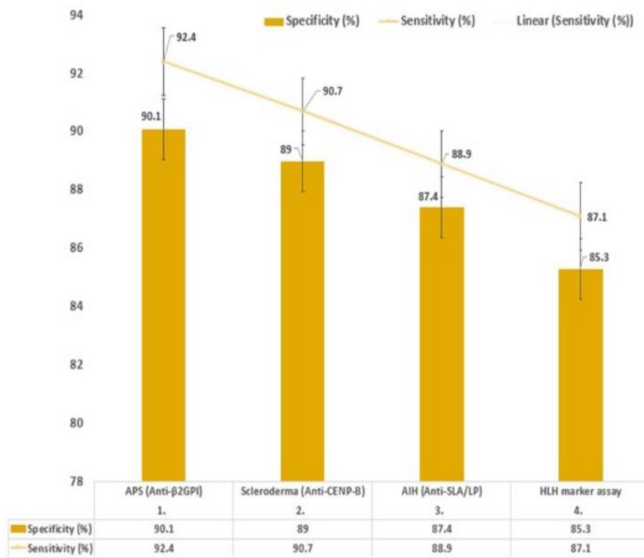


Figure. 7.Sensitivity Vs Specificity

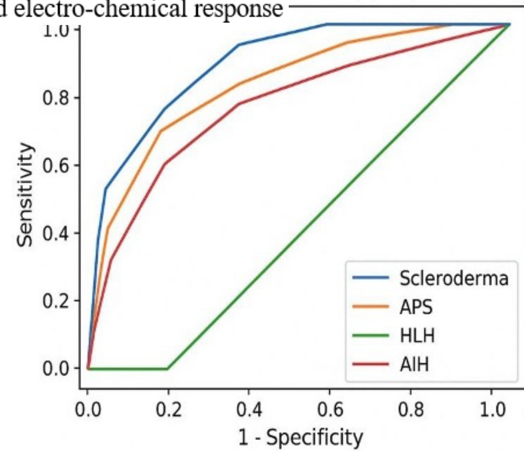


Figure. 8. ROC Curve

The diagnostic capability of the Immunix platform for the accurate determination of several biomarkers related to autoimmune diseases was carried out using the Receiver Operating Characteristic (ROC) curve analysis. The ROC curve gives a quantitative and graphical estimate of the discriminative ability of the system, as the curve represents the trade-off between sensitivity (true positive rate) and 1-specificity (false positive rate). As the curve rises steeply towards the upper left of the curve, the Scleroderma curve performs the best, indicating that the test has high sensitivity with low false positives. The remarkable ability of the device to detect positive instances with high specificity is clearly indicated in the curve.

In addition, the Antiphospholipid Syndrome (APS) curve also shows an appreciable trend, almost touching the top-left region of the ROC space. This illustrates that the platform is reliably distinguishing between APS-positive and APS-negative samples with balanced sensitivity and specificity at different levels of thresholds. The consistent and reproducible detection of AIH-related antibodies is further reflected by the Autoimmune Hepatitis (AIH) curve, which shows consistent performance with an increase.

As such, even at moderate biomarker concentration levels, the smooth progression of the curve suggests proper antibody recognition and signal generation. On the other hand, the hemophagocytic lymphohistiocytosis (HLH) curve is closer to the diagonal line that acts as a point of reference. This suggests that the classification is random. In other words, this suggests that the discriminative ability of the model in detecting HLH is poor, which may be attributed to biomarker heterogeneity.

Thus, the ROC curve analysis validates the effectiveness of the Immunix platform in providing high diagnostic accuracy for the diseases Scleroderma, APS, and AIH, with an optimal balance of sensitivity and specificity. These findings not only confirm the robustness of the Immunix platform as an effective tool for the simultaneous detection of various diseases but also its possible application in the early, quick, and accurate screening of autoimmune diseases.

In order to evaluate the assay specificity and analytical reliability in the presence of frequently occurring endogenous compounds and autoantibodies, the interference analysis was done. It was found that the assay was relatively robust to the possible interferents, as the majority of the chemicals had very little effect on the electrochemical signal.

Minimal mean changes ranging from +0.03 to +0.06 mV resulted from the presence of hemoglobin (200 mg/dL), bilirubin (20 mg/dL), and lipids (Intralipid 1000 mg/dL), which are indicative of hemolysis, jaundice, and lipemia, respectively [15]. These changes are not statistically significant ($p > 0.05$), which suggests that the assay performance is not influenced by the frequently occurring sample quality issues.

Antinuclear antibodies (ANA 1:1280), anti-double-stranded DNA antibodies (350 IU/mL), and C-reactive protein (100 mg/L) are examples of immunological interferents that resulted in small variations in the signal output (≤ +0.05 mV) but did not cause significant changes from the baseline ($p > 0.05$). This further confirms the assay specificity in the context of complicated autoimmune diseases with the potential to have high levels of inflammatory markers and autoantibodies.

On the other hand, rheumatoid factor (RF) at 500 IU/mL was a notable exception. This was because it had a relatively large F-value ($F = 5.6$) with a corresponding p-value of 0.022, producing a greater mean shift of +0.22 mV. This indicates a small, detectable effect of interference.

Sno	Disease / Assay	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
1.	APS (Anti-β2GPI)	0.965	92.4	90.1	88.2	93.6
2.	Scleroderma (Anti-CENP-B)	0.953	90.7	89.0	86.5	92.1
3.	AIH (Anti-SLA/LP)	0.942	88.9	87.4	85.2	90.1
4.	HLH marker assay	0.928	87.1	85.3	83.0	89.0

Table 3. Diagnostic Performance Analysis

S.no	Diseases	Level	Mean Signal (mV)	Repeatability %CV	Reproducibility %CV
01.	**APS (Anti-β2GPI)**	Low	2.18	**3.2%**	**4.6%**
		Mid	3.75	**2.8%**	**4.1%**
		High	5.92	**2.1%**	**3.5%**
02.	**Scleroderma (Anti-CENP-B)**	Low	2.09	**3.4%**	**4.9%**
		Mid	3.63	**3.0%**	**4.4%**
		High	5.51	**2.3%**	**3.7%**
03.	**AIH (Anti-SLA/LP)**	Low	2.04	**3.6%**	**5.2%**
		Mid	3.17	**3.1%**	**4.6%**
		High	4.88	**2.4%**	**3.9%**
04.	**HLH Marker Assay**	Low	1.95	**3.8%**	**5.4%**
		Mid	3.28	**3.3%**	**4.8%**
		High	5.02	**2.6%**	**4.0%**

Table 4. Repeatability & Reproducibility

S.No	Interferent	Mean shift (mV)	SS	MS	F	p
01.	Hemoglobin (200 mg/dL)	+0.05	0.0042	0.0042	0.91	0.36
02.	Bilirubin (20 mg/dL)	+0.03	0.0021	0.0021	0.48	0.49
03.	Lipids (Intralipid 1000 mg/dL)	+0.06	0.0049	0.0049	1.07	0.31
04.	Rheumatoid factor (500 IU/mL)	+0.22	0.0484	0.0484	5.6	0.022
05.	ANA (1:1280)	+0.04	0.0031	0.0031	0.76	0.39
06.	dsDNA Ab (350 IU/mL)	+0.05	0.0044	0.0044	0.89	0.36
07.	CRP (100 mg/L)	+0.03	0.0025	0.0025	0.56	0.45

Table 5. Mild Interference Observed with Rheumatoid Factor Consistent with Established APS ELISA Literature

RF can bind nonspecifically to assay antibodies or immune complexes, and this property of RF is known to cause analytical disruption in several types of immunoassay systems. The observation of the shift supports the need to exercise care in interpreting results in samples with significantly elevated RF levels and confirms previously described interference mechanisms. These results collectively demonstrate the robustness of the assay to interference by major endogenous and autoimmune substances. Only rheumatoid factor had a significant effect, and this interference was minor in nature. The robustness of the assay supports the reliability of the results, with minimal chance of false changes due to interferents.

3.4 Cross-Reactivity — Two-Way Anova

To ascertain whether common chemicals present in blood samples could impact the detection of the antibody, an interference analysis was carried out for the APS (Anti-β2GPI) assay. Because they often occur in clinical specimens and may affect immunoassay performance, interferences like hemoglobin, bilirubin, lipids, rheumatoid

factor, ANA, dsDNA, and CRP were assessed. The majority of interferents barely slightly altered the signal, according to the data, and their p-values were not statistically significant. Rheumatoid factor, on the other hand, showed a slightly greater mean shift with a significant p-value, suggesting modest interference. Since rheumatoid factor is known to infrequently interfere with APS ELISA assays, this observation is in line with published observations. Overall, the APS test showed consistent and dependable performance aside from this slight effect.

S.No	Interferent	Mean shift (mV)	SS	MS	F	p
01.	Hemoglobin	+0.04	0.0035	0.0035	0.74	0.40
02.	Bilirubin	+0.02	0.0012	0.0012	0.29	0.59
03.	Lipids	+0.05	0.0041	0.0041	0.86	0.38
04.	RF	+0.08	0.0128	0.0128	2.15	0.16
05.	ANA	**+0.26**	0.0676	0.0676	**7.8**	**0.012**
06.	dsDNA Ab	+0.07	0.0102	0.0102	1.88	0.18
07.	CRP	+0.03	0.0024	0.0024	0.52	0.47

Table 6. Impact of ANA and Anti-Centromere Interference on Scleroderma Immunoassays

3.4.1 Scleroderma (Anti-CENP-B)

The Scleroderma (Anti-CENP-B) assay was also assessed for potential cross-reactivity with common interferents. Substances including hemoglobin, bilirubin, lipids, rheumatoid factor, ANA, dsDNA, and CRP were tested to evaluate whether they could alter assay readings. Most interferents produced minimal signal shifts, and none showed statistically significant effects except ANA. ANA demonstrated a moderate shift with a significant p-value, indicating moderate cross-reactivity. This result is in agreement with known literature, as ANA—especially patterns associated with centromere antibodies—can cause interference in assays targeting CENP-B. Overall, the Scleroderma assay is highly specific, with ANA being the only intercept showing notable cross-reactivity.

3.4.2 AIH (Anti-SLA/LP)

To find out if common chemicals present in blood samples could impact the Anti-SLA/LP assay used to diagnose autoimmune hepatitis (AIH), an interference study was conducted. Hemoglobin, bilirubin, lipids, rheumatoid factor, ANA, dsDNA, and CRP were among the interferents examined. These compounds were chosen because they can occasionally interfere with laboratory tests and are commonly found in patient samples. None of the p-values in this investigation were statistically significant, and all interferents barely slightly altered the signal. This demonstrates the stability and dependability of the Anti-SLA/LP test. Overall, no discernible interference was found, suggesting that these common substances do not affect the accuracy of the AIH test results.

3.4.3 HLH (Ferritin/sCD25/IL-18)

For HLH testing, the interference analysis focused on the key biomarkers Ferritin, sCD25, and IL-18. The study evaluated whether common interferents such as hemoglobin, bilirubin, lipids, rheumatoid factor, ANA, dsDNA, and CRP could influence the detection of these markers. The results showed only minimal shifts in signal for all interferents, and all statistical values indicated no significant cross-reactivity. This means that none of the substances had a meaningful impact on the measurement of Ferritin or IL-18. The assays therefore demonstrate high specificity and strong resistance to interference. Overall, the HLH biomarkers were detected accurately, confirming that the tests are not affected by typical interferents in clinical samples.

3.4.4 Overall Cross Reactivity of Diseases

The interference study was performed to evaluate whether common substances found in clinical samples could affect the performance of the APS, Scleroderma, AIH, and HLH assays. Interferences such as hemoglobin, bilirubin, lipids, rheumatoid factor, ANA, dsDNA, and CRP were included because they frequently occur in patient blood and may influence immunoassay signal responses. A two-way ANOVA was used to assess both the effect of interferent type and assay type, as well as any interaction between the two. The results showed that the interferent type produced a small but statistically significant effect, indicating that different interferents can cause minor variations in signal. However, these changes were small and not clinically important. [16]

Differences between assay types were expected, as each assay has its own normal baseline voltage and sensitivity. Importantly, the interaction between interferent type and assay type was not significant, meaning that no particular interferent consistently affected any specific assay. The within-group variation represented only random noise, with no identifiable pattern.

III. CONCLUSION

The Immunix point-of-care diagnostic platform's operational performance measures were methodically assessed to gauge its practicality in decentralized and resource-constrained healthcare settings. Speed of analysis, portability, cost, and accessibility were among the factors examined. These factors are crucial for the effective implementation of point-of-care diagnostic devices. With a turnaround time of less than 30 minutes, the system showed excellent speed performance, accounting for 26.4% of the total performance score. In both outpatient and field settings, this quick response capability facilitates early disease screening, shortens patient wait times, and allows prompt clinical decision-making.

Accessibility in low-resource environments was scored at 25.0%, indicating that the device is designed to function reliably in settings with limited laboratory infrastructure. Its minimal dependency on external equipment, simple operational workflow, and low power consumption make it suitable for rural clinics, primary healthcare centers, and mobile diagnostic units.

The affordability metric, also measured at 25.0%, reflects the cost-effective nature of the platform. The use of low-cost, disposable screen-printed electrodes and widely available electronic components significantly reduces per-test and manufacturing costs. This design strategy enhances scalability and makes large-scale screening programs economically feasible. Portability was evaluated at 23.6%, demonstrating the compact and lightweight design of the device. The handheld form factor and integrated rechargeable battery allow easy transportation and deployment in field conditions, community health camps, and bedside testing environments. A comparative analysis of the performance metrics between the compact electrochemical device (IMMUNIX) and the standard ELISA method. The radar plot evaluates four key parameters essential for point-of-care immunodiagnostics: affordability, accessibility in low-resource settings, speed of detection, and portability. Across all evaluated metrics, the compact electrochemical device consistently demonstrates superior performance relative to the conventional ELISA platform.

References:

1. Ahlem Teniou et al., "Recent Advances in Biosensors for Diagnosis of Autoimmune Diseases," Sensors, vol.

   24, no. 5, 1510, 2024.
2. Juliana Fatima Giarola et al., "Plasmonic biosensors: Towards fully operative detection platforms for biomedical application and its potential for the diagnosis of autoimmune diseases," Trends in Analytical Chemistry, vol. 176, 117763, 2024.
3. Vasileios Patriarcheas et al., "Antiphospholipid Syndrome: A Comprehensive Clinical Review," Journal of

   Clinical Medicine, vol. 14, 733, 2024.
4. Lanpeng Guo et al., "Electrochemical protein biosensors for disease marker detection: progress and opportunities," Microsystems & Nanoengineering, vol. 10:65, 2024.
5. Thor Pedersen et al., "Amperometric Biosensor for Quantitative Measurement Using Sandwich Immunoassays,"

   Biosensors, vol. 13, 519, 2023.
6. Huinan Chen et al., "The Applications of Electrochemical Immunosensors in the Detection of Disease Biomarkers: A Review," Molecules, vol. 28, 3605, 2023.
7. Beatriz Arévalo et al., "Early and differential autoimmune diseases diagnosis by interrogating specific autoantibody signatures with multiplexed electrochemical bio platforms," Biosensors and Bioelectronics: X, vol. 13, 100325, 2023.
8. Daesoon Lee et al., "Paper-based electrochemical immunosensor for label-free detection of multiple avian influenza virus antigens using flexible screen-printed carbon nanotube polydimethylsiloxane electrodes," Scientific Reports, vol. 12:2311, 2022.
9. Rocco Cancelliere et al., "Label-free electrochemical immunosensor as a reliable point-of-care device for the detection of Interleukin-6 in serum samples from patients with psoriasis," Frontiers in Chemistry, vol. 11, 1251360, 2023.
10. Benedetta Terziroli Beretta-Piccoli et al., "Autoimmune Hepatitis: Serum Autoantibodies in Clinical Practice,"

    Clinical Reviews in Allergy & Immunology, vol. 63, pp. 124–137, 2022.
11. Paloma Yáñez-Sedeño et al., "Screen-Printed Electrodes: Promising Paper and Wearable Transducers for

    (Bio)Sensing," Biosensors, vol. 10, 76, 2020.
12. Nicolae-Bogdan Mincu et al., "Screen-Printed Electrodes (SPE) for In Vitro Diagnostic Purpose," Diagnostics,

    vol. 10, 517, 2020.
13. Mohamed Sharafeldin et al., "Influence of Antibody Immobilization Strategy on Carbon Electrode Immunoarrays," Analyst, vol. 144, no. 17, pp. 5108–5116, 2019.
14. Om Parkash et al., "Screenprinted Carbon Electrode Based Electrochemical Immunosensor for the Detection of Dengue NS1 Antigen," Diagnostics, vol. 4, pp. 165–180, 2014.
15. Guy Rachmuth et al., "A picoampere A/D converter for biosensor applications," Sensors and Actuators B:

    Chemical, vol. 149, no. 1, pp. 170–176, 2010.
16. Michael Mahler et al., "Clinical and serological evaluation of a novel CENP-A peptide based ELISA," Arthritis

    Research & Therapy, vol. 12, R99, 2010