An Overview of Immunosensors and Their Application

Anil Kumar Gupta, Sambhavi Animesh, and Amit Singh

Abstract

A key challenge in clinical healthcare is meeting the need to detect a disease at an early stage. Early and accurate diagnosis not only cuts the treatment cost but can also reduce disease burden, mortality rate, and social inequalities. Therefore, researchers are always searching for a method that allows rapid, simple, sensitive, selective, and cost-effective detection of the target biomarker (peptides, proteins, or nucleic acid). Immunosensors are one such point-of-care diagnostic device that can play an important role in almost all clinical healthcare fields. They are a promising alternative to the traditional immunoassays and state-of-the-art affinity sensors to diagnose clinically important analytes/antigens due to their high affinity, versatility, compact size, fast response time, minimum sample processing, and the measurements' reproducibility. For many decades now, significant advancement has been made in the immunosensor field in which the use of nanomaterials for increased sensitivity, multiplexing, or microfluidic-based devices may have the potential for promising use in clinical analysis. This chapter will provide an overview of the currently available immunosensor technology, its

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types that are currently being developed, and the limitations and future directions of immunosensor technology for the clinical laboratory.

Keywords

Immunosensor · Immobilization · Antigen · Antibody · Communicable and noncommunicable disease

1 Introduction

Biological and biochemical processes are paramount in clinical diagnostics, medical applications, bioreactors, food quality control, agriculture, industrial wastewater, mining, and the military defense industry. However, conversion of the biological data directly to an electrical signal is challenging. The application of biosensors has increased significantly due to improved procedures and gadgets, especially in the conversion of biological signals to electrical signals. Nowadays, numerous biosensors have been produced industrially and are being utilized to develop large-scale multi-valued sensing systems. The first biosensor was developed by Clark and Lyons [1] to quantify glucose level in clinical samples using electrochemical detection of oxygen molecules via glucose oxidase electrode. From that point forward, remarkable advancement has been made both in innovation and the uses of biosensors with inventive methodologies including electrochemistry, nanotechnology to bioelectronics [2, 3]. The bioelement (antibody, aptamer, etc.) immobilized on the transducer's surface binds to the target molecule and passes signals to the transducer. The superiority of biosensing elements in the generation of real-time signals is incredible. They can detect the target molecules even in the picogram quantities and are therefore considered a powerful tool to detect pathogens at their initial infection phase [4]. This unique feature of biosensors has motivated researchers to develop new biosensing technologies continuously and the industry is now worth billions of dollars [4, 5].

Immunosensors are one of the most important biosensors classes, widely accepted as an analytical instrument, especially in the healthcare section due to their excellent detection efficiency and accuracy. The latest advances in immunosensor make it possible to combine detection with the current digital technology and miniaturize them without compromising the performance [6]. This book chapter will provide an overview of the immunosensor technology currently available, its types that are currently being developed, and also address the limitations, challenges, and future directions of immunosensor technology for the clinical laboratory.

1.1 Biosensors

The biosensor is a self-contained analytical device, combined with a biological element (biosensing components) and a physicochemical component (transducer component) [4] (Fig. 1). It detects changes during the biological process and converts them to an electric signal. Typically, a biosensor consists of three basic components as follows:

1. Detector (detect the biomolecule and generate impetus).
2. Transducer (convert the impetus to output signal).
3. Signal processing system (Process the output and present it in a user-friendly form).

1.2 Classification of Biosensor

The biosensors can be categorized on the basis of physicochemical transduction action or the type of biorecognition element. It can be classified as electrochemical, mechanical, and optical biosensors according to transducers used. Further, electrochemical biosensors can be reclassified as amperometric biosensors— measure current produced during oxidation/reduction of reactant, potentiometric biosensors— measure the potential of the biosensor electrode with respect to a reference electrode, and conductometric biosensors— measure the change in conductance arising due to the biochemical reaction [7]. An overview of biosensor classification is shown in Fig. 2.

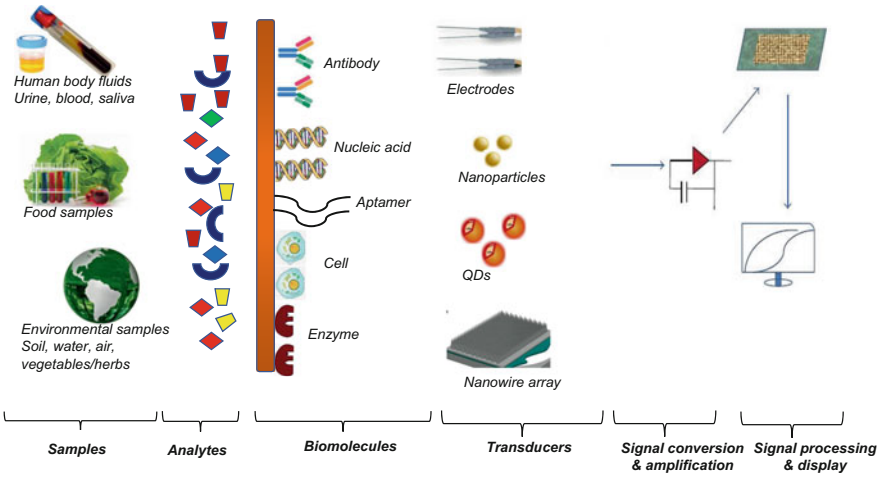

Fig. 1 Schematic diagram showing the components of a biosensor

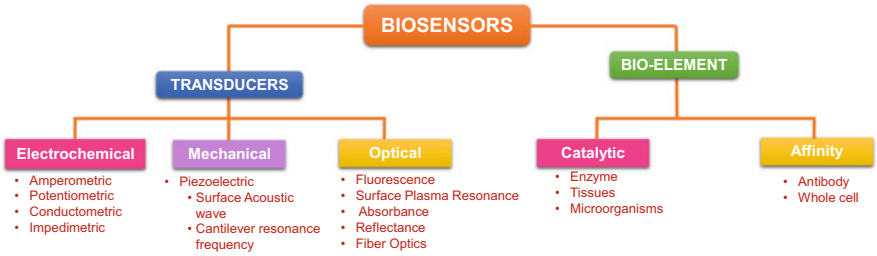

Fig. 2 Classification of biosensors

1.3 Immunosensor

The term "Immunosensor" is specifically employed to designate the entire instruments, i.e., immunoreaction-based biosensors. Immunosensors are solid-state devices in which the signals of immunochemical reaction are captured by a transducer. In the immunosensor design, the sensing component is formed by the immobilization of antigens/antibodies, and the binding events are being transformed into a measurable signal by the transducer [8].

1.4 Principles of Immunosensor

The immune system is a complex network of cells and proteins, which guards the body against infection. It keeps a record of every infectious agent (microbes) defeated once to recognize and destroy the microbe quickly; if it enters the body again. In the presence of foreign substances (*i.e.,* antigens), cells of the immune system produce specialized immunoglobulins (*i.e.,* antibodies) that bind specifically to these antigens. This phenomenon has many applications, including the development of sensors. "*A sensor that is based on the concept of immunology uses an antibody (as a bioreceptor) for specific molecular recognition of antigens and subsequently forms a stable immunocomplex are known as an immunosensor.*" An immunocomplex formation is determined and measured by coupling this reaction (signals) to a transducer's surface. The electrical device detects the signals and converts it to an electrical signal where it is processed, recorded, and viewed (Fig. 3). The produced analytical signals are directly proportional to analytes' concentrations [6, 9].

At the time of immunochemical reaction, the highly specific recognition of an antibody's variable regions with the epitopes of an antigen occurs via different types of bonding such as hydrophobic and electrostatic interactions, van der Waals force, and hydrogen bonding. The produced antigen–antibody complex is generally reversible due to a weak force holding the antibody and antigen together. The antigen–antibody complex formed would dissociate with a slight change in the reaction environment (e.g., pH or ion strength). The strength of an antibody binding to an antigen is generally characterized by its affinity constant (K). The high affinity and

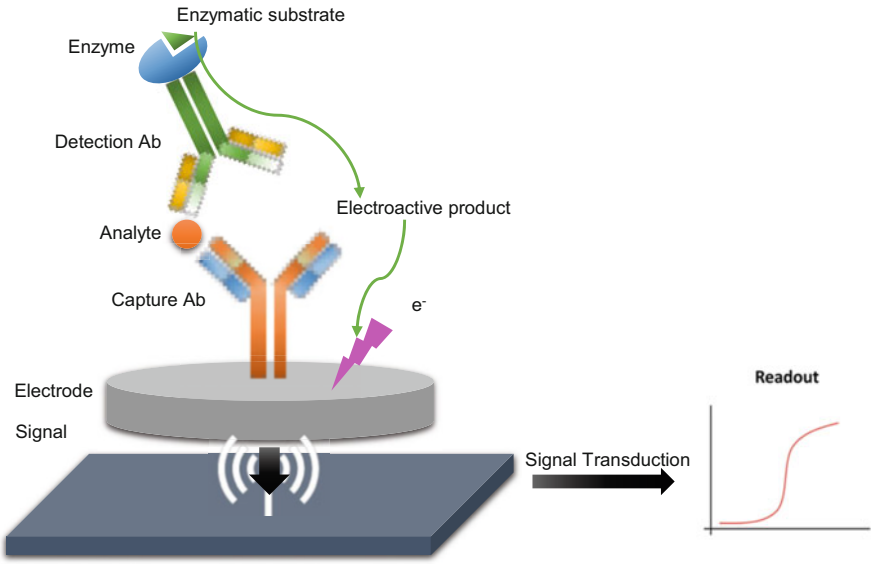

Fig. 3 A systemic diagram of immunosensor

specificity of this antigen–antibody complex determine the key feature of the immunosensor [6, 10]. An ideal immunosensor should be designed with the following specifications:

1. Should identify target antigens very quickly.
2. Should be able to generate antigen–antibody complexes without the requirement of supplementary reagents.
3. Should be able to produce results with high accuracy and reproducibility.
4. Able to detect the target in real samples easily.

1.5 Structure of Immunosensor

The immunosensor is mainly composed of three elements: bioreceptors, transducers, and electronics. The structure of the immunosensor is depicted in Fig. 3.

- Bioreceptors are biological recognition elements (antibody, enzyme, enzyme-substrate, aptamers, haptens, or nucleic acid) capable of detecting a particular target analyte such as enzyme-substrate, complementary DNA, antigen, or ligands. These elements are either integrated insight or intimately related to a physicochemical transducer.
- The second and most critical component of the immunosensor is the transducer, used to convert biochemical signals produced by the analyte's interaction with the receptor into an electrical signal. The intensity of the signals produced via biochemical reaction is directly or inversely proportional to the concentration of

the analyte. Electrochemical transducers are most commonly used to develop immunosensors. These systems provide some advantages, i.e., simple and unique design, low cost, and compact size [6].

- The electronic part is considered as the third component of the immunosensor, which is used to amplify and digitalize the physicochemical output signals from the transducer devices such as "electrochemical (potentiometric, conductometric, capacitive, impedance, amperometric), optical (fluorescence, luminescence, refractive index), and microgravimetric devices" [11, 12].

1.6 Why Immunosensor Is a Better Choice Than Other Sensors?

Since the discovery of biosensor in the early 1950s, it has become very important tools in the fields of agriculture, industrial processing, food processing, pollution control, and environmental monitoring. The immunosensors have certain advantages that make them superior to other states of the art sensors due to their compact size, low cost, quick response time, higher sensitivity, and selectivity [13]. Further, they offer easy-to-use and easy-to-automate, digitize, and miniaturize. They may bypass some inherent problems of traditional analytical methods [14]. Therefore, immunosensor is increasing focus of researchers in immunochemical studies due to their immense clinical diagnosis potential [15, 16], environmental analysis [17], and monitoring of the biological process. A great deal has been achieved in diagnosing certain diseases by measuring markers or pathogenic microorganisms responsible for the illnesses such as proteins, enzymes (glucose oxidase), microorganisms (HIV, Toxoplasmosis, Syphilis, Leishmania, Malaria), and hormones (TSH, LH) using fast and responsive immunosensor. For example, an amperometric immunosensor was recently developed, which detects *Trypanosoma cruzi* (*T. cruzi*)-specific antibodies in patient blood samples and tracks the anti-*T. cruzi* antibody decay during the treatment of chagasic patients [18, 19]. The applications of the immunosensors have been discussed in the latter part of this chapter.

2 Immunosensing Elements

An immunosensing element (biorecognition element) comprises a molecular probe to detect the target/analyte present in the samples. It is the most critical part of the immunosensor as each biorecognition element has its advantages and disadvantages that determine the overall performance of the immunosensor. The analyte specificity is mainly dependent on the selectivity and robust affinity between the biorecognition element and the target analyte [12, 20]. "A sensor based on the concept of immunology where the antibody is used as a bioreceptor for the specific molecular recognition of antigens and subsequently forms a stable immunocomplex are known as Immunosensor." The most prominent biorecognition elements used in the development of immunosensors are antibodies (Ab), antigens, and aptamers.

2.1 Antibodies

Antibodies are naturally occurring proteins of approximately ~150 kDa in size. Typically, an antibody consists of a light chain and a heavy chain which are linked by disulfide bonds to form the characteristic Y-shape. As shown in Fig. 2, the ends of each arm carry two identical variable regions (Fv) of the antibody for the recognition of the antigens, whereas the rod-like part containing constant sequences (Fc) is essential for the physiological functions of antibodies. (Fig. 4). The variable region encompasses three hypervariable areas, known as complementarity-determining regions (CDRs), responsible for the specific antibody–antigen interaction. This region encodes a unique recognition pattern which binds with the analyte with very high accuracy and affinity; therefore, they are used as a biosensing element. The diversity in CDRs allows the endless supply of Abs with different specificity and binding strength (affinity). Of the many immunoglobulins classes (i.e., IgE, IgM, IgG, etc.), the immunoglobulin G is the most prominently used class in the biosensing field [21].

The antibodies bind with its target with variable stringency which mainly depends on whether the antibodies are monoclonal or polyclonal. Monoclonal antibodies are highly specific recognizing only one epitope of a target molecule. On the contrary polyclonal antibodies can recognize totally diverse epitopes of the identical target. Because monoclonal antibodies are specific to a single epitope, they are less prone to cross-reactivity than polyclonals; however, polyclonals' cost is more economical, so polyclonals are still used. Along with the whole antibody, single-chain Fv fragments (scFv) and antibody fragment-antigen binding (Fab') units are widely used in the development of Immunosensors. An scFv is a fusion protein consisting of a variable region of heavy (V_H) and light (L_H) chains joined together by a flexible peptide linker of 10–25 amino acids [22, 23]. The Fab' is a region on an antibody consisting of one constant and one variable domain of each heavy and the light chain. It is mainly responsible for binding to the antigens [24]. Although antibodies have won enormous popularity, they still suffer from certain limitations. The antibodies are

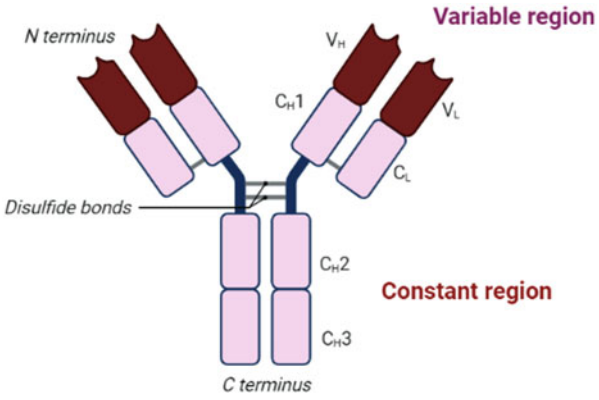

Fig. 4 Structure of immunoglobulin (IgG)

produced in animals, which is costly and time-consuming, limiting new antibodies' discovery.

Further, once an antibody is discovered, the isolation and purification procedures can be expensive. As antibodies are proteinous, they are highly sensitive to pH, ionic strength, and temperature, affecting their activity. The antibodies are produced in a living organism therefore display batch to batch variation. Additionally, the sensor regeneration is challenging in immunosensors because the dissociation of the Ag–Ab complex from the sensor surface often requires a drastic change in conditions such as low pH and high ionic strengths which denatures the antibodies. Moreover, antibodies cannot be produced against a non-immunogenic target [21, 22].

2.2 Antigens

Antigens are defined as molecules that can elicit an immune response in the body against any foreign substances. They contain distinct sites known as epitopes recognized and interacted with various immune system components such as antibodies. Sometimes antigens are immobilized on the sensor as a biosensing element to detect antibodies in the samples [25]. These immunosensors are most commonly used in serological assays to detect infections, pathogens, viruses, etc. Laila and coworkers have developed novel competitive electrochemical immunosensors for the simultaneous detection of different types of coronavirus (CoV), such as Middle East respiratory syndrome corona virus (MERS-CoV) [26]. The AuNPs electrodeposited carbon disposable array was used as an electrode. The human corona virus (HCoV) or MERS-CoV antigens were immobilized on the electrodes. The biosensor's main principle is the indirect competition between the free virus in the sample and the immobilized MERS-CoV protein. The sensor shows a linear response in the concentration range from 0.001 to 100 ng.mL^{-1} and 0.01 to 10,000 ng.mL^{-1} for MERS-CoV and HCoV, respectively. It can detect in 20 min with a detection limit as low as 0.4 pg.mL^{-1} for HCoV and 1.0 pg.mL^{-1} for MERS-CoV. The method is single-step, sensitive, and accurate [26].

The Antigens' utility as a sensing element has a significant advantage of detecting antibodies against any infection. But, it also has disadvantages similar to antibody-based sensors such as stability, specificity, and immobilization concerns. The antigens are often proteinous in nature, either purified or synthetically prepared for immobilization on the sensor platform [27]. These proteins might not undergo correct folding while expression and do not have the correct structure similar to native proteins, for which the antibody was generated in the body. These factors might affect the sensitivity and specificity of the sensor. However, careful consideration involving assays to validate the protein structure at the start of development will significantly help in creating a viable sensor.

2.3 Aptamers

Aptamers are tiny-sized, single-stranded oligonucleotides, either RNA or DNA or peptides. It folds into a well-defined 3D structure, which provides high specificity while binding to their corresponding ligands by complementary shape interactions. Aptamers are selected from a randomly synthesized initial library containing up to 10^{15} different oligonucleotides molecules through a combinatorial chemistry procedure termed Systematic Evolution of Ligands by Exponential Enrichment (SELEX) consisting of repetitive cycles of selection and amplification (Fig. 5) [28–30]. Aptamers recently emerged as a new class of biorecognition probes that can be used in the biosensor. Such biosensors are known as "Aptasensors." Aptamers offer a broad point of interest over other existing biological recognition components in terms of stability, design flexibility, robustness, and cost-effectiveness [31, 32]. As aptamers are either DNA or RNA, they can be easily tailored and more readily engineered with various reporter molecules like fluorophores, quantum dots, methylene blue, etc., without affecting their affinity. Aptamers generally undergo a change in conformation on binding with their target. This property provides the advantage of designing unique switchable aptasensors. Aptasensors are more stable than immunosensors and can be easily regenerated for reuse [31, 33–36].

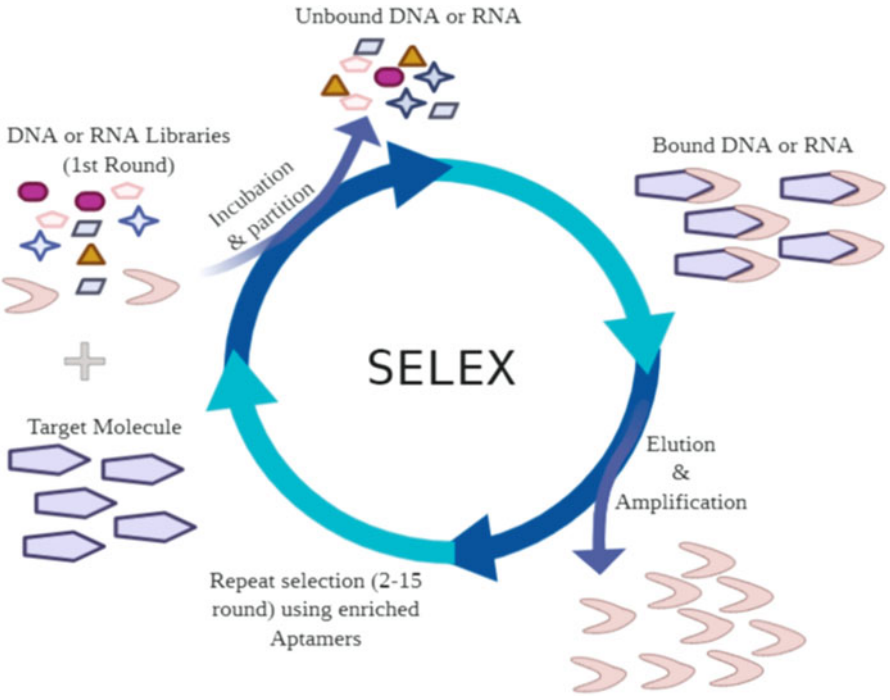

Fig. 5 Systematic evolution of ligands by exponential enrichment (SELEX)

Researchers are combining antibodies and aptamers to develop novel "Apta-immunosensors" in a sandwich format [37–39]. For example, a capacitive aptamer-antibody sensor was developed by Qureshi and coworkers for the detection of "vascular endothelial growth factor-165" (VEGF) in human serum. The anti-VEGF aptamer was immobilized on the sensor surface, followed by sandwiching with VEGF antibody-coupled magnetic beads, enhancing the response signal by 3–8-folds [38]. In another example, Zhu et al. [39] demonstrated the successful detection of "human epidermal growth factor receptor 2 (HER2)" and HER2-overexpressing breast cancer cells by an electrochemical sandwich sensor, where antibodies were used as a capture probe and aptamers as an indicator probe. In another example, Guo et al. developed aptamer-antigen-antibody sandwich biosensor based on LSPR [40]. They used Au nanorods containing coagulase binding aptamer as a capture receptor and tagged anti-thrombin protein as an LSPR signal amplification probe. The developed sensor was reusable and its LOD increased from 18.3 to 1.6 pM.

Overall, on comparing the scFv, Fab, and aptamers as biosensing element, scFv fragments-based immunosensors display the highest customizability, i.e., functional groups, immobilizing peptides, etc., due to their recombinant synthesis techniques. On the other hand, if time and cost are an issue in developing the biosensor, Fab' fragments should be chosen as they are relatively cheap and can be produced quickly from whole antibodies which takes several days. Nevertheless, if sufficient funds and time do not seem to be a factor, aptamers should be utilized as they show the best affinity toward their target analytes and are incredibly stable (excellent biosensor renewability) [41]. Additionally, aptamers generally undergo a change in conformation on binding with their target. This property provides the advantage of designing unique switchable aptasensors.

3 Immunosensor Format

Based on the detection format, Immunosensors can be direct (non-labeled) or indirect (labeled) Immunosensors.

3.1 Direct or Non-labeled Immunosensor

Direct immunosensors involve the linking of biological elements "intimately" (e.g., affixed, adsorbed, chemically bonded, adhered, coupled to, or otherwise in direct physical contact) with the transducer for the "direct detection" of the binding event to occur. In this type of immunosensor, labeling is not needed; thus, the sensors can be used for quick and real-time analysis. However, the label-free immunosensors have certain limitations of nonspecific adsorption of antibodies on the surface, leading to an increased background signal. Hence, it is vital to use a proper blocking agent such as bovine serum albumin (BSA), surfactants (Tween 20, polyethylene glycol), casein, and thionic compounds for gold surfaces [10, 42, 43].

3.2 Indirect or Labeled Immunosensors

The indirect immunosensors use a labeled secondary antibody for the detection of the signal. In an indirect detection format, signals are generated from the biorecognition elements' labels. The most unremarkably used labels are enzymes, i.e., peroxidase, alkaline phosphatase, glucose oxidase, catalase, or luciferase. Some other labels such as electroactive compounds (ferrocene, Prussian blue or In^{2+} salts), fluorophores (rhodamine, fluorescein, Cy5, ruthenium diimine complexes, phosphorescent porphyrin dyes, etc.), metallic nanoparticle (gold or silver), and quantum dots are also used [14]. Compared to label-free, labeled immunosensors have advantages like higher sensitivity and lower nonspecific adsorption, leading to lower background noise. However, it has some drawbacks, like the labeling process might affect the antigen–antibody binding efficiency [10, 42, 44, 45].

Indirect immunosensors can be further divided into two other types of formats: competitive format and non-competitive format. In competitive immunosensors, the sensor is first incubated with a mixed solution containing a known amount of labeled antigen and an unknown sample, where both compete to bind to the limited number of available antibody binding sites. The signal obtained from the labeled analyte is inversely proportional to the concentration of the sample analyte. The sample analyte is quantified by determining the amount of labeled analyte–antibody binding reaction. Competitive assays are commonly used to analyze small molecules because their small size limits the number of antibodies that can bind to the analyte due to steric hindrance [45, 46].

In a non-competitive format, the secondary antibody is labeled and the detection is in a sandwich format. Thus, the antigens' prerequisite criteria in this format are that it must possess at least two epitopes binding to two specific antibodies. In this assay, excess amounts of primary and secondary antibodies are used, and the analyte is sandwiched between two antibodies. An antibody immobilized on the solid substrate surface is called a capture antibody (primary) that captures the sample's antigen. Another one is the secondary antibody, a labeled antibody that binds to the other epitope of antigen and generates a signal for the detection [10, 45].

4 Classification of Immunosensors

The immunosensors are composed of an antigen-antibody reaction that generates an analytical signal converted into a transducer's physicochemical response. In the immunosensors, the target could be either an antigen (Ag) or an antibody (Ab). Nevertheless, the most popular approach involves detection of Ag using antibodies, but some works also report Ab detection, such as determining specific antibodies against pathogenic infection and autoimmune diseases. Immunosensors can be classified into three main classes, including electrochemical, mass-sensitive, and optical, according to the sensing platform used in the sensors [10]. However, there are immunosensors based on other transduction mechanisms like thermal changes (thermometric).

4.1 Electrochemical Immunosensors

The electrochemical immunosensors combine the antigen–antibody reactions with electrochemical measurements. The biorecognition elements, i.e., an antigen or antibody, are fabricated on an electrode's surface. The binding of the recognition element with its target biomarker/proteins results in electrochemical current/or voltage changes, which are measured [6, 47]. Thus in this immunosensor, the electrochemical signals are generated only by antigen–antibody complex and are not influenced by the concentration of unbound detecting secondary antibodies. Therefore, it is unnecessary to remove an unbound detecting agent, significantly reducing operation time [44]. Typically, an electrochemical biosensor is either a three-electrode or a two-electrode. The three-electrode format consists of a working electrode, a reference electrode, and a counter electrode.

On the contrary, the two-electrode system consists only of a working electrode and a reference electrode. However, the three-electrode system can protect the change in the reference electrode's half-cell potential because the charge from electrolysis passes through the counter electrode [47]. On the other hand, the two-electrode system is more straightforward and cheaper.

Electrochemical biosensors comprise the largest group of chemical and biological sensors [48]. Electrochemistry for analyte detection in immunosensors has several advantages; for instance, it is cost-effective, easy to operate, portable, and simple to construct. Besides, as electrochemistry is a surface-based method, minute samples are only required for detection purposes; hence, the reaction volume does not matter [49]. Further, the electrochemical immunosensors are classified into amperometric, potentiometric, impedance, and conductometric based on their transduction mode [50, 51].

4.1.1 Amperometric

Amperometric immunosensors measure the current output generated due to oxidation and reduction reactions of an electroactive species at a constant voltage. If the current is measured throughout controlled variations of the potential, it is stated as *voltammetric*. In amperometric, the measured current is proportional to the concentration of the analyte of interest. Amperometric immunosensors might be direct, where the sensing elements are non-labeled and the natural changes due to immune complex formations are detected. However, only a few applications implicate direct sensing since most of the analytes (protein) are not intrinsically able to act as redox molecules. Therefore, indirect amperometric immunosensors are most commonly used, where an electrochemically active label is needed for the analyte's electrochemical reaction at the sensing electrode. Enzymes, such as horseradish peroxidase (HRP) and alkaline phosphatase (AP), are the active labels most commonly used to catalyze the reaction of substrates to form electroactive products [52, 53]. Indirect amperometric immunosensors are more sensitive and versatile [10]. It is widely used as a cancer biomarker and cancer cell detection.

4.1.2 Potentiometric

These immunosensors mainly measure any potential changes that occur due to immunocomplex formation between antibody and antigen. Potentiometric devices measure any change in the accumulation of a charge potential at the working electrode against a reference electrode at zero current [54, 55]. In alternative words, potentiometry provides data regarding ion activity in an electrochemical reaction. The fundamental principle of all potentiometric transducers is governed by the Nernst equation [56]. According to this equation, potential changes are logarithmically proportional to the precise ion activity.

$$\text{EMF or } E\text{cell} = E^\circ \text{cell} - \frac{RT}{nF} 1nQ$$

E_{cell} represents the observed cell potential at zero current; this is sometimes referred to as the electromotive force or EMF. E°_{cell} is a constant potential contribution to the cell, R *is* the universal gas constant, T *is* the absolute temperature in degrees Kelvin, n is the charge number of the electrode reaction, F is the Faraday constant, and Q is the ratio of ion concentration at the anode to ion concentration at the cathode.

The potentiometric immunosensors have the advantages of simplicity of operation by automation and miniaturization of sensors. It also offers low LOD (between 10^{-8} and 10^{-11} M), which is important for cancer detection as biomarkers' concentration is very low in the early cancer stages. However, these sensors' major limitation is their sensitivity, which is lower than other immunosensors because, in most of the immunoaffinity reactions, the change in potential is relatively small [10]. Another challenge is the nonspecific effect of binding or signaling due to the other ions present in the sample. This often leads to a high signal-to-noise ratio, which is difficult to circumvent [57].

4.1.3 Impedimetric

In the impedimetric immunosensor, the impedance consists of a resistive and a capacitive part due to a complex interaction with a small amplitude voltage signal as a function of frequency, and the resulting current is recorded. Impedimetric immunosensors function by applying electrochemical impedance spectroscopy (EIS) to a biosensor platform using antibodies as receptors, which provide excellent sensitivity and selectivity. Unlike amperometric and potentiometric systems, impedance biosensors are label-free and do not depend on any specific enzyme for the analyte detection [15, 58–60].

4.1.4 Conductometric

Conductometric immunosensors are based on altering electrical conductivity at a constant voltage caused by immunoreaction that specifically generates or consumes ions. When a biorecognition element binds to its analyte, it changes ion species' concentration. This further leads to a change in the conductivity of the solution or current flowing through them. The signal generated due to such changes is generally

measured by an ohmmeter or multimeter. Conductometric immunosensors have several advantages, including low driving voltage, large-scale production, and miniaturization suitability without a reference electrode [61].

4.2 Optical Immunosensors

In optical biosensors, the analyte-antibody reactions are integrated with an optical transducer system, enabling the visible response whenever an analyte of interest is present in the sample. These are based on the quantification of chemiluminescence, absorbance, phosphorescence, reflectance, or fluorescence emission in the UV, visible, or near-infrared (NIR) or any color change [62–64]. Typically, in optical immunosensors, the light either comes from a diode, laser, or white-hot light bulb. Any alterations in the light's attributes reflected from or passed through the sensor are measured. Optical immunosensors have the advantage of allowing a safe non-electrical remote sensing of materials and usually do not require reference sensors since the comparative signal can be generated using the same source of light as the sampling sensor [65]. Moreover, when optical sensors respond in the visible light range, it removes the need for any equipment to read results, making them less expensive, portable, and easy to use [66, 67].

The detection schemes employed in the optical immunosensors are either label-free methods, such as surface plasmon resonance (SPR) and quartz crystal microbalance (QCM) measurements, Raman spectroscopy, and electrochemical impedance spectroscopy, or labeled such as fluorescence, chemiluminescence, electrochemiluminescence (ECL), field-effect transistors, and nanoparticles. Label-based detection often requires a combination of specific sensing elements fabricated with the detecting antibodies or the target. The color change can be visualized with the naked eye in colorimetric designs and does not require any sophisticated equipment. However, labeling makes the assays more complicated, time-consuming, and laborious. Moreover, this process is costly and often results in the denaturation of the modified biomolecules [68]. Among the various methods of optical detection, fluorescence is by far the most exploited. This is because of the existence of diverse fluorophore collections, which are highly sensitive to environmental changes. Additionally, they are easy to build and can facilitate detecting multiple compounds in a single device. But the instrument used for signal readout in fluorescence-based immunosensors is usually expensive and more suitable for laboratory settings.

On the contrary, the "label-free" sensors do not require covalent labeling to either the analyte or the biorecognition element. "Label-free" strategies are one of the foremost effective and promising methods for quicker, simpler, and additional convenient detection since they avoid the high-priced and tedious labeling method while retaining the activity and affinity of the antibodies [69]. SPR is an excellent example of a label-free optical transducer system. It is based on a phenomenon that occurs when light is reflected off thin films of metal and can be verified by the arrangement based on the Kretsch Mann configuration [70].

4.3 Piezoelectric Immunosensor

Any minor changes in the mass due to an analyte's binding to the antibody immobilized on a piezoelectric crystal can be detected by piezoelectric (PZ) immunosensors. The operation principle is based on the propagation of acoustic shear waves in the substrate of the sensor. The specific adsorption of antibody molecules influences the acoustic wave phase and velocity on the sensor surface [10]. Acoustics immunosensor is based on the piezoelectric effect and is highly effective in determining protein affinity on functionalized surfaces.

The piezoelectric crystals have a characteristic oscillation frequency in the presence of an electrical field. This frequency mainly depends on the crystal properties, such as its weight. A precisely cut quartz crystal slab is generally used for measuring very small mass quantities. The measuring device is known as quartz crystal microbalance (QCM). When QCM are coated with biorecognition elements such as antibodies, such sensors are known as QCM-based immunosensor [71]. The analyte binding causes a tiny change in mass on the electrode surface, resulting in the change of crystal frequency, which can be measured. QCM-based immunosensor is also an acoustic wave sensor that utilizes a thickness-shear mode vibration with the complete substrate's vibration.

The major drawback of piezoimmunosensors is nonspecificity due to the sensing layer's anomalous adsorption and nonspecific binding of proteins to the antibody test surface.

4.4 Others

Most biological reactions involve the release or absorption of heat. This concept is exploited in the development of an immunosensor known as thermometric immunosensors. It detects any temperature change, i.e., either released or absorbed heat due to the specific analyte-antibody/antigen reaction, and the temperature variations are converted to an electrical signal [72]. The transducer usually preferred for the thermometric sensor could be a semiconductor with an extremely high negative temperature coefficient of resistance. The thermometric immunosensors often are more stable in the long run as the analyte and transducer are distinctly placed and are not in contact with each other. It is also cost-effective and is generally unaffected by the unsteady optical or ionic impact of sample attributes. But this type of sensor has the inherent disadvantage of not being specific in its detection [72].

The temperature-based detection mode is often coupled with an enzyme thermistor because almost all enzyme reactions involve enthalpy changes [73]. In common practice, the thermometric immunosensors are combined with enzymatic reactions via the flow-injection assay (FIA) method. One such example is the thermometric immunosensor developed by Bari and coworkers [74] to detect tumor necrosis alpha (TNF-α), a protein associated with Alzheimer's disease, cancer, and inflammatory-related diseases. The calorimetric scheme consists of a microfluidic device integrated with an antimony/bismuth thermopile sensor to quantify TNF-α. The sensor is in a

sandwich format where the anti-TNF-α monoclonal antibody captures the analyte, while the glucose oxidase-conjugated secondary antibody was used for detection. The heat is generated due to the enzymatic reaction between glucose oxidase and its substrate. This heat was consequently transformed into an electrical signal by the thermoelectric sensor [74].

5 Immobilization of Immunoactive Elements

For the efficient performance of an immunosensor, biological components should be appropriately connected to the transducer. Biosensors are typically designed with a high loading of biomolecules to obtain sufficient biocatalyst activities and provide an appropriate molecular environment to enable biological activity [25]. The local chemical and thermal atmosphere can have a significant impact on the stability of the biomolecule. Various factors such as the analyte's physical chemistry characteristics, the existence of the biological elements, type of transducers, and sensing environment need to be assessed during the immobilization process (Table 1). Also, it must be essential that the biological elements should display maximum activity in their immobilized microenvironment. Generally, antigens can be immobilized by two methods, namely chemical and physical methods. The physical method is characterized by weaker, mono-covalent interactions like hydrogen bonds, hydrophobic interactions, van der Waals forces, affinity binding, and ionic binding of the catalyst with the support material. In the chemical method, the formation of covalent bonds is accomplished via the ether, thioether, and amide/carbamate bonds formed between the enzyme and support material [76]. Four methods such as adsorption, covalent bonding, cross-linking, and entrapment are commonly used for the immobilization of biomolecules [25, 77].

Table 1 Factors influencing immobilized enzymes' performance (Adopted and modified from [75])

S. No.	Factors	Implication of immobilization
1	Hydrophobic partition	Enhancement of the reaction rate of the hydrophobic substrate
2	Microenvironment of carrier	Hydrophobic nature stabilizes the enzyme
3	Multipoint attachment of carrier	Enhancement of enzyme thermal stability
4	Spacer or arm of various types of immobilized enzymes	Prevents enzyme deactivation
5	Diffusion constraints	Enzyme activity decreases and stability increases
6	Presence of substrates or inhibitors	Higher activity retention
7	Physical posttreatments	Improvement of enzyme performance
8	Physical nature of the carrier	Carriers with large pore size mitigate diffusion limitation, leading to higher activity retention

5.1 Adsorption

Adsorption is the best and economical method for the immobilization of biomolecules. However, interaction is weak, and the life of the electrode is very short. The adsorption process can be divided further into two classes: physical and chemical. Physical adsorption is weak and mediated primarily via van der Waals forces, whereas chemical adsorption is much stronger and requires covalent bond formation. The biomolecules (antibody, enzyme, protein, etc.) are resuspended in an aqueous solution and then placed on the solid support for a fixed period under appropriate environmental conditions [75]. The unabsorbed/unbound molecules are then removed from the surface by washing with buffer. It is generally considered a non-destructive method in terms of biomolecule activity due to the non-involvement of any functional group as supporting agents. However, this technique has some serious drawbacks: enzymes are loosely bound to the support by weak physical bonding, i.e., van der Waals forces. Any minor changes, i.e., temperature, pH, or ionic strength, may affect the result due to the biomolecules' desorption/leaching. In contrast, absorbed biomolecules-based biosensors have displayed poor functioning and storage stability due to nonspecific adoptions on the surface of the transducer can cause contamination and interference with the signal and liquidation of biomolecules, especially enzymes [77].

5.2 Covalent Bonding

Immobilization by covalent bonding is one of the best and widely accepted methods in the scientific community. It forms stable complexes between a functional group (–NH_2, –COOH, –OH, C_6H_4OH, and –SH.) of the biomaterial such as antibody, protein molecules (antigen), and an auxiliary matrix through a covalent bond [78]. The functional group of antibodies, which could be used as a covalent coupling agent includes amino group, carboxylic group, phenolic group, sulfhydryl group, thiol group, imidazole group, indole group, and hydroxyl group. Various chemistries have been developed for the covalent immobilization of Ab to the substrate. The establishment of covalent immobilization requires a mild environment, including low temperature, low ionic strength, and pH in the physiological range. Many traditional coating materials, such as polyethyleneimine [79], (γ-aminopropyl) trimethoxy silane [75, 80], and the copolymer of hydroxyethyl- and methyl-methacrylate, are often used as the mediate layers for immunoreactive molecule immobilization [77]. The major advantage of covalent immobilization is providing strong bindings between antibody and support matrix and reducing chances of enzyme leakage from the activated support. However, due to chemical modification, there is a higher risk of enzyme denaturalization, resulting in reduced enzyme activity in affinity reaction and poor reproducibility may be observed.

5.3 Cross-linking

Immobilization by cross-linking is an irreversible methodology performed by the formation of intermolecular cross-linkages between the antibody by covalent bonds. In this method, the biomaterial is chemically attached to solid supports/material to significantly increase the attachment [27, 81]. The most commonly used interactions are based on the use of cross-linkers that cross-links the functional groups present on the antibody to the functional groups present or induced on the substrate. An overview of the commonly used reactive group of antibodies is listed in Table 2.

The binding of a thiolated antibody to the gold-coated matrices is one of the most frequently used methods today. Because it offers a leach-proof, covalent binding of the antibody to matrices and requires minimal immobilization steps; however, it involves minor antibody modification before immobilization.

In another method, glutaraldehyde (GLD), a homo-difunctional cross-linking agent, cross-links the amino groups ($–NH_3$) available on the antibody. During immobilization, imine bonds are formed by aldehyde groups, induced on a GLD-functionalized substrate attached via lysine (amine groups) in antibody, resulting in the reversible Schiff bases. The antibody immobilization requires an initial ionic exchange in a low ionic medium with the amino groups on the substrate, followed by covalent binding, while in a high ion medium, the antibody is directly bound to slower immobilization kinetics [82]. Due to strong GLD binding with antibody molecules, leakage is minimal. However, GLD can cause significant conformational changes in antibody and could lose antibody affinity and specificity as well. This may be minimized by using inert proteins such as gelatin and bovine serum albumin during immobilization [27, 83].

Table 2 Commonly used cross-linker reactive groups for antibody (Ab) immobilization

S. No.	Reactivity	Functional group	Reactive chemical group
1	Amine-reactive	$–NH_2$	NHS ester, imidoester, epoxide isothiocyanate, aldehyde, pentafluoro-phenyl ester, hydroxymethyl phosphine
2.	Carboxyl-to-amine-reactive	–COOH	Carbodiimide
3.	Sulfhydryl-reactive	–SH	Maleimide, haloacetyl, pyridyldisulfide, thiosulfonate, vinylsulfone
4.	Aldehyde-reactive, (oxidized sugars)	–CHO	Hydrazide, alkoxyamine
5.	Hydroxyl	–OH	Isocyanate
6.	Azide-reactive	$–N_3$	Phosphine

5.4 Entrapment

In the entrapment immobilization, the biomolecules are not directly connected to polymeric materials' surface but entrapped insight into the polymeric network. It only allows crossing the substrate and products via micro/nano matrices pore, leading to a delay in the reaction. However, it retains the antibody affinity and specificity. The solution is coated on the electrode by various methods. The commonly used gels include starch gels [84], nylon, and conductive polymers such as polyaniline (PANI) [85]. The whole entrapment process is directed via two steps: (1) mixing of the antibody in a monomer solution, followed by (2) polymerization of monomer solution by the chemical reaction or changing experimental conditions. There are various methods available for the entrapment of biomolecules, depending on the type of entrapment, such as electro-polymerization, photo-polymerization, a sol–gel process for lattice fiber type, and microencapsulation for microcapsule. [75]. Although, this method offers to immobilize antibodies in their native conformation, leading to enhance their stability and protect from denaturation. However, poor substrate diffusion has been observed due to an increase of gel matrix thickness, resulting longer time consumed by the substrate to reach the enzyme active site [77]. Furthermore, the entrapped enzymes are most likely to suffer from leakage if the size of the support matrix's pores is too large.

6 Immunosensors as Diagnostic Tools

Immunosensors provided a new direction toward developing novel diagnostics in diseases, drug detection, and food quality control. Immunosensors can be designed for the detection of biomarkers, autoimmune diseases, cardiac diseases, etc.

6.1 Immunosensors for Detection of Biomarkers

The utilization of immunosensors for the detection and observation of biomarkers is presently a noteworthy area of research. Recently, the development of these novel techniques has assisted in the discovery of many new markers and provided a deeper insight into their disease role.

6.1.1 Cancer Biomarkers

Cancer is a devastating disease with the second most common cause of mortality and morbidity in developed countries. It is crucial to detect cancer at an early stage so that specific treatment may be applied as soon as possible and lead to better outcomes and prolonged cancer patients' survival. The development of novel molecular diagnostic tools has changed cancer's overall landscape in the last few decades. Enormous technological improvements in the genomics and proteomics field have identified several biomarker proteins whose over-expression can direct normal cells' oncogenic transformation into cancerous cells. A biomarker is defined

as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention" [86]. Cancer biomarkers can be of various molecular origins, including free DNA, RNA, or protein/glycoproteins (i.e., antigens, cytokines) or circulating tumor cells. Recently, cancer-derived exosomes have drawn much attention as a biomarker for the early diagnosis and drug sensitivity analysis of cancer as they carry the cargo reflective of genetic or signaling alterations in cancer cells of origin [87, 88].

Diagnostic biomarkers are those markers that help in detecting the disease, while prognostic biomarkers are indicative of disease reoccurrence. On the other hand, Predictive biomarkers measure the response to undergoing treatment. The different phases of cancer progression are marked by the changes in the different cell's specific biomarkers and their expression level. These tumor markers are considered one of the most valuable early cancer detection, classification, staging, and progression monitoring tools. Generally, some of the tumor markers are present in blood at very trace levels in the absence of a tumor. However, the markers' levels rise upon forming a small tumor, so very low limits of detection (LODs) of the developed immunosensors are essential for the early screening of a small tumor. Also, most cancers are heterogeneous and multifactorial, involving more than one marker; therefore, the use of panels of tumor markers can be more productive in their detection and diagnosis [89]. Several types of immunosensors have been developed for the detection of cancer biomarkers. In the table, immunosensors for different cancer targets are listed along with their transduction mode (Table 3). The achieved detection limits and linear detection ranges are also delineated.

Electrochemical immunosensors are most commonly used for the detection of cancer biomarkers. For example, Kim et al. [115] developed amperometric electrochemical immunosensors for diagnosing lung cancer by detecting Annexin II and MUC5AC biomarkers. The probe of the sensor was fabricated by electropolymerizing conducting polymer (poly-terthiophene carboxylic acid; poly-TTCA) onto a gold nanoparticle/glassy carbon electrode (AuNP/GCE) and a dendrimer (Den). The assay format is based on the principle of competitive reaction between label-free proteins and glucose oxidase-labeled proteins. The final sensor design was obtained by covalently attaching an antibody (anti-Annexin II) and hydrazine (Hyd), which is a catalyst for reducing H_2O_2 generated by glucose oxidase onto the Den/AuNP-modified surface. The use of dendrimer increased the sensor probe's sensitivity two or three times. The interaction of Annexin II and MUC5AC with the antibody was examined using quartz crystal microbalance, impedance spectroscopy, and amperometric ways. The detection limit of the proposed technique was 0.051 ng/mL.

Several potentiometric immunosensors are also developed for cancer detection and biomarker monitoring [49, 106, 116]. Jia and coworkers developed new techniques for detecting human phosphatase of regenerating liver-3 (hPRL-3), a prognostic biomarker of liver cancer. In this work, hPRL-3 can be detected in the concentration range of 0.04–400 nM, and the mammary adenocarcinoma cell (MDAMB231) in the concentration range of 0–105 cells/mL [49].

Table 3 Some examples of immunosensors for detection of cancer biomarkers

S. No	Biomarker	Cancer	Immunosensor type	Detection range	LOD	Reference
1.	CYFRA21-1	Lung cancer	Optical	0.05 pg/mL–100 ng/mL	0.05 pg/mL	Chiu and Yang [90]
2.	PSA	Prostate cancer	Electrochemical	0.2–1.0 ng/mL 1–40 ng/mL	20 pg/mL	Salimi et al. [91]
3.	PSA	Prostate cancer	Electrochemical	0–0.1 U/mL	0.0016 U/mL	Johari-Ahar et al. [92]
4.	PSA	Prostate cancer	Electrochemical	0.05–30 ng/mL	0.04 ng/mL	Shen et al. [16]
5.	PSA	Prostate cancer	Electrochemical	2.0 pg/mL−10.0 ng/mL	0.5 pg/mL	Yang et al. [93]
6.	PSA	Prostate cancer	Optical	5–500 ng/mL	9.9 ng/mL	Xiao et al. [97]
7.	ErbB2	Breast cancer	Electrochemical	1.0–200.0 ng/mL	0.22 ng/mL	Zhong et al. [98]
8.	ErbB2	Breast cancer	Electrochemical	0.01–100 ng/mL	0.01 ng/mL	Sharma et al. [99]
9.	p16INK4a	Cervical cancer	Electrochemical	15.62 ng/mL−0.25 μg/mL	0.49 ng/mL 28 (HeLa cells)	Duangkaew et al. [94]
10.	p16INK4a	Cervical cancer	Piezoelectric	50–1200 ng/mL	10 ng/mL	Yang et al. [95]
11.	CA15-3	Breast cancer	Electrochemical	1.0–150 U/mL	0.3 U/mL	Amani et al. [96]
12.	CA15-3	Breast cancer	Electrochemical	0.1–20 U/mL	0.012 U/mL	Li et al. [100]
13.	EGFR	Breast cancer	Electrochemical	1 pg/mL–1 μg/mL	0.88 pg/mL	Elshafey et al. [101]
14.	EGFR	Breast cancer	Electrochemical	2–14 fg/mL	2 fg/mL	Asav and Sezgintürk [102]
15.	EGFR	Breast cancer	Electrochemical	1 pg/mL–100 ng/mL	1 pg/m	Vasudev et al. [103]
16.	tPA	Breast cancer	Electrochemical	0.1–1.0 ng/mL	0.026 ng/mL	Vasudev et al. [103]
17.	UBE2C	Breast cancer	Electrochemical	500 pg/mL–5 μg/mL	7.907 pg/mL	Jayanthi et al. [104]
18.	MDM2	Brain cancer	Electrochemical	1 pg/mL–1 μg/mL	0.29 pg/mL	Elshafey et al. [101]
19.	CEA	Cancer	Thermometric	7.81–500 pg/mL	0.6 pg/mL	Ma et al. [105]
20.	CEA	Cancer	Electrochemical	1 pg/mL–1 μg/mL	0.3 pg/mL	Wang et al. [106]
21.	SOX2	Cancer	Electrochemical	25 fg/mL–2 pg/mL	7 fg/mL	

(continued)

Table 3 (continued)

S. No	Biomarker	Cancer	Immunosensor type	Detection range	LOD	Reference
						Aydın and Sezgintürk [107]
22.	RACK-1	Cancer	Electrochemical	0.01–2 pg/mL	3.1 fg/mL	Aydın et al. [108]
23.	CD146	Cancer	Electrochemical	0.0050–20 ng/mL	1.6 pg/mL	Wang et al. [109]
24.	IL6	Cancer	Electrochemical	$4.0–8.0 \times 10^2$ pg/mL	1.0 pg/mL	Wang et al. [110]
25.	DHEAS	Pediatric adrenocortical carcinoma	Electrochemical	10.0–110.0 μg/dL	7.4 μg/dL	Lima et al. [111]
26.	HE4	Ovarian cancer	Electrochemical	3–300 pM	0.06 pM	Lu et al. [112]
27.	CA 125	Ovarian cancer	Optical	0.1–40 U/mL	0.1 U/mL	Suwansa-ard et al. [113]
28.	CA 125	Ovarian cancer	Capacitive	0.05–40 U/mL	0.05 U/mL	Suwansa-ard et al. [113]
29.	CA 242	Pancreatic and colorectal cancers	Electrochemical	0.001–10,000 U/mL	1.54×10^{-3} U/mL	Du et al. [114]

Abbreviations: *UBE2C* Ubiquitin-conjugating enzymes 2C, *SOX2* SRY (sex determining region Y)-box 2, *RACK 1* Receptor For Activated C Kinase 1, *CYFRA21-1* cytokeratin fragment 19, *PSA* prostate-specific antigen, *ErbB2* epidermal growth factor receptor 2, *CA15-3* carbohydrate antigen 15-3, *CEA* carcinoembryonic antigen, *EGFR* epidermal growth factor receptor, *CD146* cluster of differentiation 146 antigen, *DHEAS* dehydroepiandrosterone sulfate, *tPA* tissue plasminogen activator, *MDM2* Murine double minute 2, *HE4* human epididymis-specific protein 4, *IL6* interleukin-6, *CA 125* cancer antigen 125, *CA 242* carbohydrate antigen 24-2

In a fascinating study, a convenient immunosensor for detecting carcinoembryonic antigen (CEA) was developed using just an ordinary thermometer as a readout [105]. The concept of generating enormous heat due to the exothermic reaction between the water and calcium oxide is exploited in this immunosensor. The immunosensor is in a sandwich format where an anti-carcinoembryonic antigen antibody acts as a capture antibody and a biotinylated antibody for detection. The biotinylated detection antibody is labeled with streptavidin-functionalized platinum nanoparticles. It catalyzes the decomposition of H_2O_2 into O_2, increasing pressure inside the reaction bottle. This further pushed the water flow into the exothermic reaction bottle containing calcium oxide. Then the water reacts with the calcium oxide to generate a large amount of heat in the exothermic reaction bottle, which was recorded by a standard thermometer.

The detection and diagnosis of cancer are very challenging due to several reasons. Firstly, cancer biomarkers are generally present in patients' biological fluids, such as blood or urine. Thus, the developed immunosensors should be sensitive enough to detect and quantify biomarkers' presence in a contaminant-ridden environment. Secondly, cancer involves multifactorial changes. At present, the most commonly used indicators for cancer diagnosis are morphological changes and histological characteristics of tumors or biomarker detection. A plethora of molecular biomarkers has recently been used for the development of single-analyte biosensors such as carcinoembryonic antigen (CEA) [117–120], α-fetoprotein (AFP) [121], epidermal growth factor receptor-2 (HER-2) [122], interleukin-6 (IL-6) [123, 124], and interleukin-8 (IL-8). But, most cancers have more than one marker associated with their incidence. Therefore, the development of multianalyte sensors will be more helpful in diagnosing and monitoring cancer patients. Then again, it has another limitation as performing such large numbers of multianalyte assays possesses various other technical and practical challenges, making it difficult to quantify a specific analyte accurately. In these cases, each antibody must be first characterized for performance and specificity by itself and again in combination with the additional reagents and antibodies. Moreover, the differences in shelf lives, stability, and binding affinities of the reagents and analyte concentrations may vary widely and may create additional difficulties [118].

Electrochemical immunosensors can be a strong candidate for performing multianalyte protein analysis because of their high sensitivity, selectivity, cost-effectiveness, quick response time, and simplicity. They can achieve excellent detection limits with tiny analyte volumes [125]. Furthermore, electrochemical sensors can be miniaturized and mass fabricated, which makes them better point-of-care diagnostics. In this regard, Wilson et al. developed an electrochemical immunosensor for simultaneous measurement of concentrations of seven important tumor markers: AFP (α-fetoprotein), ferritin, CEA (carcinoembryonic antigen), hCG-β (human choriogonadotropin β), CA 15-3 (carbohydrate antigen), CA 19-9, and CA 125 [126]. It consists of an array of immunosensing electrodes fabricated on a glass substrate, with each electrode containing a different immobilized antigen. Each electrode was capable of measuring a specific tumor marker using electrochemical enzyme-based competitive immunoassay. The secondary anti-IgG

antibody was labeled with alkaline phosphatase (AP). The hydroquinone diphosphate was added at the end of the assay, which is the enzyme-substrate. The oxidation current generated was measured simultaneously for all the electrodes after applying a potential of 320 mV. The developed multianalyte sensor had outstanding precision and accuracy and was comparable in performance to single-analyte ELISAs. In another example, Wu et al. [89] developed a simple, automated, and convenient multianalyte detection system based on screen-printed and flow-injection techniques. It is an electrochemical immunosensor that allows simultaneous detection of carcinoembryonic antigen, α fetoprotein, β-human choriogonadotropin, and carcinoma antigen 125 in clinical serum samples with concentrations up to 188 μg/L, 250 μg/L, 266 IU/L, and 334 kIU/L, respectively. The detection limits were 1.1 μg/L, 1.7 μg/L, 1.2 IU/L, and 1.7 kIU/L. The immunosensor arrays were stable for up to 1 month.

6.1.2 Cardiovascular Disease Markers

Cardiovascular diseases are the world's biggest killer for both men and women [127]. According to the World Health Organization (WHO), 17.9 million deaths are attributed to this disease in 2015, with 7.3 million being due to acute myocardial infarction (AMI) [128]. Early detection of patients with a high risk of acute myocardial infarction (AMI) is very important. Some of the indicators of elevated risk of AMI are creatine kinase MB (CK-MB), Myoglobin (Mb), myeloperoxidase (MPO), and cardiac troponins (cTn). To predict cardiovascular events; C-reactive protein (CRP) is the best-known biomarker, followed by cardiac troponin I or T (cTnI/T), myoglobin, lipoprotein-associated phospholipase A, interleukin-6 (IL-6) [129], interleukin-1 (IL-1), low-density lipoprotein (LDL), myeloperoxidase (MPO), and tumor necrosis factor-alpha (TNF-α) [111]. A cardiovascular patient's effective treatment strongly depends on a quick and rapid turnaround time, i.e., the time taken between acquiring the patient's blood sample and its results. The standard time for the diagnosis of chest pain should be between 30 min and 1 h. Therefore, handheld biosensor devices are the ideal tools for this setting, where testing can be conducted on-site, assisting in diagnosing the condition [127]. Suprun et al. developed a label-free immunosensor that can detect cardiac myoglobin in just 20 min and can be used to establish the diagnosis of acute myocardial infarction [130]. In a very exciting study, a group of researchers has developed an electric aptasensor that can detect cardiac troponin I (cTnI), a protein excreted by the heart muscle into the blood following a heart attack. It can provide the result in 1 min using just a single droplet of blood [131]. Over the last decade, many immunosensors have been developed to detect a wide variety of cardiac markers [130, 133–138]. In one such example, Khan et al. developed an impedimetric immunosensor for quick, sensitive, and selective detection of myoglobin (Mb). It incorporates a screen-printed multiwalled carbon nanotube electrode for signal amplification on which the anti (anti-Mb-IgG) antibody was immobilized.

The developed immunosensor is highly specific and sensitive, with a detection limit of 0.08 ng/mL [139]. Similarly, Ko et al. [140] developed an immunosensor for troponin I detection with a LOD of 148 pg/mL. Mattos et al. [141] developed an

amperometric immunosensor for cardiac troponin T (CTnT) detection in human serum. The antibodies are covalently bonded on a stable carboxylic film. It showed good operational stability performance, measuring it 100 times every 2 min. Recently, the detection of multi biomarkers has witnessed a significant boost. One such example is a sandwich-type antibody immunosensor for multiplexed detection of seven cardiovascular diseases (CVD) risk markers—SAA (serum amyloid A), TNF-α R1 (tumor necrosis factor-α receptor 1), IL-6R (interleukin-6 receptor), ICAM (intracellular adhesion molecule), VCAM (vascular cell adhesion molecule), MYO (myoglobin), and E-Sel (E-selectin). The multianalyte immunosensor can reliably quantify SAA, VCAM, and MYO, similar to ELISA [142].

6.1.3 Autoimmune Disease Marker

In autoimmune diseases(Ads), the immune system mistakenly attacks and damages the body's tissues, organs, and cells. It is estimated that ADs affect 5–10% of the general population [143, 144]. Due to the limited knowledge of ADs' pathogenesis, the medical treatment modalities are mainly based on managing the symptoms rather than curing the disease. Therefore, it is essential to detect and treat the disease early to obviate the symptoms' severity and the irreversible damage to organs or joints. But the biggest challenge is the sensitive detection of specific antibodies directed against various substances produced by the body. ADs are usually diagnosed based on the symptoms and the laboratory tests confirming the presence of serological and genetic biomarkers, such as autoantibodies or complement proteins [145, 146]. Some biomarkers are more sensitive and specific for a particular type of ADs. For example, systemic lupus erythematosus (SLE) diagnostic criteria include a list of autoantibodies, i.e., anti-dsDNA antibodies, lupus anticoagulants, anti-Sm antibodies, and antiphospholipid [147]. On the contrary, other autoimmune diseases involve different autoantibodies, such as anti-citrullinated protein antibody (ACPA) for rheumatoid arthritis (RA), anti-neutrophil cytoplasmic antibodies (ANCA) for inflammatory bowel disease (IBD), anti-transglutaminase in celiac disease, and ANCA-associated vasculitides [148, 149], anti-annexin II and V antibodies for antiphospholipid syndrome (APS) and systemic sclerosis (SS) [150]. Others have reviewed a more detailed spectrum of autoantibodies for ADs diagnosis [151–154].

The advent of highly sensitive immunosensors has helped in detecting these biomarkers at the early stages. As the diagnosis of autoimmune diseases mainly involves the detection of autoantibodies in the serum of affected patients, generally, the biorecognition element used in the immunosensors are the antigens [130, 132–138]. Neves et al. developed a disposable electrochemical immunosensor to detect celiac disease using the CV method [155]. In this work, the biorecognition element is immobilized on screen-printed carbon electrodes (SPCE) nanostructured with carbon nanotubes and gold nanoparticles. The carbon-metal nanoparticle hybrid conjugation aids in amplifying immunological interactions. The immobilized tTG on the transducer's surface was exposed to the sample and subsequently, alkaline phosphatase-labeled anti-human IgA or IgG antibody were added. The electrochemical signal was then generated by the anodic redissolution of enzymatically

generated silver. Because diagnosis often relies more upon qualitative results, this SPCE-based disposable biosensor may be an excellent point-of-care diagnostic device. In a similar example, Yerga et al. developed a blocking-free one-step immunosensor using 8-channel screen-printed arrays to detect celiac disease biomarkers, i.e., anti-transglutaminase IgA antibodies [156]. The dynamic linear range was 3–40 U mL^{-1}, with a limit of detection of 2.7 U mL^{-1}. The response time of the immunosensor was 80 min and was stable at least for 1 month when stored at 4 °C [156]. In another example, a sensitive and label-free impedimetric immunosensor was developed to detect anti-myelin basic protein autoantibodies in human cerebrospinal fluid and serum samples from MS patients [157].

Due to autoimmune diseases' heterogeneous nature, it is crucial for the detection of different antibodies simultaneously. For this purpose, Bleher et al. developed a label-free optical immunosensor involving multiple antigen–antibody interactions relevant to diagnosing antiphospholipid syndrome (APS) [158]. The proteinogenic antigens (β2-glycoprotein I) and amino-functionalized cardiolipin were immobilized on a glass surface using 11-aminoundecyltrimethoxysilane. The developed immunosensor could detect a broader antibody pattern of each patient within one single measurement. Each measurement is less time-consuming than standard ELISA procedures and can provide results in less than 20 min, depending on the measurement protocol.

6.2 Immunosensor for Detection of Metabolites

6.2.1 Glucose Detection

Diabetes is a metabolic disease that causes an abnormal blood sugar level, which activates several metabolic pathways related to inflammation and apoptosis events. This disease has no cure thus far. Hence, patients with diabetes systematically need to monitor their blood glucose levels to avoid complications. The glucose concentration can be monitored using a glucose meter for peripheral blood samples drawn by a finger prick. Most of the glucose meter is enzyme-based, relying on the substrate decomposition by an enzyme such as glucose oxidase to detect the product. However, since the enzyme rapidly degenerates, the sensor needs to be calibrated several times a day, and its lifetime is limited [159, 160].

Moreover, these meters are invasive and painful as the blood needs to be repetitively withdrawn for daily profiling. The antibody-based sensors provided a new direction toward the development of novel immunosensors for glucose monitoring. Electrochemical biosensors are most commonly used for the measurement of glucose. This is partially historical, but the primary reason for the success of devices of this type is that they offer suitable sensitivity and reproducibility and, importantly, can be manufactured in great volume at low cost [3].

Paek et al. [161] developed an alternative glucose analysis method based on antigen-antibody binding, which may be active over an extended period. In this study, mice were immunized with dextran chemically conjugated with keyhole limpet hemocyanin to produce monoclonal antibodies. Then, the sugar-specific

antibodies were screened. The antibodies showing typical binding characteristics toward epitopic sites and rapid reaction kinetics were selected for eventual use in blood glucose monitoring. The produced antibodies are very selective and can discriminate biological glucose compounds with a 1,4-linkage. The antibodies are immobilized on a solid surface to develop a label-free sensor system. When glucose was added to the medium, the sensor signal was inversely proportional to the glucose concentration in a range between 10 and 1000 mg dL^{-1}, which covered the clinical range. The response time was approximately 3 min for the association and 8 min for dissociation based on a 95% recovery of the final equilibrium under optimum conditions [161].

6.2.2 Cholesterol Detection

Cholesterol is an important sterol synthesized by liver cells. It is an essential component of cell membranes that acts as a precursor for synthesizing hormones, vitamin D, and bile acids. The high cholesterol level in serum is connected directly to various diseases such as cardiovascular diseases, hypothyroidism, nephrotic syndrome, diabetes, and liver diseases [162]. Several methods such as chromatographic colorimetric, enzymic colorimetric, spectrophotometric, and microphotometric are commercially available to detect cholesterol in serum. However, these methods have several disadvantages: tedious, costly, labor-intensive, less specific, and less sensitive [163]. Various type of electrochemical-based immunosensor has been designed using chemical agents such as microfluidic, nanoparticles (gold, silver, graphene, boronic acid, silica, and polymer) to detect cholesterol directly from clinical samples, i.e., urine, blood, etc., which are listed in Table 4).

Rahman et al. [171] developed a simple and cheap cholesterol biosensor by immobilizing cholesterol oxidase (ChOx) and horseradish peroxidase (HRP) onto a poly(thionine)-modified glassy carbon electrode. The immobilized HRP has shown admirable electro-catalytic activity in reducing H_2O_2 produced by the cholesterol during the enzymatic reaction of ChOx. Under standard conditions, the minimum detection limit (LOD) and sensitivity were 3.0 μM and 6.3 μM, respectively.

Gold nanoparticles have also been explored to develop biosensor for cholesterol detection. Umar and his coworker [172] developed a highly sensitive and selective amperometric cholesterol biosensor using properties of gold and bismuth subcarbonate ($Bi_2O_2CO_3$) nanoplates, which were synthesized by the hydrothermal process at a lower temperature. The invented biosensors exhibit a high and reproducible sensitivity of 139.5 μAmM^{-1} cm^{-2}, a large linear variation from 0.05 to 7.4 mM. Also, the sensor displays a fast response time of ~4 s, and a low detection limit of 10 μM ($S/N = 3$) for cholesterol sensing [172]. Some other components such as polyaniline, Au/hollowed-TiO_2, and Cu/Ni graphene have also been explored to develop an effective immunosensor.

6.2.3 Creatinine Detection

Creatinine (2-amino-1-methyl-5H-imidazol-4-one) is the end product of disruptive creatine metabolism. The quantification of creatinine level in human blood and urine is clinically important since it partially represents the nephritic, muscular, and

Table 4 Some examples of creatinine immunosensors

S. No.	Electrode type	Linear range (nM)	Limit of detection (nM)	Sensitivity	Test accuracy (%)	Reference
1	ChOx/PDMS/NiO/Pt	0.12–10.23	0.1	45 $\mu AmM^{-1}\ cm^{-2}$	1.25	Kaur et al. [164]
2	ChOx/CHER/AuNPs/SPCE	0.012–10.23	0.0078	–	5.2	Huang et al. [165]
3	ChOx/PBNPs/SPCE	0.15	0.2	2.1 $\mu AmM^{-1}\ cm^{-2}$	Not determined	Cinti et al. [166]
4	ChOx/GO/AuNPs/SPC	0.000025–12.93	0.000002	0.084 $\mu AmM^{-1}\ cm^{-2}$	4.95	Huang et al. [165]
5	Apo-ChOx/PTBA/FAD/PGE	0.0008–0.0048	0.0003	0.21 $\mu A\mu M^{-1}$		Huang et al. [167]
6	Apo-ChOx/PABA/FAD/PGE	0.0008–0.0056	0.0004	0.022 $\mu A\mu M^{-1}$	4.17	Huang et al. [167]
7	ChOx/Poly (CBNP)/PGE	0.0025–0.0275	0.0002	1.49 $\mu A\mu M^{-1}$	4.4	Vidal et al. [168]
8	ChOx/PB/GCE	8–4.5	4	0.54 $\mu AmM^{-1}\ cm^{-2}$	6.7	Singh et al. [169]
9	ChOx/Epoxyresin/P	1.0–8.0 mM	0.1	0.63 μA/mM	Not determined	Pundir et al. [170]
10	ChOx/PB sol–gel	0.001–0.08	0.00012	0.329 $\mu A\mu M^{-1}$	Not determined	Vidal et al. [168]

thyroid functions. Unlike urea, creatinine density in the body fluids is not affected by protein intake; thus, its level is a more accurate and reliable indicator of kidney function [173]. The commonly used methods are Jaffé's reaction and enzyme colorimetry to detect creatinine in clinical samples [174]. However, colorimetric methods are adversely affected by the presence of numerous metabolites/drugs in body fluids, whereas enzymic assays are cumbersome, complex, and costly.

In the past decades, various biosensors such as electrochemical, potentiometric, amperometric, and nano-sensor have been developed around the globe. However, they show variable sensitivity and accuracy [175].

Yang and his coworkers developed an electrochemical using gold nanoparticles and iron oxide-mediated sensor for the detection of creatine in clinical samples. The sensor was not useful clinically due to poor sensitivity and complex steps required for signal conversion [98]. Tang et al. explored Surface Plasmon Resonance Imaging and Near-Infrared Quantum Dots technology for the detection of creatinine. The sensitivity and specificity were quite better but observed poor reproducibility and test accuracy [176].

In this sense, electrochemical amperometric immunosensors can overcome these limitations due to fast miniaturization and rapid, precise, sensible detection. In label-free electrochemical immunosensors, the sensing platform involves the transport of the redox species to produce the amperometric signal, which is directly proportional to the diffusion of species on the electrode surface hindered by immobilized antigen/antibody. The redox species are essential for label-free electrochemical detection and are the most common approach to detect redox species in electrolyte solutions [177]. The key weakness of this approach is that it involves several steps, including multiple washes, which hindered their utility in point-of-care detection [178, 179]. Many strategies based on nanomaterials have been applied in the development of immunosensors to generate a new version of immunosensor to enhance their performances and accuracy [99]. Since then, many nanomaterials metal oxide nanoparticles, noble metal nanoparticles, and carbon-based nanomaterials have been explored to develop novel sensing platforms [180]. Recently, Erika Trindade et al. [181] developed an electrochemical immunosensor for creatine detection using redox probe-free probe technology. The sensor displays a positive response from 0.1 to 1000 ng/mL and the limit of detection (LOD) was 0.03 ng/mL, which shows high accuracy and specificity.

6.3 Immunosensors for Detection of Infectious Disease

Infectious diseases have been increased rapidly in recent years, causing high fatality rates due to incorrect diagnosis, delay in therapy, and many other complications [182, 183]. Globally, it accounts for approximately 40% of the overall 50 million annual deaths and is the main cause of death in many developing nations. Pathogenic microorganisms such as bacteria, viruses, and fungi are responsible for infections. Most infections can spread easily with the possibility of outbreaks or pandemics [184].

To identify a pathogen, rapid and accurate diagnostic devices/instruments are the prime concern. An accurate, rapid diagnosis can minimize hospitalization needs with a high impact on medical costs. Most of the existing POC tests consist of immunoassays: agglutination, immunochromatographic, and immunofiltration tests [185]. The diagnostic devices based on immunosensor could be more useful at home or in doctors' offices, as it permits rapid diagnostics, allowing the quick establishment of treatment and fast recovery by the patients. Most of the biosensors listed in the literature and available in the market are based on electrochemical techniques. The majority of these devices use nucleic acids as biorecognition components derived from the pathogenic agent, based on hybridization processes with a complementary DNA strand immobilized onto transducers, intercalating redox mediators in double-stranded DNA, or label-free detection mainly based on impedimetric measurements [186].

6.3.1 Influenza

Influenza is the most common acute respiratory infection caused by influenza type A, B, and C viruses. "Influenza viruses are classified into type A (infecting a large variety of species together including humans, pigs, horses, ocean mammals, and birds), type B (mostly infects humans), type C (almost exclusively infect humans), and type D (only infect cattle) types." Among these, influenza A viruses are the foremost serious and are liable for seasonal epidemics [187]. Extensive efforts have been made in the detection of the flu virus by health organizations, government agencies, academia, and independent laboratories worldwide. Over the past few decades, these efforts are starting to drive a move in strategy, i.e., from culture-based serological assays to genetic characterization methods and new optical and electrical biosensors. Among these methods, the polymerase chain reaction (PCR) and related techniques, including reverse transcription PCR and real-time PCR (rtPCR) have been broadly connected in biomedical research facilities for recognizing and measuring gene expression profile of the flu virus [188].

Various immunosensors have also been developed in the last two decades for rapid POC detection. In probe molecules-based detection, specific antibodies (monoclonal/polyclonal) are attached to the sensing plates, which bind to corresponding targets via antibody–antigen interactions [189]. Recently, the use of monoclonal antibodies in the development of immunosensor has increased dramatically. Many studies have also shown human monoclonal antibodies with neutralization ability and its assorted reactivity for subtyping influenza HA proteins or entirety infection particles [145, 186, 190].

Many impedimetric immunosensors were developed by exploring the principle of high-affinity antibody–antigen interactions for influenza viruses. Su et al. [191] demonstrated the utility of specific monoclonal antibodies for the detection of influenza A virus from swabs samples using the wash-free magnetic bioassay method. The LOD was 0.3 nM for nucleoprotein and 250 TCID50/mL for the spiked protein of influenza virus A.

Jarocka et al. [192] developed an impedimetric immunosensor with immobilized recombinant HA antigens, which can detect up to picograms of anti-HA antibodies

against influenza A H5N1 viruses in biological samples. It provides excellent sensitivity and very low limits of detection. The polyclonal antibodies were produced against the M1 protein (biomarker for influenza virus) and were used to detect all possible subtypes of influenza A viruses via electrochemical impedance analysis. The sensor shows quick and excellent activity with 1.0 fg/mL of LOD in saliva, corresponding to 5–10 viruses per sample [193].

6.3.2 Tuberculosis

Tuberculosis (TB) is a serious infectious disease caused by the aerobic, gram-positive bacterium *Mycobacterium tuberculosis* (*M. tuberculosis*). It is generally curable if diagnosed correctly and in a timely manner. However, the lack of effective and accessible point-of-care (POC) tests hindered the systematic screening of TB [182, 183, 194, 195]. Tuberculosis required more emphasis on the development of newer, more rapid, and sensitive diagnostic methods. Till now, various diagnostic methods have been developed for rapid detection of *M. tuberculosis*, like PCR, ELISA, Line probe assay, automated culture system, flowcytometry, etc. [182]. Since last two decades, various immunosensor technologies, i.e., screen-printed carbon electrodes [196], graphene-polyaniline mediated electrochemical [197], immunofluorescence microtip sensor [198], nanoparticles mediated sensors [199, 200] were developed for the detection of tuberculosis. Although, these sensors provide quick and fast detection of antibodies specific to *M.tuberculosis*, that could be used as POC test in primary health care center [183, 201–204].

6.3.3 Sexually Transmitted Diseases (STDs)

Sexually transmitted diseases (STDs) are viral or bacterial infections, transmitted from one person to another via unprotected sexual contact. HIV/AIDS, hepatitis B, herpes, and human papillomavirus seem to be the most common viral STDs, whereas gonorrhea, chlamydia, and syphilis are diagnosed as the largest bacterial infections [205]. A series of immunosensor has been developed against organisms responsible for STDs, i.e., HIV [206], *Neisseria gonorrhoeae* [207], syphilis [208], Chlamadia [209], etc.

6.3.3.1 Human Immunodeficiency Virus

The "human immunodeficiency virus (HIV) causes acquired immunodeficiency syndrome (AIDS)" and is transmitted primarily by unprotected sexual intercourse or the use of contaminated syringes [205]. A rapid, label-free capacitive immunosensor has been developed by Teeparuksapun et al. for the detection of p24-Ag (HIV-1 capsid protein) in serum soon after infection. The linear association was observed from 2.4×10^{-6} to 2.4×10^{-3} pg/mL with a LOD of 7.9×10^{-8} pg/mL [210]. Another electrochemical ELISA-type immunosensor was developed using HIV-1 gp41 and HIV-2 gp36 to detect HIV-1 and HIV-2 antibodies from patient serum/plasma. The sensor detects over a wide range of antibody concentration range (0.001–1 μg/mL), with a LOD of 1 ng/mL (6.7 pM) for both HIV-1 and HIV-2 [211].

6.3.3.2 Hepatitis

Hepatitis is an inflammatory condition of the liver caused by several types of hepatitis viruses designated from A to E. Hepatitis B and C are chronic diseases responsible for cirrhosis and liver cancer [212]. An optical immunosensor was designed at the beginning of 2005 to detect hepatitis C virus-specific antibodies using the photo immobilization method. Since then, significant progress has been made toward the advancement of sensing device development [213].

Recently, an electrochemical immunosensor was invented based on Fe_3O_4 nanoflowers (Fe_3O_4 NFs) and heterogeneous chain reaction (HCR) signal amplification technology for the fast and efficient detection of hepatitis B surface antigen (HBsAg) in serum samples. The developed sensor displays a wide linear detection range of 0.5 pg mL^{-1}–0.25 ng mL^{-1}. The low detection limit was 0.16 pg mL^{-1} with admirable constancy, accuracy, and reproducibility [214].

6.4 Immunosensors for Drug Safety

Therapeutic monitoring is essential for drug adjustment to reach optimal efficiency and minimal toxicity of the drug. Ideally, the concentration of drugs should be quantified at the location of the receptor but owing to its inaccessibility; drug concentrations are measured in body fluids such as serum, plasma, saliva, urine, or cerebrospinal fluids [215]. The most widely used drug detection techniques such as HPLC, GC-MS, LC-MS, radioimmunoassay, and chemiluminescence are time- and reagent-consuming, require trained staff, and complex pretreatment of biological samples. Thus, there is a need to develop a modern, minimally invasive, and handheld drug detection system that could be useful in homes, laboratories, and clinics.

Yang et al. [93] developed a simple immunosensor to detect clenbuterol using gold nanoparticles and QDs for enhanced signals. Using EIS and ECL emission, AuNP/ovalbumin-clenbuterol/anti-clenbuterol-QDs sensor was characterized that achieved LOD of 0.0084 ng/mL in the range of 0.02–50 ng/mL. An impedimetric immunosensor mediated 3-mercaptopropionic acid SAMs was developed to detect ketamine, a drug used for anesthesia. The developed sensor can detect 0.41 pmol/L of ketamine in clinical samples [216]. A similar approach has also been used to develop a sensor for detecting ciprofloxacin, a drug widely used for the treatment of pulmonary, urinary, or digestive infections, with a LOD of 10 pg/mL being obtained [217].

Developing innovative technologies for the rapid quantification of the drug is often essential in managing drug formulations in the pharmaceutical industry. A label-free immunosensor was developed using graphite oxide as an immobilization platform for antibodies specific to acetaminophen. The sample preparation steps were examined by several electrochemical techniques such as SWV, EIS, and EQCM, and the LOD found by SWV measurements was 0.17 μM [19].

Another major social and health issue is drug abuse and nobbling. The most commonly used doping agents are “beta-blockers, steroidal hormones, growth

hormones, theophylline and derivatives, peptides and methamphetamines." There is a growing interest in developing quick, sensitive, and specific screening tests, especially in sports, for the on-site detection of doping agents. Currently, numerous kits are commercially available to detect such drugs from body fluids such as saliva and urine. For example, Oratect, a gold particle-based ICTs immunoassay is used to detect marijuana (THC), cocaine, amphetamines, opiates, and methamphetamines [218]. A simple and sensitive electrochemical sensor was invented by scientists for the real-time detection of two lying drugs: morphine and methamphetamine. The sensor was designed using the immobilization of 3-mercaptopropionic acid on a gold electrode, which helped bind antibodies specific to the targeted drugs. "The sensors precisely detected morphine and methamphetamine in the linear range of 4–80 pg/L and 20–200 pg/L, respectively, with a LOD of 0.27 pg/L and 10.1 pg/L" [189].

6.5 Immunosensors for National Security

Immunosensors are used in military and defense to detect biological or chemical warfare agents, including a wide variety of synthetic chemicals, natural or animal toxins, and bacterial exotoxins capable of damaging or killing humans [219]. For example, "*Bacillus anthracis, Francisella tularensis, Brucella* sp., *Yersinia pestis, staphylococcal enterotoxin B,* botulinum toxin, and orthopoxviruses are typical biological warfare agents." Immunosensors are also used to detect various types of explosives/bombs and thus save millions of lives worldwide [220, 221]. "Military explosives mainly constitute nitroexplosives, such as 2,4,6-trinitrotoluene (TNT), dinitrotoluene (DNT), and hexogen (RDX)". Due to its low vapor pressure at room temperature, the identification of nitroexplosive vapors remains a problem. Immunosensors may also be used to identify volatile nitro vapors at a highly sensitive and precise location. Immunosensors can also track soldiers' health, response to the dietary shift, fatigue, environmental factors, etc., to improve soldier efficiency. Immunosensors will play an essential role in future military operations because of their sensitivity, selectivity, lower costs, scale, weight, and versatile on-site deployment [222].

7 Challenges and Prospects of Immunosensor

The number of immunosensors and their implementation have been expanded with modern approaches such as "magnetic nanoparticles, quantum dots, carbon nanotubes, noble metal nanoparticles, or hybrid nanomaterials," either as labels or immobilization platforms [175]. These materials provide important advantages, such as strong biocompatibility that preserves bioreceptor function, higher surface-to-volume ratio, i.e., rises in the number of immobilized bioreceptors, and exceptional conductivity and optical proprieties. The higher sensitivity and specificity have made it possible to use these immunosensors for in vitro as well as in vivo applications. Even though significant advancement has been made in the field of immunosensors,

novel techniques are still required to boost the sensitivity, specificity, and simplicity of these devices that would satisfy the exigent criteria of clinical diagnosis or industry. The vast majority of immunosensor mentioned in this chapter and used today are focused on using antibody, antigens, and aptamer as a recognition component. The high selectivity is a key advantage of the immunosensor, while inconsistency, cost, and the need to use them in combination with a mediator system limit their uses in clinical science. Another challenge is a need for miniaturization and integration on a computer platform that can detect biomarkers or pathogens in real time. In the last decade, remarkable progress has been made in the field of nanotechnology; novel sensor could be designed with new technologies of nanoparticles and nanostructured surfaces for a wide range of antigen detection using electrochemical strategy. Further, portable, cost-efficient, and accurate sensors may be produced via multiplexing of various antigens/protein detection devices, especially those coupled with microfluidics that could guarantee accurate detection, especially panels of cancer biomarkers detection in blood, urine, saliva, or other body fluids. Though incorporating electrochemical immunoassays in microfluidic platforms can produce a scalable platform for the construction of devices for clinical diagnostics, ultimately, the development and advancement of these systems would lead to faster clinical decision-making, reducing the patient's stress, and lower costs for healthcare.

8 Summary

We have discussed the various aspects of the immunosensor in this chapter. Although, the concept of straightforward detection of the binding event is simple and elegant, the advancement of such a gadget is overwhelming. The concept is very elegant, but requires a multidisciplinary approach that combines the expertise of immunologists, immuno-chemists, engineers, and materials scientists to develop the intimate interface between the biologic component and the transducer. Some critical parameters; (1) selection of high-affinity antibodies would be a critical parameter, failing with loss in sensitivity or specificity of immunosensor; (2) interface component also could become contaminated on contact with body fluids, leading to increased response times; (3) Behavior of biological material with selected transducer should be understood well under experimental conditions.

It is worth noting that scientists should also think about commercializing expect such as affordable and reliable P.O.C. devices to facilitate biomedical care in developing countries. Intrinsically, it will add an extra advantage to public health and reduce the healthcare sector's financial load.

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