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Review

Applications of electrochemical immunosensors for early clinical diagnostics

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ABSTRACT

Cancer and cardiovascular diseases are the major threats to global health. Hence, there is a growing demand for a range of portable, rapid and low cost biosensing devices for the detection of these diseases. Electrochemical immunosensors are simple, rapid, reliable and inexpensive devices and they have sensitive detection limits to monitor both levels of the biomarkers in normal and patient serum. Due to the specific binding of antibody to its corresponding antigen, immunosensors based on antibody–antigen interaction are one of the most widely used analytical techniques in the quantitative detection of these diseases. The changed levels of markers in patients are associated with diseases. In this article the biosensors and biomarkers, which were commonly used in terms of monitoring the diagnosis and treatment of cancer and cardiac diseases, are reviewed. In addition, the developed biosensors are compared in terms of precision, reproducibility, regeneration, stability and specificity.

Contents

1. Introduction

The pioneering study of Clark and Lyons, more than four decades ago, shed light on some analytical researchers in designing of biosensors, which perform as economical and fast tools for clinical, chemical, environmental and pharmaceutical studies. Because of its simple use and portability in relatively complex samples, biosensors offer a potential alternative to advanced bioanalytical systems [1].

A biosensor is composed of two components, a bioreceptor and a transducer. First part, the bioreceptor is a biomolecule that recognizes the target analyte, and second part the transducer converts the recognition event into a measurable signal [2]. Immunosensors are antibody–antigen based affinity biosensors, in which the detection of antigen as a target analyte is a result of the specific binding of the antigen to particular region of an antibody on the electrode surface [3]. Also, in electrochemical immunosensors antibody acts as a bioreceptor and antigen acts as a target analyte and transducer can be able to quantify the antigen concentration by using amperometric, potentiometric, impedimetric or conductometric signals. Fig. 1 shows a schematic presentation of an electrochemical immunosensor.

In recent years, optical and electrochemical detection methods have been used in early clinical diagnosis. Optical detection transduction method is less sensitive when coupled with radioimmunoassay, has short half-life of radioactive agents, concern of health hazards, and has disposal problems. On the other hand, electrochemical

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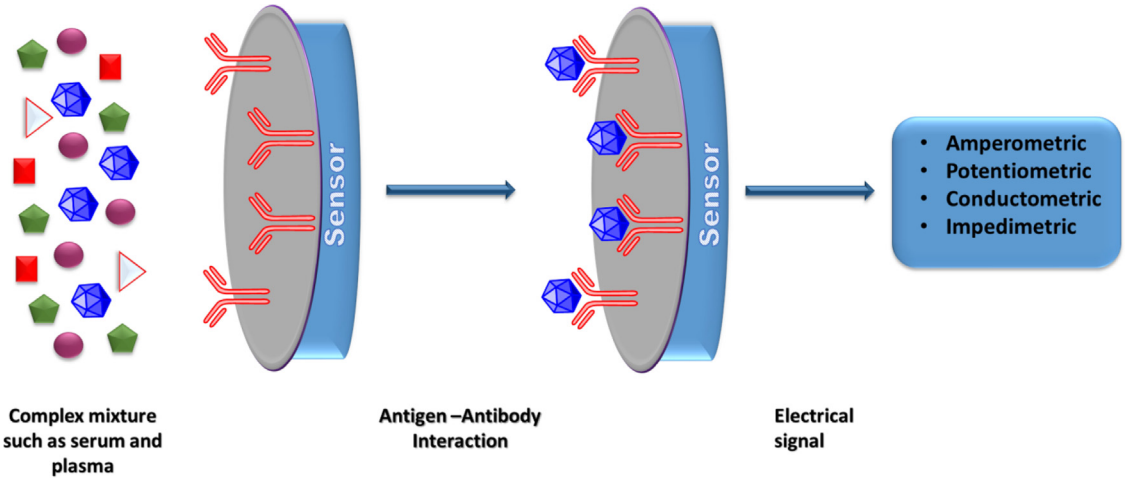

Fig. 1. Schematic representation of electrochemical immunosensor.

detection method shows no similar problems and electrochemical immunoassays and immunosensors enable fast, simple, and economical detection [4].

Electrochemical immunosensors are used as care devices since they are portable, simple, easy to use, cost effective and disposable in most cases [5,6]. Compared with traditional immunoassay methods, electrochemical immunosensors are specific, simple and convenient, and can offer multitarget analyses and miniaturization. They can perform in situ, real-time, automation detection [7].

Specificity for any given biomarker is often achieved by use of antibodies. The specific binding of an antibody to its target antigen in a complex mixture such as serum and plasma provides the detection and quantification of diseases at levels as low as picograms (pg) [8]. Also, this is another advantage of immunosensors.

In this study the biosensors and biomarkers, which were commonly used in terms of monitoring the diagnosis and treatment of cancer and cardiac diseases, are reviewed. And also, it focuses on recent development for tumor markers and cardiac markers testing and monitoring in clinical diagnosis. However, a wide range of researches have been published in this area. This review is limited to recent publication within the past nine years. Most reviews have been organized around only tumor or cardiac biomarkers and their diagnosis of biosensors. Herein we present different biomarkers used in several publications of electrochemical immunosensors for tumor and cardiac diseases and highlighted the major clinically relevant parameters, such as their detection limit/range and designing of bioassay. Moreover, in this review, we emphasize on the opportunities for further improvement in tumor and cardiac diseases diagnostic and treatment monitoring.

2. Electrochemical immunosensors

An antibody based biosensor was applied for the first time in the 1950s, leading the possibility of immune-diagnosis [4]. These types of biosensors have high specificity and low limit of detection due to their extreme antibody affinity to their antigen. Antibodies are proteins produced by the immune system. However, antigens can be a variety of different molecules, from protein to DNA, lipids, etc [6].

In developing immunosensors, the immobilization of antibody is an important step because antibody acts as the recognition element for antibody–antigen reaction. The performance of the detection and antigen binding capacity can be increased by a proper antibody surface. Thus the choice of the antibody immobilization method is very important in the design of an immunosensor. Several methods including physical [9–10] and chemical adsorption have been used for the preparation of oriented antibody molecular layers on the surface of the transducer. Self-assembly (SAM) technique has been used as chemical adsorption method for immobilization of antibody [11–13]. In this technique, a self-assembly monolayer is fixed on the surface through chemical bonds. Then the antibody is covalently attached to the monolayer by using cross-linkers.

Transducer types used in immunosensors are electrochemical (amperometric, potentiometric, conductometric, capacitative), optical (fluorescence, luminescence, refractive index), piezoelectric or calorimetric. The electrochemical immunosensors rely on the measurements of currents and/or voltage to detect binding between antibody and antigen.

In potentiometric measurements, the potential difference between a working and a reference electrode is determined by a voltammeter when there is no significant current flowing through them. The potential difference is measured due to the oxidation and reduction of the species in sample solution. The transducer may be an ion selective electrode (ISE) based on thin film or selective membranes as recognition elements. Analytical information is obtained when the ISE convert the biorecognition process into a potential signal.

In amperometric measurements, a current occurs as a result of electrochemical oxidation or reduction of an electroactive species. This type of measurement is taken by maintaining a constant amplitude voltage at working electrode (gold, platinum, and carbon) related to reference electrode, under a fixed potential, current pass through sample [8].

In impedimetric measurements, when biorecognition elements occur at the modified surfaces, the interfacial properties change. Thus impedimetric immunosensors can be used to determine quantitative parameters of electrochemical properties. Electrochemical reactions, known as electron transfers at the electrode surface, involve electrolyte resistance, adsorption of electroactive species, charge transfer at the electrode surface, and mass transfer from the bulk solution to the electrode surface. Each reaction process represented by an electric circuit consists of resistance, capacitors, or constant phase elements combined in parallel or in series. The most favorite model of electric circuit for a simple electrochemical reaction is the Randles–Ershler electric equivalent circuit model, consisting of electrolyte resistance (R_s), charge-transfer resistance (R_{ct}) at the electrode/electrolyte interface, double-layer capacitance (C_{dl}), and mass transfer resistance (R_{mt}), also Warburg impedance (W) [2,14].

In conductometric measurements, there is a relationship between a biorecognition event and conductance. While a reaction, a change in the ionic species concentration leads to change

the solution's electrical conductivity or current flow. A conductometric biosensor consists of two metal electrodes (usually platinum or silver) separated by a certain distance. An ohmmeter (or multimeter) is used to measure the change in conductance between the metal electrodes [15].

3. Tumor and cardiac markers

The National Cancer Institute (NCI) defines a biomarker as "a biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process or of a condition or disease. A biomarker may be used to see how well the body responds to a treatment for a disease or condition". Biomarkers can derive from various molecular origins, including DNA (i.e., specific mutation, translocation, amplification, and loss of heterozygosis), RNA, or protein (i.e., hormone, antibody, oncogene, or tumor suppressor) [16]. Different diseases are known to produce or alter the levels of different biomarkers. Therefore, sensitive and rapid diagnosis methods are very important for their detection. Tumor biomarkers are potentially one of the most valuable tools for early cancer detection, accurate pretreatment staging, determining the response of cancer to chemotherapy treatment, and monitoring disease progression. Also in tumor process, changed levels of tumor markers in patients are associated with certain tumor [17]. In addition, the early and quick diagnosis of cardiovascular disease is important not only for patient survival but also saving cost and a lot of time. Discussion of some of the major tumor and cardiac markers and developed for immunosensors for these diseases are presented below.

3.1. Tumor markers

Cancer is defined as abnormal and uncontrolled cell growth due to an accumulation of specific genetic and epigenetic defects and it is one of the main causes of mortality worldwide. Many factors including exposure to carcinogenic chemicals, radiation, genetic and environmental factors cause cancer [16]. There are more than 200 distinct diseases associated with cancer affecting different parts of the body and so clinical test of cancer is very complex [5]. In clinical analysis sensitive, precise, and accurate assays for the determination of tumor markers with low concentration level in complex biological samples are necessary for effective early diagnosis and treatment of cancer [18–20].

In the absence of a tumor, the tumor marker is of low level. Even a small tumor forms, levels of some markers rise. Thus the limits of detection (LODs) are important for early screening of a small tumor [7]. Methods and strategies based on biochemistry, immunology and molecular biology have been developed and used for the determination of tumor markers in human serum. Immunoassay techniques, which show highly specific molecular recognition, are frequently used methods in clinical quantitative detection of tumor markers. Radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), fluoroimmunoassay, chemiluminescent immunoassay, and electrochemiluminescent immunoassays are present among these techniques. Beside the sensitivity, precision and selectivity advantages, there are several disadvantages of these techniques. For instance they have radiation hazards, high cost, require qualified personnel and sophisticated instrumentation and they are also time consuming. Moreover, existing diagnostic tests (e.g., microtiter-plate ELISAs) are not sensitive enough for screening cancer at an early stage and can only detect proteins at levels corresponding to advanced stages of disease [7,21]. Compared with conventional immunoassay methods, electrochemical immunosensor offer several advantages, such as high sensitivity, fast analysis, simple pretreatment, small analyte volume, simple instrumentation, and minimal manipulation and therefore they are largely used [17,22].

Biomarkers can be presented intracellular or extracellular. Intracellular markers need to be collected and enriched if their concentration is very low [5]. In Table 1, the most common biomarkers is listed and in Table 2, the thresholds of some basic tumor markers in human serum are listed [7,23].

Carcinoembriyonic antigen (CEA) is a cell surface glycoprotein with a molecular weight of about 200 kDa [24,25] used as a biomarker for clinical diagnosis of colon tumors, breast tumors, ovarian carcinoma [25–27], gastric, pancreatic, lung carcinomas [18,25,26]. Therefore, CEA is a broad spectrum multi-tumor marker for clinical diagnosis [18]. The level of CEA for a non-smoker adult is < 2.5 ng/mL and for a smoker < 5.0 ng/mL [26,28]. A rising CEA level indicates progression or recurrence of the cancer [26,28,29,30].

Prostate-specific antigen (PSA) is one of the first tumor biomarkers for screening and diagnosing prostate cancer [16,31]. PSA is a 32–33 kDa single-chain glycoprotein [31]. A very low amount of this substance reaches the bloodstream forming free PSA (fPSA) and PSA complexed to a-1-antichymotrypsin (PSA-ACT). Total PSA (tPSA) is the sum of f-PSA and PSA-ACT complex in serum. tPSA level, in healthy males, is usually less than 4 ng/mL and increases in serum during prostate cancer [32].

Tumor marker, α-1-fetoprotein (AFP) is an oncofetal glycoprotein [33,34,35,36] with a single-chain alpha globulin containing

Table 2
The thresholds of basic tumor markers in human serum [5,7].

Tumor markers	Thresholds	Tumor markers	Thresholds
NSE	12.5 mg/L	CEA	3 μg/L
PSA	4 μg/L	CA125	35 kU/L
GST	3.2 U/L	CA153	25 kU/L
ALP	0	CA27-29	36.4 kU/L
CT	100 ng/L	CA549	11 kU/L
SCCA	1.5 μg/L	CA19-9	37 kU/L
Ferritin	250 μg/L	CA50	14–20 kU/L
hCG	5.0 IU/L	CA242	20 kU/L
AFP	10 μg/L	CA72-4	6 kU/L

Table 1
Common biomarkers utilized for cancer detection [16].

Type of cancer	Biomarker
Breast	BRCA1, BRCA2, CA 15-3, CA 125, CA27.29, CEA, NY-BR-1, ING-1, HER2/NEU, ER/PR
Colon	CEA, EGF, p53
Esophageal	SCC
Liver	AFP, CEA
Lung	CEA, CA 19-9, SCC, NSE, NY-ESO-1
Melanoma	Tyrosinase, NY-ESO-1
Ovarian	CA 125, HCG, p53, CEA, CA 549, CASA, CA 19-9, CA 15-3, MCA, MOV-1, TAG72
Prostate	PSA

590 aminoacids and 3.4% carbohydrate and has molecular weight of approximately 70 kDa [34]. In the blood of healthy adults, AFP presents less than 20 ng/mL [35]. The concentration increases in response to hepatocellular carcinoma [33,34,37,38], teroblastoma [34,37,38] and especially liver carcinoma.

Carbohydrate antigen 19-9 (CA 19-9) is a vital carbohydrate tumor marker [39,40]. It is commonly used for many malignancies, such as colorectal, gastrointestinal, hepatic carcinomas and pancreatic and biliary cancer [39].

Myeloctomatosis oncogene (c-Myc) oncoprotein, a product of c-Myc proto-oncogene, regulates the transcription of genes involved in normal cell growth, differentiation, and apoptosis [41]. The level of c-Myc oncoprotein increases when human cancers including breast cancer, lung cancer, melanoma and lymphoblastic leukemia develop [42].

Cancer antigen 125 (CA125) is a membrane mucin-like glycoprotein greater than 200 kDa, and has been used for monitoring the epithelial ovarian tumors [43,44].

Neuron specific enolase (NSE), a widely used biomarker for small-cell lung cancer, neuroblastoma, and neuroendocrine cancers, increases up to 1 μg/mL in serum of serious cancer patients [45,46].

Epidermal growth factor receptor (EGFR, ErbB-1; HER1 in humans) is a cell trans-membrane glycoprotein located at the cell surface. EGFR is useful in many tumors of epithelial origin, such as non-small cell lung cancer (NSCLC), breast, head and neck, gastric, colorectal, esophageal, prostate, bladder, renal, pancreatic, and ovarian cancers [47].

Cancer antigen 15-3, as a breast-associated mucin, and it is used as a biomarker for breast carcinoma patients with distant metastases [44,48,49].

Murine double minute 2 (MDM2) is a proto-oncogene, which can be amplified and over-expressed in a wide range of neoplasm tumors, such as sarcomas, lymphomas, breast cancers, lung cancers, and testicular germ cell tumors, 10% of glioblastomas and astrocytomas, and 40% of oral squamous cell carcinomas [50].

Squamous cell carcinoma antigen (SCCA), which is subfraction of the tumor antigen TA-4, is a tumor marker of cervical cancer. It is also associated with other types of cancer with epithelial or endodermal origins, including lung cancer, head and neck cancer, melanomas, and hepatocellular carcinoma. Squamous cell carcinoma antigen (SCC-Ag) is a glycoprotein with isoforms ranging from 45 to 55 kDa. The serum level of SCC-Ag is increased when the tumor size gets bigger or the disease reoccurs [51].

Human chorionic gonadotrophin (hCG) is an important diagnostic marker of pregnancy and one of the most important carbohydrate tumor markers. It is increased when abnormal placental invasion and placental immaturity occurs [52,53].

Human epididymis-specific protein 4 (HE4) is a new ovarian biomarker, originally predicted to be a protease inhibitor involved in sperm maturation. When HE4 over expresses in ovarian neoplastic tissue its level rises in the serum of patients with ovarian cancer. HE4 is not only expressed in the early stages of the disease, but is also an early indicator of disease recurrence [54].

HER-3 is a type of transmembrane growth factor receptor, which can activate intracellular signaling pathways in response to extracellular signals. Because of HER-3 being an incomplete receptor functionally, it is a dependent protein. Studies show that tumor progression in breast, ovarian, and pancreatic cancers, gastric carcinoma, malignant melanoma and metastases, and head and neck squamous cell carcinoma are linked to over expression of HER-3 [55].

VEGF is an important regulator of angiogenesis and vascular permeability, and is a powerful mitogen for endothelial cells. In breast cancer, VEGF is used as a marker of unfavorable prognosis. However, angiogenesis is essential for growth and tumor progression. It has been observed that patients with metastasis have higher levels of serum VEGF [12].

The developed immunosensors for cancer diagnosis in literature are summarized in Table 3.

Limbut et al. [56] developed an immunosensor for AFP detection. They used self-assembled thiourea monolayer (SATUM), self-assembled thioctic monolayer (SATAM) and 3-mercaptopropionic acid (SAMPAM) for covalent coupling of anti-AFP. They reported that all systems have given the same linear range with nearly the same sensitivity and detection limits. They obtained that all the systems had good stability, could be regenerated and reused up to 48 times with good reproducibility. On the other hand thiourea, which is cheaper than thioctic acid and 3-mercaptopropionic acid, is certainly a good alternative to be applied for the immobilization of antibodies on gold surfaces [56].

Precision, reproducibility, regeneration, stability, and specificity are the important parameters in terms of monitoring the quality of a newly developed biosensor.

The precision of the electrochemical immunosensor was evaluated by calculating the intra- and inter-assay variation coefficients (CVs). The CVs of intra- and inter-assay were similar; therefore precision and reproducibility of the immunosensors were acceptable. Zhong et al. [22] developed an electrochemical immunosensor for CEA detection. They calculated the CVs of intra- and inter-assays. The intra-assay precision of the analytical method was evaluated by analyzing four concentration levels five times per run. The CVs of intra-assay were 5.7, 4.9, 4.1 and 6.5% at 0.1, 10, 100 and 300 ng/mL CEA, respectively. Similarly, the CVs of the inter-assay were 5.3, 7.8, 6.7 and 6.1% at 0.1, 10, 100 and 300 ng/mL CEA, respectively. Another method for reproducibility of the immunosensor is calculating of relative standard deviation (RSD) in different electrodes for each concentration of antigen [22]. Yu et al. [82] and Han et al. [95] developed an electrochemical immunosensor for CEA detection. For all these immunosensors, the reproducibility of the response was examined by analysis of the same concentration of CEA (20 ng/mL) using independent determinations. The immunosensors have acceptable reproducibility with a relative standard deviation of 0.45 and 2.45% (less than 5.0%), respectively.

Yang et al. [111] and Li et al. [100] developed an electrochemical immunosensor for PSA detection. To evaluate the reproducibility of the immunosensors, they prepared a series of five electrodes for the detection of 1 ng/mL PSA and the relative standard deviation (RSD) of their measurements for five electrodes were 7.9 and 6.7%, respectively.

Regeneration is a key factor in their application and development of immunosensors; therefore many immunoanalysts are interested in reusing of immunosensors. Even though the antibody–antigen linkage can be broken under drastic conditions (e.g., in alkaline or acidic solutions or with chaotropic agents), the immobilized immunoreagents could also suffer from the functional damage or even be released from the immunosorbents [43,60]. Tang et al. [43] regenerated the immunosensor by dipping into versatile solution consisting of glycine–HCl (0.2 mol/L, pH 3.6) and NaCl (0.25 mol/L) for 5 min and then rinsing with a phosphate buffer solution of pH 7.0. Because of effectively dissociating the antigen–antibody complex and peeling the captured antigens off the immunosensors, they preferred glycine. Additionally, NaCl provides properly high ion-strength to partition of antibodies and antigens easily. After treatment with glycine solution and NaCl, anti-CA125 is still bound on the film surface and therefore again ready for antigen–antibody reaction. And they reported that no remarkable decrease was observed in current response after five measurements [43]. Zhuo et al. [93] rinsed immunosensor with 0.05 M NaOH and then used 0.05 M HCl, 4 M urea, 0.2 M glycine–hydrochloric acid (Gly–HCl) buffer solution to break bond between CEA and anti-CEA and the regenerated immunosensors were used to detect the same concentration. After first seven times regeneration by urea solution kept 95.97% of the original amperometric responses, first

Table 3
The developed immunosensors for cancer diagnosis in literature.

Biomarker	Immobilization strategies	Assay principle	Linear range	Limit of Detection	Reference
Alpha- fetoprotein (AFP)	Au/3-MPA/EDC/NHS/anti-AFP/1-dodecanethiol/AFP Au/thioctic acid/EDC/NHS/anti-AFP/1-dodecanethiol/AFP Au/thiourea/glutaraldehyde (glu)/anti-AFP/ethanolamine/1-dodecanethiol/ AFP	Capacitive	0.001–100 μg/l	10 ng/L	[56]
AFP	SPCE/chitosan (Chi)/HRP-labeled anti-AFP/AFP	Amperometric	0–150 ng/mL	0.74 ng/mL	[57]
Carcinoembriyonic antigen (CEA)	GCE/MWCNTs-Chi/AuNPs/anti-CEA/BSA/CEA	Differential pulse voltammetry	0.3–20 ng/mL	0.01 ng/mL	[17]
CEA	SPCE/Chi/colloidal Au/CEA/HRP-labeled anti-CEA	Amperometric	0.5–25 ng/mL	0.22 ng/mL	[58]
CA 19-9	Graphite electrode/cellulose acetate (CA)/CA 19-9+CA 125+thionine/HRP-labeled antibodies	Differential pulse voltammetry	0–144 U/mL	0.2 U/mL	[59]
Carcinoma antigen (CA 125)			0–150 U/mL	0.4 U/mL	
PSA	GCE/MWCNTs/ionic liquid/chitosan nanocomposite (MWCNTs/IL/Chi)/AuNPs-incorporated polyamidoamine dendrimer (AuNPs-PAMAM)/phtaloyl chloride (Ph)/anti-PSA+thionine/PSA/HRP-labeled anti-PSA	DPV impedimetric	0.05–80 ng/mL 5–25 ng/mL	0.001 ng/mL 0.5 ng/mL	[60]
CEA	ITO/polydopamine (PDA)/mesoporous silica nanoparticles (MSNs)-Ab1 (anti-CEA)/BSA/CEA/HRP-Ab2-AuNPs	Amperometric	10^{-2}–40 ng/mL	1.7 10^{-3} ng/mL	[61]
CEA	Au/o-aminophenol/glutathione (GSH) monolayer modified AuNPs/EDC/NHS/ anti-CEA/CEA	Impedimetric	0.5–20 ng/mL	0.1 ng/mL	[62]
CEA	ITO/reduced graphene oxide (rGO)/Thi/AuNps/anti-CEA/BSA/CEA	SWV	0.01–300 ng/mL	0.065 pg/mL	[63]
AFP	ITO/rGO/Prussian blue (PB)/AuNPs/anti-CEA/BSA/CEA			0.885 pg/mL	
CEA	ITO/polyvinyl alcohol (PVA)/ferrocene carboxylic acid encapsulated mesoporous silica (FCA-MPS)/anti-CEA/BSA/CEA	DPV	0–45 ng/mL	0.2 ng/mL	[64]
AFP	ITO/PVA/HRP-MPS/anti-AFP/BSA/AFP		1–90 ng/mL	0.5 ng/mL	
PSA	Glass fiber pad/anti-PSA-Quantum Dots (QD) (ZnS and CdSe)conjugates/ nitrocellulose membrane with second anti-PSA/BSA/PSA	SWV	0.05–4 ng/mL	0.02 ng/mL	[65]
PSA	Au nanowire (Au NW)/anti-PSA doped polypyrole/BSA/PSA	DPV	10 fg/mL–10 ng/mL	0.3 pg/mL	[66]
CA 125	GCE/Chi-poly(diallydimethylammonium chloride) (PDDA)-Prussian blue (PB)/ AuNPs/anti CA125/BSA/CA 125	DPV	2–100 U/mL	0.71 U/mL	[67]
AFP	Pt/gelatin/AgNPs/anti-AFP/glu/AFP	Potentiometric	2–197 μg/L	0.8 μg/L	[37]
CEA	Au/4-aminothiophenol (4ATp)/nano Au/monoclonal anti-CEA/BSA/CEA/HRP/ anti-CEA/AuNPs/Thi-4Atp@AuNPs	Amperometric	2 pg/mL–40 ng/mL	0.7 pg/mL	[18]
PSA	Au/cys/glu/capture anti-PSA/BSA/PSA/functionalized SiNPs (with anti-PSA and alkaline phosphatase (ALP)/glycine containing AgNO3 and ascorbic acid-2-phosphate	Amperometric	1–35 ng/mL	0.76 ng/mL	[31]
CEA	GCE/AuNPs decorated thionine/infite coordination polymer (AgNPs/Thi/ICP)/ anti-CEA/BSA/CEA	Amperometric	50- fg/mL–100 ng/mL	0.5 fg/mL	[68]
CEA	GCE/[Ag-AgO2]/SiO2/BSA/nano-Au/anti-CEA/BSA/CEA	Amperometric	0.5–160 ng/mL	0.14 ng/mL	[24]
AFP	Au/1,1′-bis-(2-phosphoethyl)-4,4′-bipyridinium dibromide-titanium dioxide nanoparticle)/AuNPs/anti-AFP/BSA/AFP	Amperometric	1.25–200 ng/mL	0.6 ng/mL	[33]
CEA	GCE/AuNPs-Thi-graphene oxide/anti-CEA/BSA/CEA	Amperometric	10–500 pg/mL	4 pg/mL	[69]
AFP	GCE/sulfhydryl viologen-AuNPs (SV-AuNPs)/anti-AFP/BSA/AFP	Amperometric	0.25–200 ng/mL	0.23 ng/mL	[70]
AFP	GCE/Gold nanowires (AuNWs)/ZnO nanorods (NRs)/Chi/anti-AFP/glycin-HRP-labeled AFP	Amperometric	0.5–160 ng/mL	0.1 ng/mL	[34]
CEA	Au/thiourea/anti-CEA/ethanolamine/CEA	Capacitance	0.01–10 ng/mL	10 pg/mL	[25]
AFP	GCE/nano-Au film/MWCNT-PDDA/DNA/Thi/nano-Au/anti-AFP/BSA/AFP	Amperometric	0.01–200 ng/mL	0.04 ng/mL	[35]
CEA	GCE/nano-Au film/Chi/nano-Au/anti-CEA /BSA/CEA	Amperometric	0.2–120 ng/mL	0.06 ng/mL	[71]
CA 19-9	3 DOMM Au electrode/Au-SiO2@Fe3O4 nanospheres/streptavidin/biotinylated-Ab1/BSA/CA 19-9/HRP-Ab2/Au@SB-15(Santa Barbara Amorphous 15)	Amperometric	0.05–15.65 U/mL	0.01 U/mL	[21]

Table 3 (*continued*)

Biomarker	Immobilization strategies	Assay principle	Linear range	Limit of Detection	Reference
AFP	GCE/Chi-TiO2-Gr films/AuNPs/anti-AFP/BSA/AFP	Amperometric	0.1–300 ng/mL	0.03 ng/mL	[72]
CEA	GCE/negatively charged polysulfonilic acid (PSAA)/toluene blue (TB)/nano-Au/anti-CEA/HRP/CEA	Amperometric	0.5–120 ng/mL	0.2 ng/mL	[73]
CEA	GCE/negative charged PDDA-wrapped CNTs (PDCNTs)/negative charged poly (sodium-*p*-styrene-sulfonate) (PSS)/gold nanoclusters/anti-CEA/BSA/CEA	Amperometric	0.1–160 ng/mL	0.06 ng/mL	[74]
CA 125	GCE/carbon nanofiber (CNF)/EDC/NHS/CEA antigen/HRP labeled anti-CEA/CEA	Amperometric	2–75 U/mL	1.8 U/mL	[75]
AFP	Carbon ionic liquid electrode (CILE)/amino functionalized graphene sheets (GR-NH2)/AuNPs/anti-AFP/BSA/AFP	Amperometric	1–250 ng/	0.1 ng/mL	[76]
c-Myc	Au/cys/glu/primary c-Myc antibody (C_{ab})/6-mercapto-1-hexanol (MCH)/ethylenediamine/target c-Myc oncoprotein (C_{ag})/AuNP labeled c-Myc antibodies (AuNPs-C_{ab})	Impedimetric	43 pM–43 nM	1.5 pM	[42]
CEA	Au/1,6-hexanedithiol (HDTS)/AuNPs/Staphylococcal protein (SPA)/anti-CEA/BSA/CEA	Impedimetric	1 pg/mL–100 ng/mL	0.1 pg/mL	[10]
CA 19-9	GCE/Chi-MWCNTs/GOD/Fe3O4@SiO2-Au@mSiO2/anti-CA 19-9/CA 19-9	CV	0.01–1.11 U/mL	0.004 U/mL	[39]
		DPV	11.11–476.11 U/mL		
CEA	GCE/PB/AuNPs/anti-CEA/CEA/horseradish peroxidase conjugated anti-CEA anti-CEA antigen (HRP-anti-CEA)/NCAuPt	Amperometric	0.01–200 ng/mL	0.11 pg/mL	[77]
CA 125	Gold rod electrode/11-mercaptoundecanoic acid/1-dodeccanethiol etholic acid/anti-CA 125/CA 125	Capacitive	0.05–40 U/mL	0.05 U/mL	[78]
CEA	SPE/polyethyleneimine wrapped multiwalled carbon nanotube (MWCNT-PEI)/anti-CEA/ethanolamine + BSA/CEA/anti-CEA tagged ferrocene carboxylic acid encapsulated liposomes	Voltammetric	5.10^{-12}–5.10^{-7} g/mL	5.10^{-12} g/mL	[79]
CEA	SPCEs/CNTs-Chi/AuNPs/anti-CEA(Ab1)/BSA/CEA/SiO2 nanosphere + GOD + anti-CEA(Ab2)	Amperometric	5 pg/mL–2 ng/mL	3.2 pg/mL	[80]
	SPCEs/CNTs-Chi/AuNPs/anti-AFP (Ab1)/BSA/AFP/SiO2 nanosphere + GOD + anti-AFP(Ab2)	Amperometric	5 pg/mL–1 ng/mL	4.0 pg/mL	
CEA	GCE/thionine functionalized CNTs(Thi-CNTs/AuNPs/anti-CEA(Ab_1)/BSA/CEA/HRP labeled signal antibody-AuNPs-PAN@CNT nanocomposite (HRP-Ab_2-AuNPs-PAN@CNT)	Amperometric	0.02–80 ng/mL	0.008 ng/mL	[81]
CEA	GCE/nano-Au/nickel hexacyanoferrates nanoparticles (NiHCFNPs)/nano-Au/anti-CEA/BSA/CEA	Amperometric	0.5–160 ng/mL	0.1 ng/mL	[28]
CEA	Carbon ionic liquid electrode (CILE)/rGO/poly(L-Arg)film/AuNPs/anti-CEA/BSA/CEA	Amperometric	0.5–200 ng/mL	0.03 ng/mL	[82]
CA 125	GCE/Au-CA 125/cellulose acetate (CA)/HRP-labelled CA 125 antibody/CEA	Amperometric	0–30 U/mL	1.73 U/mL	[83]
CEA	GCE/sulfonated graphene sheets(HSO_3-GS)/Thi/Chi-PdCu/anti-CEA/BSA/CEA	Amperometric	0.01–12 ng/mL	4.86 pg/mL	[27]
CEA	GCE/gold nanoparticle-graphite composite (Au-GN)/HRP labelled anti-CEA antibody (HRP-anti CEA)/CEA	Amperometric	0.1–80 ng/mL	0.04 ng/mL	[26]
CA 125	CPE/AuNPs-Thi mixture/anti CA-125/BSA /CA 125	Amperometric	10–30 U/mL	1.8 U/mL	[43]
CEA	Nano Fe_3O_4 modified CPE/(3-mercaptopropyl)trimethoxysilane (MPTS)/nanogold/CEA/BSA/HRP labeled anti-CEA	Amperometric	1–55 ng/mL	0.13 ng/mL	[84]
CEA	ITO/hyper branched polyester(HPDMPA-COOH)/EDC/NHS/Ab_1/BSA/CEA/HRP labeled antibody conjugated AuNPs (HRP-Ab_2-AuNPs)	Amperometric	0.1–80 ng/mL	2.36 pg/mL	[85]
CEA	GCE/$(AuNPs\text{-}AgNPs)_n$/Thi/AuNPs/anti-CEA/BSA/CEA	Amperometric	10 pg/mL–100 ng/mL	3 pg/mL	[19]
AFP	Pt disk electrode/poly(3,4-ethylenedioxythiophene)(PEDOT)/AuNPs/Azure I/ZnSe QDs/anti AFP/HRP/AFP	Amperometric	5.10^{-5}–250 ng/mL	1.1 fg/mL	[38]
CEA	Magnetic carbon paste electrode (MCPE)/magnetic beads (MB)/o-phenylene diamine (PPD)/AgNPs/anti-CEA/BSA/CEA	Amperometric	0.01–40 ng/mL	0.001 ng/mL	[86]
Neuron specific enolase (NSE)	GCE/SWCNT/EDC/NHS/NSE/BSA/anti-NSE primary antibody/alkaline phosphatase conjugated secondary antibody labeled gold nanoparticle (AP-anti IgG-AuNPs)/α-naphthyl phosphate (α-NP)	Amperometric	0.1 ng/mL–2 μg/mL	0.033 ng/mL	[46]
CEA	GCE/Au-TiO_2/anti-CEA/BSA/HRP-Ab_2 labeled Pt hallow nanospheres (HRP-Ab_2-HPtNPs)/CEA	Amperometric	0.02–120 ng/mL	12 pg/mL	[30]
PSA		Amperometric	–	–	[87]

Table 3 (*continued*)

Biomarker	Immobilization strategies	Assay principle	Linear range	Limit of Detection	Reference
	Au/4-(2-(4-acetylthio)phenyl)ethynyl) benzoic acid (APBA)/EDC/NHS/PAMAM/ Ab1(monoclonal antibody)/PSA/polyclonal antibody labeled biotin (Ab_2)/ alkaline phosphatase-labeled avidin				
CEA+AFP	GCE/Chi-AuNPs/capture anti-CEA ($Ab_{1,1}$) +capture anti-AFP ($Ab_{1,2}$)/BSA/ CEA+AFP/biofunctional carboxyl graphene nanosheets –toluidine blue-$Ab_{2,1}$(CGs-TB- $Ab_{2,1}$)+ CGs-TB-$Ab_{2,2}$	Amperometric	5–60 ng/mL	0.01 ng/mL–0.05 ng/mL	[88]
Tumor necrosis factor α (TNF-α)	GCE/hydrogel+chitosan/AuNPs/primary anti-TNF-α antibody/TNF-α/ALP and Ab_2 modified polystrene sphere (PS-ALP-Ab_2)	Amperometric	1 pg/mL–10 ng/mL	0.5 pg/mL	[89]
Carbohydrate antigen15-3 (CA 15-3)	GCE/N-doped graphene-anti CA 15-3/BSA /CA 15-3	Amperometric	0.1–20 U/mL	0.012 U/mL	[90]
Epidermal growth receptor (EGFR)	Au/AuNPs/Cys (1,4-phenylenediisothiocyanate) (PDITC)/protein G/ ethanolamine/anti EGFR/EGFR	Impedimetric	1 pg/mL–1 μg/mL	0.34 pg/mL	[47]
CA 125	GCE/MWCNT-Nafion/tris (2,2′-bipyridyl) cobalt Co $(bpy)_3^{+3}$/AuNPs/anti-CA 125/ BSA/CA 125	Amperometric	1–150 U/mL	0.36 U/mL	[91]
CEA	Au/cys/*p*-phthaloyl chloride/thionine/HRP-labeled anti-CEA/CEA	Amperometric	0.6–200 ng/mL	0.2 ng/mL	[92]
AFP	GCE/MWCNts/Au-PtNPs/anti-AFP/HRP/AFP	Amperometric	0.5–200 ng/mL	0.17 ng/mL	[20]
CEA	Au/cys/AuNPs/SiO2/thionine nanocomposite/AuNPs/anti-CEA/CEA	Amperometric	1–100 ng/mL	0.34 ng/mL	[93]
CA 15-3	Au/amino functionalized Fc-COOH-doped SiNPs/glu/anti CA 15-3/BSA/CA 15-3	Amperometric	2–240 U/mL	0.64 U/mL	[48]
CEA	GCE/AuNPs/Chitosan nanocomposite/Ab_1/BSA/CEA/Ab_2-AuNPs/DNA/methylene blue (MB)	Amperometric	0.1–2 pg/mL	0.05 pg/mL	[94]
CEA	GCE/TiO_2 doped chitosan-branched ferrocene complex film (CS-Fc-TiO2)/gold nanoparticles-graphene (Au-Gra) nanohybrid/anti-CEA/BSA/CEA	Amperometric	0.01–80 ng/Ml	3.4 pg/mL	[95]
PSA	GCE/MWCNTs-ionic liquid (ILs)-thionine (IL/MWCNTs/Thi)/anti-PSA/PSA/HRP-labeled PSA	Amperometric	0.2–40 ng/mL	20 pg/mL	[96]
AFP	GCE/AuNPs/graphene nanosheet nanocomposite (Au-Gra)/anti-AFP (Ab_1)/BSA/ AFP/SWCNTs-bienzyme (HRP+ GOD)-Ab_2	Impedimetric	0.001–60 ng/mL	0.33 pg/mL	[97]
CEA	GCE/1,4-phenylenediamine(H_2N-Ph-NH_2)/AuNPs/anti-CEA/BSA/CEA	Amperometric	10 pg/mL–100 ng/mL	3 fg/mL	[98]
AFP	GCE/SBA-15/1-naphthyl phosphate (1-NP)/alkaline phosphatase (ALP)-labeled antibody antibody/ionic chitosan hybrid (IL-Chi)/AFP	Amperometric	1–150 ng/mL	0.8 ng/mL	[99]
CA 19-9	Graphite electrode (GE)/titania sol-gel/CA 19-9/HRP labeled CA 19-9 antibody	Amperometric	3–20 U/mL	2.68 U/mL	[40]
PSA	GCE/MWCNTs/anti-PSA(Ab_1)/PSA/anti-tPSA IgG-HRP conjugate	Amperometric	0.08–0.5 μg/L	0.03 μg/L	[32]
CA 15-3	GCE/functionalized Ab_1 loaded graphene (GS-Ab_1) ionic liquid (IL)/BSA/CA 15-3/ Ab_2-f-TiO_2-Cd+2 nanocomposite	Amperometric	0.02–60 U/mL	0.008 U/mL	[5]
PSA	GCE/graphene sheet (GS)-cobalt hexacyanoferrate nanoparticles (CoNP)-1-pyrene butanoic acid, succinimidyl ester (PBSE) composite film/anti-PSA/BSA/ PSA	Amperometric	0.02–2 ng/mL	0.01 ng/mL	[100]
PSA	GCE/silver hybridized mesoporous silica nanoparticles (Ag@MSNs)/glu/anti-PSA/ BSA/PSA	Amperometric	0.05–50 ng/mL	15 pg/mL	[101]
AFP	GCE/Pd nanoplates/anti-AFP/BSA/AFP	Amperometric	0.01–75 ng/mL	4 pg/mL	[36]
Murine double minute 2 (MDM2)	Polycrystalline Au/cys/PDITC/MDM2 antibody/ethanolamine/MDM2	Impedimetric	1 pg/mL–1 μg/mL	0.29 pg/mL	[50]
CEA	GCE/platinum-thionine-graphene nanocomposite (PTGOs)/anti-CEA/BSA/HRP-PtNW-CEA bioconjugates	Amperometric	0.01–60 ng/mL	5 pg/mL	[102]
CEA	GCE/o-aminobenzoic acid (o-ABA)/EDC/NHS/anti-CEA (Ab_1)/ethanolamine/CEA/ BSA/SWCNT-$(PDDA/ALP)_4$-PDDA-poly(sodium 4-styrenesulfonate) (PSS)-Ab_2	Amperometric	0.1–1000 pg/mL	0.04 pg/mL	[103]
CEA	GE/magnetic beads coated capture antibodies (MBs-Ab_1)/CEA/Ab_2-AuNPs-HRP	Amperometric	5–60 ng/mL	5 ng/mL	[1]
CEA	GCE/PB/Fe_3O_4-Chi suspension/AuNPs/HRP labeled anti-CEA/CEA	Amperometric	0.1–220 ng/mL	10 pg/mL	[104]
CEA	GCE/PB/AuNPs/anti-CEA/BSA/HRP conjugated anti-CEA secondary antibody attached on nanogold enwrapped graphene nanolabels (HRP-anti-CEA-NGGN)	Amperometric	0.05–350 ng/mL	0.01 ng/mL	[22]
CEA	GCE/graphene film/Chi/glu/Ab_1/blocking solution/CEA/Ab_2 functionalized AuNPs mesoporous carbon foam (Ab_2/Au/MCF)	Amperometric	0.05 pg/mL–1 ng/mL	0.024 pg/mL	[105]
AFP	GCE/AuNPs/2-aminoethanethiol (AET)/PAMAM/EDC/NHS/capture anti-AFP (Ab_1)/BSA/AFP/HRP conjugated Ab_2	Voltammetric	5–500 ng/mL	3 ng/mL	[106]
CA 125	Screen printed graphite electrode (SPGE)/AuNPs/11-mercaptoundecanoic acid (MUA)/EDC/NHS/MCH/anti-CA 125/rabbit IgG(rIgG)/CA 125	Impedimetric	0–100 U/mL	6.7 U/mL	[107]
CA 15-3		Chronoamperoetry			[49]

Table 3 (*continued*)

Biomarker	Immobilization strategies	Assay principle	Linear range	Limit of Detection	Reference
	GCE/capture anti CA 15-3 (Ab_1) immobilized graphene sheet(GS-Ab_1)/BSA/CA 15-3/nanoporous PtFe alloys labeled Ab_2 (NP-PtFe-Ab_2)		0.002–40 U/mL	3.10^{4-} U/mL	
NSE	GCE/AuNPs/nickel hexacyanoferrate nanoparticles (NiHCFNPs)/Au-Gra/anti-NSE/BSA/NSE	Amperometric	0.001–100 ng/mL	0.3 pg/mL	[45]
PSA	Au/11-mercapto-1-undecanol (MU)/MUA+ 3-aminophenylboronic acid (APBA)/HRP-labeled anti-PSA/BSA/PSA	Amperometric	2–120 ng/mL	1.1 ng/mL	[108]
AFP	GCE/AuNPs and CNT doped chitosan (AuNPs/CNT/Chi) film/AFP/BSA/ALP labeled antibody	Amperometric	1–55 ng/mL	0.6 ng/mL	[109]
CEA	GCE/PB-graphene hybrid nanosheets (PBGPs) doped chitosan (CS-PBGP)/AuNPs/anti-CEA/BSA/CEA/anti-CEA-gold-silver hallow nanospheres (AuAgHSs)–GOD	Amperometric	0.005–50 ng/mL	1 pg/mL	[110]
PSA	Electrode/GS/1-pyrenebutanoic acid, succinimidyl ester (PBSE)/Ab1/BSA/PSA/QD functionalized GS-Ab2	Amperometric	0.005–10 ng/mL	3 pg/mL	[111]
AFP	GCE/GS/mesoporous silica (MPS) with trimethylchlorosilane (TMCS)/CNTs/anti-AFP/AFP	Amperometric	0.1–100 ng/mL	0.06 ng/mL	[112]
AFP	GCE/GS-Thi composite film/glu/anti-AFP (Ab1)/BSA/AFP/PB modified hydroxyapatite loaded with HRP and secondary anti-AFP antibody (PB@HAP-HRP-Ab2)	Amperometric	0.02–8 ng/mL	9 pg/mL	[113]
PSA	GCE/GO-Ab1/BSA/PSA/ferrocene (Fc) incorporated polystyrene spheres (Ps-Fc)-chitosan-Ab2	Amperometric	0.01–20 ng/mL	1 pg/mL	[114]
AFP	GCE/Au-Chi/anti-AFP/BSA/AFP/HRP conjugated anti AFP-silver nanowire-graphene hybrid nanomaterials (HRP-anti-AFP-AuNW-GPs)	Amperometric	0.05–400 ng/mL	5 pg/mL	[115]
Squamous cell carcinoma antigen (SCCA),	GCE/reduced graphene oxide-tetraethylene pentamine (rGO-TEPA)/Ab1/BSA/SCCA/Au@mesoporous carbon CMK-3-Ab2	Amperometric	0.03–20 ng/mL	0.01 ng/mL	[116]
CEA			0.05–20 ng/mL	0.013 ng/mL	
PSA	GCE/polyethyleneimine functionalized 3,4,9,10-perylenetetracarboxylic acid (PEI-PTCA)/AuNPs/Ab_1/BSA/PSA/biotinylated ALP/streptavidin (SA)/anti-PSA/Ab2/Au modified nickel hexacyano ferrates (Au@NiNPs)/onion like graphene sheets (O-GS)	Amperometric	0.01–50 ng/mL	3.4 pg/mL	[117]
fPSA	GCE/PEI-PTCA/AuNPs/Ab1/BSA/fPSA/bio-ALP/SA/anti-fPSA/Au modified prussian blue nanoparticles	Amperometric	0.02–10 ng/mL	6.7 pg/mL	
CEA	GCE/MWCNTs/EDC/NHS/Ab1/CEA/GOD-Aushell@GOD nanospheres	Amperometric	0.02–5 ng/mL	6.7 pg/mL	[118]
CEA	Au/Nafion-cysteine composite membrane/AuNPs/anti-CEA/BSA/CEA	Amperometric	0.01–100 ng/mL	3.3 pg/mL	[119]
Mucin-16 (MUC-16)	SPE/gold nanoelectrode ensemble (GNEE)/2-mercaptopropionic acid/2-mercaptoethanol/sulfo-NHS/EDC/anti-MUC-16/ethanolamine+BSA/MUC-16	Amperometric	0.001–300 U/mL	5.10^{4-} U/mL	[120]
CEA	GCE/three dimensional macro porous gold nanoparticles-graphene composites (3D-AuNPs/GN)/Ab1/BSA/CEA/HRP labeled anti-CEA (HRP-Ab2)/Thi/nanoporous silver (NPs)	Amperometric	0.001–10 ng/mL	0.35 pg/mL	[121]
SCCA	GCE/nitrogen doped graphene sheets (N-GS) in chitosan/glu/Ab1/BSA/SCCA/Pt-FeO4-Ab2	Amperometric	0.05–18 ng/mL	15.3 pg/mL	[51]
Human chorionic gonadotropin (hCG)	GCE/GS/acid treated MWCNTs/Au/Ab1/BSA/hCG/HRP-Ab2/Au/Thi/MCM-41	Amperometric	0.005–500 mIU/mL	0.0026 mIU/mL	[53]
Human epididymis-specific protein-4 (HE4)	ITO/chitosan-titanium carbide (TiC)/AuNPS/anti-HE4/BSA/HE4/biotin labeled HE4	Amperometric	3–300 pM	0.06 pM	[54]
CEA	GE/GS/EDC/NHS/Ab1/BSA/CEA/mesoporous platinum nanoparticles labeled antibody (M-PtNP-Ab2)	Amperometric	0.02–20	7 ng/mL	[122]
CA 125			0.05–20	0.002 U/mL	
CA 153			0.008–24	0.001 U/mL	
Apurinic/apyrimidinic endonıuclease-1/redox factor-1 (APE-1)	GCE/ethylenediamine/protein A(PA)/EDC/NHS/Ab1/BSA/APE-1/biotinylated ALP/nanochain modified streptavidin/Ab2/nickel hexacyanoferrates nanoparticle-decorated Au nanochains (Ni-AuNCs)	Amperometric	0.1–100 pg/mL	3.9 fg/mL	[123]
Vascular endothelial growth factor (VEGF)	Au/3-mercaptopropionic acid (3-MPA)/EDC/NHS/VEGF-R1/BSA/VEGF	Impedimetric	10–70 pg/mL	10 pg/mL	[12]
HER-3	Carbon nanotubes modified screen printed electrode/EDC/NHS/anti-HER-3/BSA/HER-3	Impedimetric	2–14 fg/mL	2 fg/mL	[124]
HER-3	ITO/APTES/glu/anti-HER-3/BSA/HER-3	Impedimetric	40–200 fg/mL	40 fg/mL	[55]
HER-3	Au/4-aminothiophenol/glu/anti-HER3/BSA /HER3	Impedimetric	0.4–2.4 pg/mL	0.28 pg/mL	[125]
HER-3	Au/hexandithiol(HDT)/AuNPs/Cys/glu/anti-HER-3/BSA/HER-3	Impedimetric	0.2–1.4 pg/mL	0.2 pg/mL	[126]

four times regeneration by Gly–HCl solution kept 96.21%, first three times regeneration by HCl solution kept 95.97%, first two times regeneration by NaOH solution kept 95.97%. Thus they chose urea solution as a suitable reagent for recycling the immunosensors [93].

Specificity is vital point of using biological molecules as recognition elements to develop immunosensors. To evaluate the specificity of the electrochemical immunosensor, control experiments are performed by adding other possible interferents. Yang et al. [30] studied specificity of immunosensor in the solution with 20 ng/mL CEA and with interference substance such as, α-1-fetoprotein (20 ng/mL), hepatitis B surface antigen (20 ng/mL), human chorionic gonadotropin (20 mIU/mL), carcinoma antigen 125 (20 ng/mL), prostate specific antigen (20 ng/mL), immunoglobulin G (20 ng/mL) and BSA (100 ng/mL). The ratios of current responses with CEA to the interfering substance were 5.69, 5.64, 5.74, 5.67, 5.75, 5.73 and 5.71, respectively [30]. Another specificity study was performed by Ravalli et al. [107] and they evaluated the experiment by using electrochemical impedance spectroscopy. And they observed that relative change in impedance originated from the specific monoclonal anti CA125 antibody and CA125 interactions, not from the inferences. So anti CA125 showed a good selectivity when compared with CA 125 [107].

Usually long time stability of the immunosensors was studied on a 30-day period. Feng et al. [81] measured the stability of the immunosensor by testing the response value. The response value of the immunosensor at 5 days, 10 days, 15 days, 20 days and 30 days maintained 98.8, 97.7, 95.9, 93.7, 90.9% of its initial response (C_{CEA} = 10 ng/mL), indicating the immunosensor had good stability. According to them, the Thi-CNTs sensing platform prevents the leak of hydrophilic thionine from the electrode, but also avoid the addition of mediator to the solution which may affect the activity of the antibody [81].

Liu et al. [38] developed an immunosensor for AFP detection. Its current response maintained about 96.2% of the original value after the storage periods of 40 days. They reported that the good stability of the immunosensor depended on two factors: the first one was the microenvironment of the layer-by-layer assembly film; the second was the polymer PEDOT, which offered a stable substrate for the immobilization of molecules [38].

3.2. Cardiac markers

Cardiovascular disease (CVD) is a kind of disease that affects heart and blood vessels. Many factors such as genetic, gender, age, high blood pressure and cholesterol, diabetes, obesity and overweight, smoking and stress cause CVD. It develops when a plaque builds up in the walls of the arteries. This plaque narrows the arteries and makes it difficult for blood to flow through and causes a heart attack or stroke [127,128].

Cardiovascular disease (CVD) leads to human death and World Health Organization (WHO) estimated that 17.3 million (30%) of all global deaths in 2008 were linked with CVD, 7.3 million of which were heart disease and 6.2 million were stroke [129]. Over 80% of the world's deaths from CVDs occur in low- and middle-income countries [127]. The number of people, who die from CVDs, mainly from heart disease and stroke, will increase to 23.3 million by 2030 [127,130].

The current diagnostic methods routinely used for CVD are electrocardiography (ECG), chest X-ray, echocardiography, cardiac catheterization, CT heart scan and blood tests [131]. For successful prognosis of the disease, early and quick diagnosis is vital for patients. The diagnosis of CVD has been based on the WHO criteria, whereby patients must meet at least two of three conditions: characteristic chest pain, diagnostic electrocardiogram (ECG) changes, and elevation of the biochemical markers in their blood samples. Although ECG is an important management tool for guiding therapy, it might not be always useful in every case because some patients might have normal electrocardiograms. Therefore, measurement of cardiac markers is critical in assisting the diagnosis of CVD [128].

There are several important characteristics of a biomarker including (a) high clinical sensitivity and specificity, (b) quick release of biomarker in the blood enabling early diagnosis, (c) capability to remain elevated for longer time in the blood, and (d) ability to be assayed quantitatively. For the accurate disease diagnosis, different types of biomarkers should be analyzed simultaneously because it is difficult to select a specific marker for the diagnosis of CVD [127].

Clinically used cardiac markers are summarized in Table 4 [127].

Myoglobin is a non-enzymatic cardiac protein and is the earliest marker of the acute myocardial infarction (AMI). As a cardiac biomarker, myoglobin is used together with troponin to help diagnosis of heart attack. Because of its small size of 17.8 kDa, it is released into plasma in major amount within 3 h of the onset of AMI and the plasma concentrations usually return to normal within 24 h. Normal serum myoglobin levels range from 30 to 90 ng/mL. After 1 h of the onset of myocardial infarction, serum myoglobin level can raise to 200 ng/mL or even higher. During a peak hour myoglobin level can raise to 900 ng/mL [132].

Human cardiac troponins (cTnT and cTnI) is known 'gold standard' because of their specificity and sensitivity to serological diagnosis and prognosis of acute myocardial infarction and acute coronary syndrome (ACS) [133,134]. cTnI has not been identified outside of the myocardium, but, cTnT is expressed in skeletal

Table 4
Clinically cardiac markers.

Cardiac Biomarker	Type of cardiovascular diseases	Cut-off levels
Troponin I (cTnI)	Detection of acute myocardial infarction (AMI)	0.01–0.1 ng/mL
Troponin T (cTnT)	Detection of AMI	0.05–0.1 ng/mL
Myoglobin	Early detection of AMI	70–200 ng/mL
C-reactive protein (CRP)	Early detection of inflammation/cardiac risk factor	$< 10^3$ ng/mL low risk 1–3 × 10^3 ng/mL intermediate risk > 3–15 × 10^3 ng/mL high risk
Creatine kinase MB subform (CK-MB)	Early detection of AMI	10 ng/mL
B-type natriuretic peptide (BNP)	Acute coronary syndromes/diagnosis of heart failure/ventricular overload	
N-terminal pro-B-type natriuretic peptide (NT-proBNP)	Acute coronary syndromes/diagnosis of heart failure/ventricular overload	0.25–2 ng/mL
Myeloperoxidase (MPO)	Detection of inflammation	> 350 ng/mL stratification risk
Heart fatty acid binding protein (11-FABP)	Myocardial necrosis	≥ 6 ng/mL stratification risk
TNF-α	Inflammation/cardiac risk factor	< 0.0036 ng/mL low risk ≤ 0.0036 ng/mL high risk
Interlukin-6 (IL-6)	Inflammation/cardiac risk factor	Low < 0.0013 ng/mL Mid 0.00138–0.002 ng/mL High > 0.002 ng/mL
Fibrinogen		Low < 3.58 × 10^6 ng/mL Mid 3.58–4.20 × 10^6 ng/mL

muscle to a smaller extent. Especially, the cTnT levels increase 2–4 h after the acute myocardial infarction symptoms and could be evaluated up to 14 days after the acute episode of myocardial damage [133]. cTnI starts to rise rapidly in the blood stream within 3–4 h after onset of acute myocardial infarction and remains to rise up to 4–10 days. The level of cTnI in normal patients levels of cTnI are around 10 ng/mL, but the cTnI concentration of AMI in patients goes up to 112 ng/mL (range 20–550 ng/mL) at 18 h.

C-reactive protein (CRP) is one of the plasma proteins known as acute-phase proteins, an indicator of a viral and bacterial infection, however low concentrations of CRP may also predict the risk of heart attack and stroke. CRP levels can rapidly increase up to 1000-fold over normal trace amounts with bacterial infection, trauma, surgery and other inflammatory events and decrease to normal within 12–14 days. The CRP level is typically less than 2 mg/L for healthy individuals. If CRP is higher than 3 mg/l, a person is at high risk of developing a cardiovascular disease [135].

Myeloperoxidase (MPO) is an abundant mammalian phagocyte hemoprotein which caused suddenly cardiac death. In acute coronary syndrome patients (ACS), MPO serum levels predict an increased risk for subsequent cardiovascular events [136].

P-selection, known as CD62P is a granule membrane protein 140 (GMP-140), and platelet activation dependent granule activation protein (PADGEM), was first discovered in endothelial cells in 1989 as platelet activation marker and categorized as one member of the cell adhesion molecules (CAM) family [137].

Soluble lectin-like oxidized low density lipoprotein receptor (sLOX-1) is an extracellular domain of lectin like oxidized

Table 5
The developed immunosensors for cardiovascular disease diagnosis in literature.

Biomarker	Immobilization strategies	Assay principle	Linear range	Limit of detection	Reference
Human cardiac troponin (cTnT)	NH_2-CNT-SPEs/polyethylene terephtalate (PET)/NHS-EDC-anti-cTnT/glycine/cTnT	Amperometric	0.0025–0.5 ng/mL	0.0035 ng/mL	[139]
cTnT	Au/polyethyleneimine (PEI)/carboxylated CNTs (COOH-CNT)/anti-cTnT/glycine/cTnT	Amperometric	0.1–10 ng/mL	0.033 ng/mL	[140]
CRP	Au/nanocrystalline diamond film (NCD)/mercaptopropionic acid (MPA)/EDC/NHS/anti-CRP/ethanolamine/CRP	Capacitance	25–800 ng/mL	-	[135]
cTnT	GCE/*o*-aminobenzoic acid (poly-*o*-ABA)/EDC/NHS/anti-cTnT/ethanolamine/cTnT	Amperometric	0.05–5 ng/mL	0.016 ng/mL	[141]
Myeloperoxidase (MPO)	GCE/*N*,*N*-dimethylformamide (DMF)-MWCNTs/1-ethyl-3-methylimidazolium tetrafluoroborate (EMIMBF4)/chitosan-cerium oxide (CeO2)/anti-MPO/BSA/MPO	Amperometric	5–300 ng/mL	0.2 ng/mL	[136]
P-selection	SPCE/CNT/gold nanobone /GNB)/3,4-ethylenedioxythiophene (EDOT)/EDC/NHS/anti-P-selection/BSA/P-selection/α-P-selection Ab-tagged liposome containing K4Fe(CN)6	Amperometric	10^{-13}–10^{-5} g/mL	0.85 pg/mL	[137]
Myoglobin	Au/4-aminothiophenol(4-ATP)/sulfosuccinimidyl 4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate(sulfo-SMCC)/half-antibody fragment/myoglobin	Impedimetric	10^{-14}–10^{-7} M	10–14 M	[142]
cTnT	SPE/polyethylene terephtalate (PTE)/anti-cTnT/biotin/glutaraldehyde (glu)/streptavidin microsphere/glycine/cTnT/HRP- conjugated anti-cTnT	Amperometric	0.1–10 ng/mL	0.2 ng/mL	[133]
Cardiac troponin I (cTnI)	İnterdigitated array (IDA) chip/poly(dimethylsiloxane) (PDMS)/NHS/BSA/anti-cTnI/protein G/cTnI/alkaline phosphatase (AP)-labeled anti-CTnI/enzyme substrate (PaPP)	Amperometric	0.2 ng/mL–10 μg/mL	148 pg/mL	[143]
cTnI	PDMS-GNP composite/anti-cTnI and anti-CRP (Ab1)/BSA/CdTe and ZnSe quantum dots-anti-cTnI and anti-CRP (Ab2)	Amperometric	0.01–50 μg/l	5 amol	[144]
Myoglobin (Mb)	Au/11-mercaptoundecanoic acid (MUA)+3-mercaptopropionic acid (MPA)/NHS-EDC/anti-Mb/BSA/Mb	Impedimetric	10–650 ng/mL	5.2 ng/mL	[132]
Mb	Graphite electrode/didodecyldimethylammonium bromide (DDAB)-Au/EDC/NHS/anti-human myoglobin (anti-HMB)/glycine/Mb	SWV	10–1780 ng/mL	10 ng/mL	[145]
hFABP	Au/MUA+3-mercaptopropanol (MPOH)/EDC/NHS/anti-hFABP/BSA/hFABP	Impedimetric	98 pg/mL–100 ng/mL	117 pg/mL	[146]
cTnI	SPE/AuNPs/anti-cTnI/BSA/cTnI	Capacitance	0.2–12.5 ng/mL	0.2 ng/mL	[147]
Mb	Crystal (E)-1-decyl-4-(4-decyloxyphenyl) diazenyl)pyridinium bromide (Br-Py) coated GCE)/polyethyleneimine-cp Coated gold nanoparticles film/anti-Mb/glycine/Mb	Amperometric	9.96–72.8 ng/mL	6.29 ng/mL	[148]
Soluble lectin-like oxidized low-density lipoprotein receptor I (SLOX-1) cTnI	Au/6-mercaptohexadecanoic acid/biotin-caproyl-DPPE/neutravidin/biyotinylated anti-SLOX or anti-cTnI/SLOX-1 or cTnI	Impedimetric	10^{-13}–10^{-7} M	10^{-13} M	[134]
Mb	Au-SPE/Myoglobin solution/(Mb imprinted) M1 film/proteinase K/poly(*o*-aminophenol) (PAP)/Myoglobin	Impedimetric Amperometric	0.5–53.5 μg/mL	1.5 μg/mL 0.8 μg/mL	[149]
Mb	ITO glass/APTES/AuNPs/MUA-MPA/antiMb/BSA/Mb	Impedimetric	0.01–1.65 μg/mL	1.4 ng/mL	[150]
CRP	Au-SPE/carboxylic-modified magnetic beads (HOOC-MBs)/EDC/sulfo-NHS/ethanolamine /biotinylated anti-CRP/streptavidin HRP (strp-HRP)/CRP	Amperometric	0.07–1000 ng/mL	0.021 ng/mL	[151]

low-density lipoprotein receptor-1 and releases as a soluble form. sLOX-1 is a useful marker for early diagnosis of acute coronary syndrome (ACS) [138] (Table 5).

Ko et al. [143] developed an immunosensor for cTnI detection. The detection limit from the calibration curve was calculated as 148 pg/mL. First, the proper orientation of antibodies by protein G that binds the Fc portion of the antibodies might lead to an effective recognition of the analyte. If the antibodies are randomly immobilized on the surface of the PDMS channel, the amount of immunoreaction between antibodies and antigens may be reduced because the Fab portion of antibodies may not sufficiently orient toward the target antigens. Therefore, the immobilization of the antibodies was performed through the interaction between Fc portion of the antibodies and protein G. Second, the best packing density of antibodies increased the sensitivity of their device by avoiding low numbers of antibodies immobilized onto the channel surface and steric hindrance. Third, avoiding the electrode fouling also contributed to the low limit of detection of this device [143].

Mattos et al. [141] developed an *o*-aminobenzoic acid film-based immunosensor for CTnT detection. It showed good performance in terms of operational stability, measuring it 100 times every 2 min. The relative standard deviation was approximately 3% [141].

Mattos et al. [141] and Lu et al. [136] kept the immunosensors they developed in the refrigerator at 4 °C and one found to be 91,6% of initial response after 18 days and the other 90% after 20 days.

Ávila et al. [151] used magnetic beads (MBs) for amperometric immunosensor development. MBs have demonstrated to be a useful tool to improve their sensitivity, reduce time of analysis of complex samples without pre-enrichment or purification steps. Au-SPEs and carboxylic-modified magnetic beads (HOOC-MBs) were used for the first time. Biotinylated anti-CRP reacted with streptavidin HRP (strp-HRP). The electrochemical detection of the enzymatic reaction product was measured disposable Au/SPE using 3,3′,5,5′-tetramethylbenzidine (TMB) as the electron transfer mediator and H_2O_2 as the enzyme substrate. They evaluated the selectivity of the magnetoimmunosensor toward various non-target compounds at their concentrations of 5 mg/mL BSA, 500 ng/mL D-dimer and 4000 g/mL heparin and two other non-target cardiac proteins of 7.5 ng/mL of amino-terminal pro-B-type natri-uretic peptide (NT-proBNP) and 500 ng/mL of cardiac troponin T (cTnT). The tests were performed either in the absence or in the presence of the potential interfering compounds by comparing with the magnetoimmunosensor for 500 ng/mL CRP. The presence of BSA, D-dimer and heparin did not interfere significantly in the CRP determination. Moreover, the target cardiac protein can be accurately determined in the presence of other two cardiac proteins (cTnT and NT-proBNP) at a concentration much higher than that normally found in these biological samples. A great selectivity achieved by using two specific antibodies in the design of the sandwich magnetoimmunosensor [151].

4. Future outlook

The antibodies for construction of immunochemical biosensors–immunosensors have been extensively used more than 35 years. Immunosensors are miniaturized analytical devices which selectively detect analytes and provide a concentration as a result of signal. In this review, attention is focused on the electrochemical immunosensors for tumor and cardiac biomarkers in clinical diagnosis.

In literature, the developed immunosensors were evaluated for the analysis of clinical serum samples and received a good corre lation with the enzyme linked immunosorbent assay (ELISA) [17,18,31,34,35,37,67,68,70]. Huang et al. [17] developed an immunosensor for CEA determination. They compared the results obtained from five human serum samples with the purposed method and ELISA. They reported that a good correlation between the results obtained by the proposed immunosensor and ELISA [17].

Kavosi et al. [60] evaluated the feasibility of their immunosensor for real sample analysis by using standard addition methods. They observed no significant difference results of two methods [60].

Zhang et al. [21] compared their biosensor response with the commercialized electrochemiluminescent method. They found no significant difference [21].

The electrochemical immunosensor can be miniaturized to a small pocket size device which makes them applicable for home use or the doctor's surgery as point of care (POC). For example, glucose biosensor is the most widely used an electrochemical biosensor which is based on a screen-printed amperometric disposable electrode. This type of biosensor has been widely used throughout the World for glucose testing in the home diagnosis. The recognition element of prostate cancer is anti-PSA antibody. The developed immunosensor for PSA offers an attractive possibility to screen prostate cancer in real time and on site. Also, electrochemical immunosensors offer a great selectivity and sensitivity for early clinical analysis. However, moving technology to commercial products may require more time and investments.

5. Conclusion

Biosensors, especially immunosensors, are of great value for use in clinical testing, because they are based on antigen–antibody reactions which are highly sensitive and specific. Moreover, they have vital role in measuring specific compounds in biol;ogical matrices, such as blood and plasma. Electrochemical immunosensors are simple, rapid, reliable and inexpensive devices. Thus they can be used in clinical diagnosis with confidence.

In this review, recent researches on biomarkers used for detecting cancer and cardiovascular diseases are summarized. Carcinoembriyonic antigen(CEA), prostate-specific antigen (PSA), α-1-fetoprotein (AFP), carbohydrate antigen 19-9 (CA 19-9), myeloctomatosis oncogene (c-Myc), cancer antigen 125 (CA125), neuron specific enolase (NSE), epidermal growth factor receptor (EGFR), cancer antigen 15-3, murine double minute 2 (MDM2), Squamous cell carcinoma antigen (SCCA), human chorionic gonadotrophin (hCG), human epididymis-specific protein 4 (HE4), HER-3, vascular endothelial growth factor (VEGF) are commonly used biomarkers in cancer diagnosis and Human cardiac troponins (cTnT and cTnI), myoglobin, C-reactive protein (CRP), myeloperoxidase (MPO), P-selection, soluble lectin-like oxidized low density lipoprotein receptor (sLOX-1) are commonly used cardiovascular disease biomarkers.

In developing immunosensors, the immobilization of antibody is an important step because antibody acts as the recognition element for antibody–antigen reaction. The performance of the detection of antigen binding capacity can be increased with a proper antibody surface. Thus the choice of the antibody immobilization method is very important in the design of an immunosensor. Several methods including physical and chemical adsorption have been used for the preparation of oriented antibody molecular layers on the surface of the transducer. The sensitivity of biomarkers is important for early diagnosis of diseases. Furthermore, the coupling of electrodes with nanostructured materials such as silicon nanowires, gold nanop;articles, carbon nanotubes, magnetic particles, quantum dots offer multiplexing capability for simultaneous measurements of biomarkers.

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References

[1] B. Jin, P. Wang, H. Mao, B. Hu, H. Zhang, Z. Cheng, Z. Wu, X. Bian, C. Jia, F. Jing, Q. Jin, J. Zhao, Biosens. Bioelectron. 55 (2014) 464–469.
[2] A. Sassolas, L.J. Blum, B.D. Leca-Bouvier, Biotechnol. Adv. 30 (2012) 489–571.
[3] Q. Xu, J.J. Davis., Electroanalysis 26 (2014) 1249–1258.
[4] V. Perumal, U. Hashim, J. Appl. Biomed. 12 (2014) 1–15.
[5] I.E. Tothill, Semin. Cell Dev. Biol. 20 (2009) 55–62.
[6] M. Diaz-Gonzalez, X. Munoz-Berbel, C. Jimenez-Jorquera, A. Baldi, C. Fernandez-Sanchez, Electroanalysis 26 (2014) 1154–1170.
[7] J. Wu, Z. Fu, F. Yan, H. Ju, TrAC, Trends Anal. Chem. 26 (2007) 679–688.
[8] R.K. Iles, H. Kallichurn, J. Bioeng. Biomed. Sci. 2 (2012) 1–5.
[9] B. Rezaei, N. Majidi, H. Rahmani, T. Khayamian, Biosens. Bioelectron. 26 (2011) 2130–2134.
[10] J. Zhou, L. Du, L. Zou, Y. Zou, N. Hu, P. Wang, Sens. Actuators, B 197 (2014) 220–227.
[11] T. Yang, S. Wang, H. Jin, W. Bao, S. Huang, J. Wang, Sens. Actuators, B 178 (2013) 310–315.
[12] M.K. Sezgintürk, Biosens. Bioelectron. 26 (2011) 4032–4039.
[13] D.E. Souto, J.V. Silva, H.R. Martins, A.B. Reis, R. Luz, L.T. Kubota, Biosens. Bioelectron. 46 (2013) 22–29.
[14] X. Yuan, C. Song, H. Wang, J. Zhang, Electrochemical Impedance Spectroscopy in PEM Fuel Cells Fundamentals and Applications, Springer-Verlag, London, USA, 2010.
[15] A.C. Mongra, A. Kaur, R.K. Bansal, Int. J. Eng. Res. Appl. 2 (2012) 743–749.
[16] B. Bohunicky, S.A. Mousa, Nanotechnol. Sci. Appl. 4 (2011) 1–10.
[17] K. Huang, D. Niu, W. Xie, W. Wang, Anal. Chim. Acta 659 (2010) 102–108.
[18] W. Jiang, R. Yuan, Y. Chai, L. Mao, H. Su, Biosens. Bioelectron. 26 (2011) 2786–2790.
[19] X. Sun, Z. Ma, Anal. Chim. Acta 780 (2013) 95–100.
[20] Y. Li, R. Yuan, Y. Chai, Z. Song, Electrochim. Acta 56 (2011) 6715–6721.
[21] Q. Zhang, X. Chen, Y. Tang, L. Ge, B. Guo, C. Yao, Anal. Chim. Acta 815 (2014) 42–50.
[22] Z. Zhong, W. Wu, D. Wang, D. Wang, J. Shan, Y. Qing, Z. Zhang, Biosens. Bioelectron. 25 (2010) 2379–2383.
[23] J Wu, Z Zhang, Z Fu, H. Ju, Biosens. Bioelectron. 23 (2007) 114–120.
[24] Y. Yuan, R. Yuan, Y. Chai, Y. Zhuo, L. Mao, S. Yuan, J. Electroanal. Chem. 643 (2010) 15–19.
[25] W. Limbut, P. Kanatharana, B. Mattiasson, P. Asawatreratanakul, P. Thavarungkul, Anal. Chim. Acta 561 (2006) 55–61.
[26] L. Zhu, L. Xu, N. Jia, B. Huang, L. Tan, S. Yang, S. Yao, Talanta 116 (2013) 809–815.
[27] Y. Cai, H. Li, Y. Li, Y. Zhao, H. Ma, B. Zhu, C. Xu, Q. Wei, D. Wu, B. Du, Biosens. Bioelectron. 36 (2012) 6–11.
[28] Y. Yuan, R. Yuan, Y. Chai, Y. Zhuo, X. Miao, J. Electroanal. Chem. 626 (2009) 6–13.
[29] K. Matsuoka, S. Sumitomo, N. Nakashima, D. Nakajima, N. Misaki, Eur. J. Cardiothorac. Surg. 32 (2007) 435.
[30] H. Yang, R. Yuan, Y. Chai, L. Mao, H. Su, W. Jiang, M. Liang, Biochem. Eng. J. 56 (2011) 116–124.
[31] B. Qu, X. Chu, G. Shen, R. Yu, Talanta 76 (2008) 785–790.
[32] N.V. Panini, G.A. Messina, E. Salinas, H. Fern´andez, J. Raba, Biosens. Bioelectron. 23 (2008) 1145–1151.
[33] W. Liang, R. Yuan, Y. Chai, Y. Li, Y. Zhuo, Electrochim. Acta 53 (2008) 2302–2308.
[34] X. Lu, H. Bai, P. He, Y. Cha, G. Yang, L. Tan, Y. Yang, Anal. Chim. Acta 615 (2008) 158–164.
[35] X. Ran, R. Yuan, Y. Chai, C. Hong, X. Qian, Colloids Surf. B 79 (2010) 421–426.
[36] H. Wang, H. Li, Y. Zhang, Q. Wei, H. Ma, D. Wu, Y. Li, Y. Zhang, B. Du, Biosens. Bioelectron. 53 (2014) 305–309.
[37] Z. Qiang, R. Yuan, Y. Chai, N. Wang, Y. Zhuo, Y. Zhang, X. Li, Electrochim. Acta 51 (2006) 3763–3768.
[38] K. Liu, J. Zhang, Q. Liu, H. Huang, Electrochim. Acta 114 (2013) 448–454.
[39] F. Hu, S. Chen, R. Yuan, Sens. Actuators, B 176 (2013) 713–722.
[40] D. Du, F. Yan, S. Liu, H. Ju, J. Immunol. Methods 283 (2003) 67–75.
[41] I.J. McEwan, K. Dahlman-Wright, J.e Ford, A.P.H. Wright, Biochemistry 35 (1996) 9584–9593.
[42] J. He, Y. Tian, Z. Cao, W. Zou, X. Sun, Sens. Actuators, B 181 (2013) 835–841.
[43] D. Tang, R. Yuan, Y. Chai, Anal. Chim. Acta 564 (2006) 158–165.
[44] I. Diaconu, C. Cristea, V. Hârceagă, G. Marrazza, I. Berindan-Neagoe, R. Săndulescu, Clin. Chim. Acta 425 (2013) 128–138.
[45] J. Han, Y. Zhuo, Y. Chai, Y. Yuan, R. Yuan, Biosens. Bioelectron. 31 (2012) 399–405.
[46] S. Yu, X. Cao, M. Yu, Microchem. J. 103 (2012) 125–130.
[47] R. Elshafey, A.C. Tavares, M. Siaj, M. Zourob, Biosens. Bioelectron. 50 (2013) 143–149.
[48] C. Hong, R. Yuan, Y. Chai, Y. Zhuo, Anal. Chim. Acta 633 (2009) 244–249.
[49] Y. Li, C. Xu, H. Li, H. Wang, D. Wu, H. Ma, Y. Cai, B. Du, Q. Wei, Biosens. Bioelectron. 56 (2014) 295–299.
[50] R. Elshafey, C. Tlili, A. Abulrob, A.C. Tavares, M. Zourob, Biosens. Bioelectron. 39 (2013) 220–225.
[51] D. Wu, H. Fan, Y. Li, Y. Zhang, H. Liang, Q. Wei, Biosens. Bioelectron. 46 (2013) 91–96.
[52] K. Handschuh, J. Guibourdenche, V. Tsatsaris, M. Guesnon, I. Laurendeau, D. Evain-Brion, T. Fournier, Endocrinology 148 (2007) 5011–5019.
[53] J. Lu, S. Liu, S. Ge, M. Yan, J. Yu, X. Hu, Biosens. Bioelectron. 33 (2012) 29–35.
[54] L. Lu, B. Liu, Z. Zhao, C. Ma, P. Luo, C. Liu, G. Xie, Biosens. Bioelectron. 33 (2012) 216–221.
[55] M.Ç. Canbaz, M.K. Sezgintürk, Anal. Biochem. 446 (2014) 9–18.
[56] W. Limbut, P. Kanatharana, B. Mattiasson, P. Asawatreratanakul, P. Thavarungkul, Biosens. Bioelectron. 22 (2006) 233–240.
[57] H. Yu, F. Yan, Z. Dai, H. Ju, Anal. Biochem. 331 (2004) 98–105.
[58] J. Wu, J. Tang, Z. Dai, F. Yan, H. Ju, N.E. Murr, Biosens. Bioelectron. 22 (2006) 102–108.
[59] J. Wu, Z. Zhang, Z. Fu, H. Ju., Biosens. Bioelectron. 23 (2007) 114–120.
[60] B. Kavosi, A. Salimi, R. Hallaj, K. Amani, Biosens. Bioelectron. 52 (2014) 20–28.
[61] X. Wang, J. Miao, Q. Xia, K. Yang, X. Huang, W. Zhao, J. Shen, Electrochim. Acta 112 (2013) 473–479.
[62] H. Tang, J. Chen, L. Nie, Y. Kuang, S. Yao, Biosens. Bioelectron. 22 (2007) 1061–1067.
[63] X. Jia, Z. Liu, N. Liu, Z. Man, Biosens. Bioelectron. 53 (2014) 160–166.
[64] J. Lin, Z. Wei, C. Mao, Biosens. Bioelectron. 29 (2011) 40–45.
[65] Y. Lin, J. Wang, G. Liu, H. Wu, C.M. Wai, Y. Lin, Biosens. Bioelectron. 23 (2008) 1659–1665.
[66] J. Moon, Y.H. Kim, Y. Cho, Biosens. Bioelectron. 57 (2014) 157–161.
[67] S. Chen, R. Yuan, Y. Chai, Y. Xu, L. Min, N. Li, Sens. Actuators, B 135 (2008) 236–244.
[68] W. Lu, X. Cao, L. Tao, J. Ge, J. Dong, W. Qian, Biosens. Bioelectron. 57 (2014) 219–225.
[69] F. Kong, M. Xu, J. Xu, H. Chen, Talanta 85 (2011) 2620–2625.
[70] W. Liang, W. Yi, S. Li, R. Yuan, A. Chen, S. Chen, G. Xiang, C. Hu, Clin. Biochem. 42 (2009) 1524–1530.
[71] X. He, R. Yuan, Y. Chai, Y. Shi, J. Biochem. Biophys. Methods 70 (2008) 823–829.
[72] K. Huang, J. Li, Y. Wu, Y. Liu, Bioelectrochemistry 90 (2013) 18–23.
[73] X. Li, R. Yuan, Y. Chai, L. Zhang, Y. Zhuo, Y. Zhang, J. Biotechnol. 123 (2006) 356–366.
[74] X. Gao, Y. Zhang, H. Chen, Z. Chen, X. Lin, Anal. Biochem. 414 (2011) 70–76.
[75] L. Wu, F. Yan, H. Ju, J. Immunol. Methods 322 (2007) 12–19.
[76] K. Huang, D. Niu, J. Sun, J. Zhu, J. Electroanal. Chem. 656 (2011) 72–77.
[77] X. Cao, N. Wang, S. Ji, L. Guo, K. Li, Biosens. Bioelectron. 39 (2013) 226–230.
[78] S. Suwansa-ard, P. Kanatharana, P. Asawatreratanakul, B. Wongkittisuksa, C. Limsakul, P. Thavarungkul, Biosens. Bioelectron. 24 (2009) 3436–3441.
[79] S. Viswanathan, C. Rani, A.V. Anand, J.A. Ho, Biosens. Bioelectron. 24 (2009) 1984–1989.
[80] G. Lai, J. Wu, C. Leng, H. Ju, F. Yan, Biosens. Bioelectron. 26 (2011) 3782–3787.
[81] D. Feng, L. Li, X. Fang, X. Han, Y. Zhang, Electrochim. Acta 127 (2014) 334–341.
[82] T. Yu, W. Cheng, Q. Li, C. Luo, L. Yan, D. Zhang, Y. Yin, S. Ding, H. Ju, Talanta 93 (2012) 433–438.
[83] L. Wu, J. Chen, D. Du, H. Ju, Electrochim. Acta 51 (2006) 1208–1214.
[84] D. Tang, B. Xia, Microchim. Acta 163 (2008) 41–48.
[85] J. Miao, X. Wang, L. Lu, P. Zhu, C. Mao, H. Zhao, Y. Song, J. Shen, Biosens. Bioelectron. 58 (2014) 9–16.
[86] H. Chen, D. Tang, B. Zhang, B. Liu, Y. Cui, G. Chen, Talanta 91 (2012) 95–102.
[87] M. Namgung, S. Jung, C.M. Chung, S. Oh, Ultramicroscopy 109 (2009) 907–910.
[88] X. Chen, X. Jia, J. Han, J. Ma, Z. Man, Biosens. Bioelectron. 50 (2013) 356–361.
[89] Y. Hou, T. Li, H. Huang, H. Quan, X. Miao, M. Yang, Sens. Actuators, B 182 (2013) 605–609.
[90] H. Li, J. He, S. Li, A.P.F. Turner, Biosens. Bioelectron. 43 (2013) 25–29.
[91] S. Chen, R. Yuan, Y. Chai, L. Min, W. Li, Y. Xu, Electrochim. Acta 54 (2009) 7242–7247.
[92] Z. Dai, J. Chen, F. Yan, H. Ju, Cancer Detect. Prev. 29 (2005) 233–240.
[93] Y. Zhuo, R. Yu, R. Yuan, Y. Chai, C. Hong, J. Electroanal. Chem. 628 (2009) 90–96.
[94] F. Kong, X. Zhu, M. Xu, J. Xu, H. Chen, Electrochim. Acta 56 (2011) 9386–9390.
[95] J. Han, Y. Zhuo, Y. Chai, L. Mao, Y. Yuan, R. Yuan, Talanta 85 (2011) 130–135.
[96] A. Salimi, B. Kavosi, F. Fathi, R. Hallaj, Biosens. Bioelectron. 42 (2013) 439–446.
[97] F. Yang, J. Han, Y. Zhuo, Z. Yang, Y. Chai, R. Yuan, Biosens. Bioelectron. 55 (2014) 360–365.
[98] X. Sun, Z. Ma, Biosens. Bioelectron. 35 (2012) 470–474.
[99] J. Lin, C. He, S. Zhang, Anal. Chim. Acta 643 (2009) 90–94.
[100] T. Li, M. Yang, H. Li, J. Electroanal. Chem. 655 (2011) 50–55.
[101] H. Wang, Y. Zhang, H. Yu, D Wu, H. Ma, H. Li, B. Du, Q. Wei, Anal. Biochem. 434 (2013) 123–127.
[102] B. Su, D. Tang, J. Tang, Y. Cui, G. Chen, Biosens. Bioelectron. 30 (2011) 229–234.
[103] Y. Xiang, Y. Zhang, B. Jiang, Y. Chai, R. Yuan, Sens. Actuators, B 155 (2011) 317–322.
[104] H. Chen, J. Tang, B. Su, G. Chen, J. Huang, D. Tang, Anal. Chim. Acta 678 (2010) 169–175.
[105] D. Lin, J. Wu, H. Ju, F. Yan, Biosens. Bioelectron. 52 (2014) 153–158.
[106] M. Giannetto, L. Mori, G. Mori, M. Careri, A. Mangia, Sens. Actuators, B 159 (2011) 185–192.
[107] A Ravalli, G. Pilon dos Santos, M. Ferroni, G. Faglia, H. Yamanaka, G. Marrazza, Sens. Actuators, B 179 (2013) 194–200.
[108] S. Liu, X. Zhang, Y. Wu, Y. Tu, L. He, Clin. Chim. Acta 395 (2008) 51–56.
[109] J. Lin, C. He, L. Zhang, S. Zhang, Anal. Biochem. 384 (2009) 130–135.

[110] J. Tang, D. Tang, Q. Li, B. Su, B. Qiu, G. Chen, Anal. Chim. Acta 697 (2011) 16–22.
[111] M. Yang, A. Javadi, S. Gong, Sens. Actuators, B 155 (2011) 357–360.
[112] J. Lin, Z. Wei, H. Zhang, M. Shao, Biosens. Bioelectron. 41 (2013) 342–347.
[113] Y. Dai, Y. Cai, Y. Zhao, D. Wu, B. Liu, R. Li, M. Yang, Q. Wei, B. Du, H. Li, Biosens. Bioelectron. 28 (2011) 112–116.
[114] L. Ding, J. You, R. Kong, F. Qu, Anal. Chim. Acta 793 (2013) 19–25.
[115] J. Tang, D. Tang, B. Su, Q. Li, B. Qiu, G. Chen, Electrochim. Acta 56 (2011) 8168–8175.
[116] D. Wu, A. Guo, Z. Guo, L. Xie, Q. Wei, B. Du, Biosens. Bioelectron. 54 (2014) 634–639.
[117] J. Han, Y. Zhuo, Y. Chai, R. Yuan, W. Zhang, Q. Zhu, Anal. Chim. Acta 746 (2012) 70–76.
[118] Z. Song, R. Yuan, Y. Chai, W. Jiang, H. Su, X. Che, X. Ran, Biosens. Bioelectron. 26 (2011) 2776–2780.
[119] Y. Liao, R. Yuan, Y. Chai, Y. Zhuo, X. Yang, Anal. Biochem. 402 (2010) 47–53.
[120] S. Viswanathan, C. Rani, C. Delerue-Matos, Anal. Chim. Acta 726 (2012) 79–84.
[121] G. Sun, J. Lu, S. Ge, X. Song, J. Yu, M. Yan, J. Huang, Anal. Chim. Acta 775 (2013) 85–92.
[122] Z. Cui, D. Wu, Y. Zhang, H. Ma, H. Li, B. Du, Q Wei, H. Ju, Anal. Chim. Acta 807 (2014) 44–50.
[123] J. Han, Y. Zhuo, Y. Chai, Y. Xiang, R. Yuan, Y. Yuan, N. Liao, Biosens. Bioelectron. 41 (2013) 116–122.
[124] E. Asav, M.K. Sezgintürk, Int. J. Biol. Macromol. 66 (2014) 273–280.
[125] M.N. Sonuç, M.K. Sezgintürk, Talanta 120 (2014) 355–361.
[126] M.Ç. Canbaz, Ç.S. Şimşek, M.K. Sezgintürk, Anal. Chim. Acta 814 (2014) 31–38.
[127] World Health Organization, Global Status Report on Noncommunicable Diseases 2010, World Health Organization, Geneva, 2011.
[128] A. Qureshi, Y. Gurbuz, J.H. Niazi, Sens. Actuators, B 171– (172) (2012) 62–76.
[129] World Health Organization, Global atlas on Cardiovascular Disease Prevention and Control, World Health Organization, Geneva, 2011.
[130] C.D. Mathers, D. Loncar, PLoS Med. 3 (2006) 442.
[131] A. Kakoti, P. Goswami, Biosens. Bioelectron. 43 (2013) 400–411.
[132] R.V. Sharma, V.K. Tanwar, S.K. Mishra, A.M. Biradar, Thin Solid Films 519 (2010) 1167–1170.
[133] B.V.M. Silva, I.T. Cavalcanti, A.B. Mattos, P Moura, M.D.P.T. Sotomayor, R. F. Dutra, Biosens. Bioelectron. 26 (2010) 1062–1067.
[134] M.M. Billah, H.C.W. Hays, C.S. Hodges, S. Ponnambalam, R. Vohra, P.A. Millner, Sens. Actuators, B 173 (2012) 361–366.
[135] A. Quershi, K.S. Saravan, Y. Gurbuz, M. Howell, W.P. Kang, J.L. Davidson, Procedia Chem. 1 (2009) 1079–1082.
[136] L. Lu, B. Liu, C. Liu, G. Xie, Bull. Korean Chem. Soc 31 (11) (2010).
[137] J.A. Ho, A.F. Jou, L. Wu, S. Hsu, Methods 56 (2012) 223–229.
[138] A. Pirillo, A.L. Catapano, Dis. Markers 35 (2013) 413–418.
[139] B.V.M Silva, I.T. Cavalcanti, M.M.S. Silva, R.F. Dutra, Talanta 117 (2013) 431–437.
[140] S.L.R. Gomes-Filho, A.C.M.S. Dias, M.M.S. Silva, B.V.M. Silva, R.F. Dutra, Microchem. J. 109 (2013) 10–15.
[141] A.B. Mattos, T.A. Freitas, L.T. Kubota, R.F. Dutra, Biochem. Eng. J. 71 (2013) 97–104.
[142] M.M. Billah, C.S. Hodges, H.C.W. Hays, P.A. Millner, Bioelectrochemistry 80 (2010) 49–54.
[143] S. Ko, B. Kim, S. Jo, S.Y. Oh, J. Park, Biosens. Bioelectron. 23 (2007) 51–59.
[144] F. Zhou, M. Lu, W. Wang, Z. Bian, J. Zhang, J. Zhu, Clin. Chem. 56 (2010) 1701–1707.
[145] E. Suprun, T. Bulko, A. Lisitsa, O. Gnedenko, A. Ivanov, V. Shumyantseva, A. Archakov, Biosens. Bioelectron. 25 (2010) 1694–1698.
[146] D. Stan, C. Mihailescu, R. Iosub, C. Moldovan, M. Savin, I. Baciu, Thin Solid Films 526 (2012) 143–149.
[147] V. Bhalla, S. Carrara, P. Sharma, Y. Nangia, C.R. Suri, Sens. Actuators, B 161 (2012) 761–768.
[148] E. Zapp, E. Westphal, H. Gallardo, B. Souza, I.C. Vieira, Biosens. Bioelectron. http://dx.doi.org/10.1016/j.bios.2014.03.026.
[149] F.T.C. Moreira, S. Sharma, R.A.F. Dutra, J.P.C. Noronha, A.E.G. Cass, M.F. Sales, Sens. Actuators, B 196 (2014) 123–132.
[150] Rajesh V. Sharma, S.K. Mishra, A.M. Biradar, Mater. Chem. Phys. 132 (2012) 22–28.
[151] B.E. Ávila, V. Escamilla-Gómez, S. Campuzano, M. Pedrero, J.P. Salvador, M. P. Marco, J.M. Pingarrón, Sens. Actuators, B 188 (2013) 212–220.