Review | DOI:10.5281/zenodo.18056046
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NANOSAM
Journal of NanoScience in Advanced Materials

A Review on Nanostructured Electrochemical Immunosensors for the Determination of HER2, a Breast Cancer Biomarker

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The amplification or overexpression of HER2, which is observed in approximately 15–20 % of breast cancer patients, is known to be associated with unfavorable prognosis in patients with breast cancer. Current techniques which have been approved for the detection of HER2 status such as in-situ hybridization (ISH) and immunohistochemistry (IHC) are costly, time-consuming, have limited sensitivity and selectivity, and also very invasive, limiting their applicability at the clinic in certain settings. However, electrochemical biosensors are most appropriate based on their relative simplicity with respect to instrumentation and analysis, low cost per assay, high selectivity and superior sensitivity, reliability, fast response time and their potential for miniaturization and scalability. Electrochemical immunosensors have shown promise as fast, easy to fabricate, low-cost, and sensitive sensors for the determination of HER2 mRNA/protein levels. These biosensors take advantage of chemical stability, high surface area, and biocompatibility of antibodies, their high affinity and specificity, and unique conductive properties of nanomaterials to selectively and sensitively determine HER2 levels in biological fluids. In this review, we covered electrochemical immunosensors developed for the determination of HER2 levels in biological fluids such as human serum. We highlighted specific sensing capabilities and analytical performance parameters of these immunosensors, also detailing the design of these electrochemical HER2 immunosensors.

1. Introduction

Breast cancer is the most common malignancy in women worldwide, and a highly heterogeneous disease whose prognosis and treatment is defined/determined by the expression of 3 receptors–estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2; a tyrosine kinase receptor encoded by the gene ERBB2) [1, 2]. ER and PR are also known as hormone receptors (HRs). The amplification or overexpression of HER2 (found in approximately 15–20 % of breast cancer patients) is known to be associated with extremely poor survival in patients with breast cancer [3, 4]. When HER2 is activated, it signals via the phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways to promote cell growth and differentiation. In tumor cells in which HER2 is overexpressed or amplified (HER2-positive, HER2+), this activation leads to uncontrolled cell proliferation, inhibition of cell death and metastasis [5]. HER2 is a validated, actionable molecular target, with HER2-targeted therapies showing clinical success that culminated in regulatory approvals for the treatment of patients with HER2+ breast cancer [6–11]. Thus, the level of HER2 is used at the clinical screening, monitoring and management of breast cancer. HER2 protein concentration in blood of healthy people is approximately 2–15 ng/mL and increases in the blood of breast cancer patients by 15–75 ng/mL. The prognosis of patients with HER2 + breast tumors has improved significantly with the introduction of HER2-targeted neoadjuvant/adjuvant therapies [12]. The overall most effective treatment strategy include multi-drug chemotherapy combined with dual HER2 blockade [13]. For instance, in the first-line treatment of advanced HER2+ breast cancer, the standard treatment regimen (taxane-trastuzumab-pertuzumab) provides a median progression-free survival of approximately 18–21 months and a median overall survival of around 57–65 months

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Keywords: Nanostructure: cancer; HER2; immunosensor; electrochemistry

Received: 27 September 2025 | Accepted: 01 November 2025 | Published online: 30 December 2025

[14, 15]. Based on these facts, the identification of HER2 status in patients with breast cancer is of clinical importance in order to determine optimum treatment strategies for the highest clinical efficacy. Therefore, the development of fast and low-cost analytical techniques for accurate HER2 detection in body fluids such as serum is of high clinical importance in the early breast cancer diagnosis, monitoring the effectiveness of treatment and assessing likelihood of remission post treatment.

Current techniques that have been approved by FDA for HER2 status detection involve analyses based on DNA (in-situ hybridization, ISH) and protein (immunohistochemistry, IHC) samples [16]. For instance, IHC is used at the clinic to determine HER2 status (HER2+ vs HER2-) in tumor samples from patients with breast cancer by using antibodies against HER2 protein. However, these techniques are costly, time consuming, have limited sensitivity and selectivity, and also very invasive, limiting their applicability in certain settings. Both of these methods are not suited for point-of-care diagnostics. Both requires skilled-qualified staff to execute the appropriate multi-step procedures. Therefore, the development of a costly-effective, fast, simple, sensitive, selective and non-invasive method for the HER2 detection is required for breast cancer screening, monitoring and management. For this purpose, various methodologies have been developed, including methods based on fluorescence, chromatography, mass spectroscopy and electrochemistry [17–20]. By comparison, electrochemical biosensors are most appropriate due to their relative simplicity in terms of instrumentation and analysis, low cost per assay, high selectivity and superior sensitivity, reliability, quick response and potential for miniaturization [21–25]. Electrochemical immunosensors based on nanomaterials and antibodies have shown promise as fast, easy to fabricate, low-cost, and sensitive sensors for the determination of HER2 protein levels. These biosensors take advantage of chemical stability, high surface area, and biocompatibility of antibodies, their high affinity and specificity, and unique conductive properties of nanomaterials to selectively and sensitively determine HER2 levels in biological fluids [26–28].

In the present review, we covered electrochemical immunosensors developed for the determination of HER2 levels in biological fluids. We highlighted specific sensing capabilities and analytical performance parameters of these sensors, also mentioning the design of these electrochemical HER2 immunosensors (Figure 1).

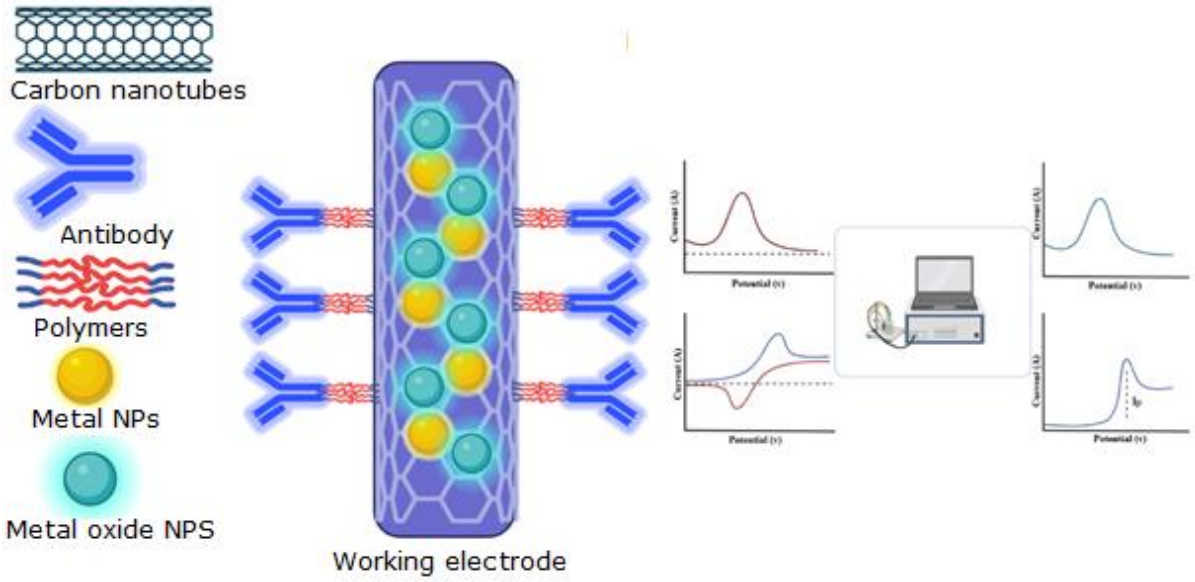

Figure 1. Immunosensors prepared from different nanomaterials used in HER2 determination.

2. Electrochemical immunosensors for the determination of HER2

A sensitive sandwich-type voltammetric immunosensor for the detection of the breast cancer biomarker human epidermal growth factor receptor 2 (HER2) was proposed by Yola (2021) [29]. The proposed immunosensor is based on gold nanoparticles decorated with a copper-organic skeleton (AuNPs/Cu-MOF) and platinum-doped graphitic carbon nitride (g-C_3N_4). The developed immunosensor had a wide linear range of 0.01–1.00 pg/mL and a low detection limit of 3.00 fg/mL. The author reported that the immunosensor

demonstrated satisfactory selectivity, stability, and reproducibility in the determination of HER2.

An electrochemical immunosensor based on electrospun carbon nanofiber (ECNF) for HER2 detection was proposed by Adabi et al. (2020) [30]. The ECNF-based electrode was modified with Au nanoparticles, cysteamine molecules, carbon nanotubes, and antibody molecules. The linear response range of the proposed immunosensor was reported as 5 to 80 ng/mL, and the detection limit was 0.45 ng/mL. The proposed biosensor was compared with the commercial kit and according to the results obtained, it was reported that the proposed immunosensor provides non-invasive, sensitive and rapid analysis for HER2 determination.

An electrochemical immunomagnetic assay for the analysis of the extracellular domain of HER2 in human serum and cancer cells was reported by Freitas et al. (2020) [31]. This study, using magnetic beads, determined two linear working ranges: 5.0 to 50 ng/mL and 50 to 100 ng/mL. The assay, which is selective for the target protein and viable breast cancer cells, reported a detection limit of 2.8 ng/mL. Same research group also used quantum dots (QDs) as nanolabels for the detection of HER2 in human serum samples [32]. They developed an electrochemical immunosensor in which bare screen-printed carbon electrodes were selected as the transducer onto which a sandwich immunoassay was developed. The affinity process was detected through the use of an electroactive label, core/shell CdSe@ZnS QDs, by differential pulse anodic stripping voltammetry in 2 h, with an actual hands-on time of shorter than 30 min, without the need for laborious electrode surface modifications. This immunosensor was found to respond linearly to HER2 concentration in a broad concentration range of 10–150 ng/mL, having a good precision and a LOD of 2.1 ng/mL (corresponding to a detected amount (sample volume = 40 µL) of 1.18 fmol) which is around 7 times lower compared to the established cut-off concentration value (15 ng/mL). The applicability of this HER2 sensor was investigated by the analysis of spiked human serum samples. The reliability of this biosensor for the selective detection of HER2 protein was proven by the analysis of CA15-3 (another breast cancer biomarker) and several other human serum proteins [32]. Authors stated that this sensor is the first electrochemical immunosensing strategy based on QDs as electrochemical label for in situ detection of extracellular domain of HER2.

Electrochemical immunosensors based on screen-printed electrodes (SPEs) have an important place in various analyses due to their low cost, large-scale production, and high-throughput analysis. Tallapragada et al. (2017) developed a screen-printed electrode-based immunosensor for the detection of HER2 antigen in human serum samples [33]. The SPE-based immunosensor, prepared in a sandwich format, was fabricated without any surface modifications to the working electrode. The sensor has a linear working range of 5-20 ng/mL and 20-200 ng/mL, a detection limit of 4 ng/ml, and a quantification limit of 5 ng/mL.

A highly sensitive and innovative electrochemical immunosensor for detecting the extracellular domain of HER2 was reported by de Silva et al. (2025) [34]. The developed immunosensor had a linear detection range of 1.0–150 ng/mL, a detection limit of 0.98 ng mL^{-1}, and a quantification limit of 3.25 ng/mL. Applied in human plasma samples, the sensor was presented as a cost-effective, sensitive, and robust tool for early breast cancer detection.

Immunosensor for HER2 detection was proposed by Xia et al. (2023) by designing polyethylenimine-functionalized MoS_2 nanoflowers (PEI-MoS_2 NFs) with good electrical conductivity and abundant active binding sites [35]. The proposed sandwich-type electrochemical immunosensor for the determination of HER2, an important biomarker in the clinical evaluation of breast cancer, exhibited a wide linear range from 100 fg/mL to 10 µg/mL and a detection limit of 15.64 fg/mL. The authors stated that the immunosensor they produced showed satisfactory selectivity, stability and reproducibility.

A disposable gold electrode modified with gold nanoparticles-decorated Ti_3C_2Tx (Au/MXene) was reported by Sakthivel et al. (2024) [36]. The sensor, applied to human serum samples, had a linear response between 0.0001 and 50.0 ng/mL and a low detection limit of 0.757 pg/mL. The authors reported that the sandwich-type immunosensor they fabricated exhibited good reproducibility and stability.

Electrochemical detection of HER2 using nitrogen-doped carbon quantum dots (N-CQDs) on coated graphite sheet (GS) substrate (N-CQDs/GS) was reported by Amir et al. (2024) [37]. The biosensor, which has a linear response range of 0.1–1.0 ng/mL and a lower detection limit of 4.8 pg/mL, was applied for the detection of HER2 protein in blood samples of breast cancer patients and demonstrated high specificity and stability.

A different approach was used to develop an immunosensor for HER2 detection by Dervisevic et al. (2021) [38]. This proposed method used high-density gold-coated silicon microneedle arrays (Au-Si-MNA) and, demonstrated a linear range between 10 and 250 ng/mL and a detection limit of 4.8 ng/mL. The authors reported that the proposed immunosensor is amenable to modification with

various biological recognition elements and can be used for the detection of different cancer biomarkers.

A highly sensitive electrochemical immunosensor based on a nanohybrid platform for the determination of HER2 in human serum samples was reported by Olorundare et al. (2023) [39]. The proposed immunosensor demonstrated a good linear response of 1 pg/mL–50 ng/mL and a low detection limit of 0.29 pg/mL. The authors also stated that their system could be expanded to detect other cancer biomarkers.

An electrochemical sandwich immunosensor prepared using secondary HER2 antibody conjugated with lead sulfide quantum dots (Ab2-PbS QDs) as a label was reported by Lah and coworkers (2019) [40]. The immunosensor, applied with high recoveries in spiked samples, exhibited a linear working range of 1–100 ng/mL and a low detection limit of 0.28 ng/mL. Based on the obtained data, the authors stated that their sensor can be used to detect HER2 in human serum.

Others developed a label-free electrochemical immunosensor for the detection of HER2 protein levels using the core-shell magnetic metal-organic frameworks (MOFs) (Fe_3O_4@TMU-21) and used this sensor in the analysis of HER2 in human serum samples [41]. The surface of the Fe_3O_4 nanoparticles was functionalized by authors using methacrylic acid to immobilize TMU-21. The fabrication of the immunosensor platform was stated to be based on Fe_3O_4@TMU-21, and multi-walled carbon nanotubes (MWCNT). Their electrocatalytic activity towards the reduction of hydrogen peroxide (H_2O_2) was applied to signal immunosensor. In the presence of HER2 protein, the reduction current of H_2O_2 in the solution was decreased, suggesting the establishment of antigen-antibody complexes on the electrode, blockinging the electron transfer of reduction of H_2O_2 at the electrode surface. A linear relationship between the logarithm of the HER2 concentrations and amperometric responses was found to be within the range of 1.0–100 pg/mL, and the detection limit was 0.3 pg/mL. Another study reported the development of antibody-based label-free electrochemical immunosensors for HER2 [42]. The sensor in this study employed a gold electrode functionalized with carboxylic acid molecules (11-mercaptoundecanoic acid, 3-mercaptopropionic acid), activated by coupling agents and modified with anti-HER2 antibodies. Using differential pulse voltammetry (DPV), the immunosensor achieved a LOD of 2.3 fM in the concentration range of 0.10–100 nM. Authors also studied if the enhancement of selectivity and sensitivity in the detection of HER2 can be achieved via conjugation of PG and antibodies onto a modified gold electrode using L-cysteine (CYS). Protein G (PG) was incorporated into the immunosensor in order to further enhance detection limits and sensitivity. Protein G and Protein A mainly function to orient antibodies in a way that improves antigen-binding efficiency, enhancing the electrochemical response of the immunosensor. Again, a gold electrode was used as the main component, this time functionalized with CYS (used as an effective linker) and PG. The PG-enhanced sensor showed improved sensitivity and a LOD of 0.51 fM, with a linear concentration range of 1 fM–0.10 nM. The sensor displayed a high selectivity for HER2 and was shown to be applicable in the analysis of HER2 in human serum samples highlighting its potential at clinic [42].

Some other reports found that aptamer-enhanced ultrasensitive electrochemical detection of HER-2 can be achieved using a novel nanohybrid substrate material based on ZnO tetrapod-K_4PTC nanohybrids [43]. These nanohybrids were used for electroanalytical aptasensing of HER2 levels. Authors stated that the K_4PTC layer, rich in carboxylate groups, is critical for aptasensing since it enables precise binding interactions with aptamer molecules which target HER2 aptamer. On the other side, the ZnO tetrapod cores were stated to boost the electronic conductivity of the sensor, facilitating efficient charge transfer and making the sensitive detection of HER2 possible. The authors studied the analytical performance of this bio-nanoelectrode for the detection of HER2 using square wave voltammetry (SWV) analysis. They found that this nano-biosensor exhibits linear response in the concentration range of 1 fg/mL–10 µg/mL, and the overall limit of detection and sensitivity of the developed sensor is approximately 0.58 fg/mL and 2.08 µA/fg/mL/mm^2, respectively. The shelf life of the sensor substrate was also found be longer than a month in normal storage conditions and it was shown to have a fast response time of around 10 s.

Ma (2021) developed a dual-mode electrochemical immunosensor based on Au@Ag NRs as double signal indicator for HER2 [44]. In this study, the MnO_2 nanosheets having many base edges provided high external surface areas for in situ grown of Au nanoparticles (MnO_2 NSs/Au NPs). MnO_2 NSs/Au NPs as the substrate materials were used to immobilize primary antibody against HER2 and to accelerate the interface electron transfer. The core–shell Au@Ag nanorods (Au@Ag NRs) were utilized as dual signal indicator. The Au@Ag NRs were shown to be able to catalyze the reduction of H_2O_2 and to amplify current signal through chronoamperometry. Furthermore, Au@Ag NRs performed strong oxidation current signals for electron transfer between Ag and Ag^+ via differential pulse voltammetry. Due to multifunction of Au@Ag NRs, highly sensitive and accurate detection of HER2 was obtained by means

of DPV and CA. Further, the immunosensor displayed a wide concentration linear range of 50 fg/mL −100 ng/mL and 100 fg/mL−100 ng/mL, with low LODs of 16.7 and 33.3 fg/mL through DPV and CA, respectively. The successful detection of HER2 in human serum was achieved using this sensor [44].

Joshi et al. reported the development of different morphological variants of palladium (Pd) nanostructures supported onto oxidized carbon nanotubes (CNTs) to enable label-free electrochemical immunosensing of HER2 protein, using an easy, convenient, and robust 1-step electroreduction methodology [45]. The data obtained in this study suggested the suitability of hierarchical palladium nanostructures supported onto carbon nanotubes [Pd(−0.1V)/CNT] for sensitive detection of HER2. In other words, morphological relevance of Pd nanostructures facilitated the immobilization of antibodies against HER2 on the sensor surface, reliably and efficiently. The high surface area of these nanostructures enabled a highly sensitive electrochemical response toward HER2 with detection limit of 1 ng/mL and a wide concentration range of 10−100 ng/mL. The simplicity of surface modification, sensitivity, and reliable electrochemical response in human blood plasma samples showed the potential of Pd nanostructuring for chip-level point-of-care screening of HER2-positive breast cancer patients.

In another study, an ultrasensitive dual-signal ratio electrochemical aptamer biosensor was developed for the detection of HER2 in human serum samples [46]. This biosensor was based on ZIF-67 @polydopamine (PDA) nanocomposite and Cu/UiO-66 @ 3, 3′,5,5′-tetramethylbenzidine (TMB) nanocomposite. PDA was selected as the electroactive material with electrochemical redox activity, and ZIF-67 with a high specific surface area, forming the ZIF-67@PDA+Apt as the nanoprobe to capture HER2. Cu/UiO-66 is a bimetallic compound having high stability, specific surface area, and strong adsorption onto aptamer chains, and the Cu/UiO-66@TMB+Apt nanocomposite was utilized as a probe for signal labeling. When HER2 was present, the capture of HER2 by the ZIF-67@PDA+Apt probe led to the weakening of the conductivity of the electrode; however, by decreasing the electrochemical signal from the PDA, changing the probe-Cu/UiO-66@TMB+Apt signaling resulted in the enhancement of the TMB electrochemical signal. This sensor sensitively detected HER2 proteins within 30 min with the concentration range of 0.75–40 pg/mL and a LOD of 44.8 fg/mL. These dual signal ratio biosensors were found to have a low limit of detection, short response time, which are able to accurately detect targets in complex biological samples [46].

Harahsheh et al. developed an aptasensor for HER2 using screen-printed electrodes modified with AuNPs [47]. In this study, the authors immobilized HER2 aptamer via electrostatic adsorption on the surface of a screen-printed electrode, which was modified with AuNPs (having approximately 20 nm diameter) to support the aptamer immobilization. This aptasensor was shown to display a quick response with a binding time of just 5 min, and to have a log-linear response in a broad concentration range from 0.001 to 100 ng/mL. Besides, this sensor was found to have a high sensitivity and improved detection limit reaching 52.85 μA/ng/mL, and 0.001 ng/mL, respectively, with a relative standard deviation of less than 5%. Furthermore, the aptasensor exhibited a stable response for 3 days with RSD of ~ 4%. This aptasensor was reported to be efficient for HER2 detection with around 8% extent of the interference. Authors of this study stated that the incorporation of Au NPs with SPEs provides support features showing promising results in the development of aptasensors.

In another work, authors reported the impedimetric aptasensing of HER2 via a glassy carbon electrode (GCE) modified with AuNPs in a composite comprised of electrochemically reduced graphene oxide and single-walled carbon nanotubes (SWCNTs) (ErGO-SWCNT/AuNP) [48]. They were incorporated into an aptamer-based assay to apply the synergy contributions on the enhancement of signal characteristics. In this study, an aptamer for HER2 (anti-HER2 aptamer containing a sequence of 86 bases) was immobilized onto the GCE. The immobilized aptamer was shown to selectively recognize HER2 on the electrode interface, resulting in an increased charge transfer resistance (R_{ct}) of the electrode when ferri/ferro-cyanide was used as the electrochemical probe. The sensor was shown to have a low LOD of 50 fg/mL and a concentration range of 0.1 pg/mL ng/mL. The applicability of the sensor was shown by determination of HER2 levels in serum samples. The performance characteristics of the mentioned sensors are summarized in Table 1.

Table 1. Performance characteristics of immunosensors developed for HER2 determination.

Sensing platforms	Capturing element/assay protocol	Technique	Sample	Linear range	LOD	Stability (days)	Ref
CZTS NPs/5.0wt%Pt/g-C_3N_4	sandwich- assay	CV	human plasma	0.01–1.00 pg/mL	3.00 fg/mL	30	[29]
Ab/CNT/Cys/Au/CNF	electrospun carbon nanofiber	CV	human serum	5–80 ng/mL	0.45 ng/mL	14	[30]
SPCE/ HOOC-MBs	sandwich-assay	LSV	human serum	5.0–50 ng/mL 50–100 ng/mL	2.8 ng/mL	60	[31]
CdSe@ZnS QDs	bare spce	DPV	human serum	10–150 ng/mL	2.1 ng/mL	7	[32]
Screen printed carbon electrodes with no modification	sandwich-assay	CV	human serum	5–20 ng/mL 20–200 ng/mL	4.0 ng/mL	7	[33]
AuNPEthaline/ poly(an-co-8-ansa)Ethaline-$HClO_4$/GCE	sandwich-assay	LSV	human plasma	1.0–150 ng/mL	0.98 ng/mL	60	[34]
La-MOF-PbO_2	sandwich-assay	CV/DPV	human serum	100 fg/mL–10 µg/mL	15.64 fg/mL	20	[35]
NG/CuMnCoOx/Ab2/HER2-ECD /Ab1/Au/MXene/ DGE	sandwich-assay	CV/DPV	human serum	0.0001–50.0 ng/mL	0.757 pg/mL	10	[36]
N-CQDs/GS		DPV	human serum	0.1–1.0 ng/mL	4.8 pg/mL	10	[37]
Au–Si-MNA		DPV	artificial interstitial fluid	10–250 ng/mL 50–250 ng/mL	4.8 ng/mL 25 ng/mL	-	[38]
nanoD/AuNPs		DPV	human serum	1.0 pg/mL–50 ng/mL	0.29 pg/mL	15	[39]
Ab2-PbS QDs	sandwich-assay	SWV	human serum	1.0–100 ng/mL	0.28 ng/mL	-	[40]
Fe_3O_4@ TMU-21	label-free	Amperometric/ CV	human serum	1.0 pg/mL –100 ng/mL	0.30 pg/mL	15	[41]
Au/CYS/ PG/Ab	label-free	DPV	human serum	185 fg/mL–18.5 ng/mL	0.51 fM/mL	30	[42]
ZnOT-K_4PTC nanohybrids	point-of-care	SWV	human serum	1 fg/mL–10 µg/mL	0.58 fg/mL	30	[43]
MnO_2 NSs/Au NPs	dual signal indicator	DPV	human serum	50 fg/ml–100 ng/mL 100–fg/ml 100 ng/mL	16.7 fg/mL 33.3 fg/mL	-	[44]
Pd(-0.1V)/CNT/SPEs	label-free	DPV	human plasma	10–100 ng/mL	1 ng/mL	7	[45]
ZIF-67 @PDA+Apt	dual-signal ratio	DPV	human serum	0.75–40 pg/mL	44.8 fg/mL	11	[46]
Screen-printed electrode modified with gold nanoparticles	sandwich- assay	DPV		0.001–100 ng/mL	0.001 ng/mL	3	[47]
ErGO-SWCNT/AuNP	direct	CV	human serum	0.1 pg/mL –1.0 ng/mL	50 fg/mL	30	[48]

CV: cyclic voltammetry, LSV: linear sweep voltammetry, DPV: differential pulse voltammetry, SWV: square wave voltammetry

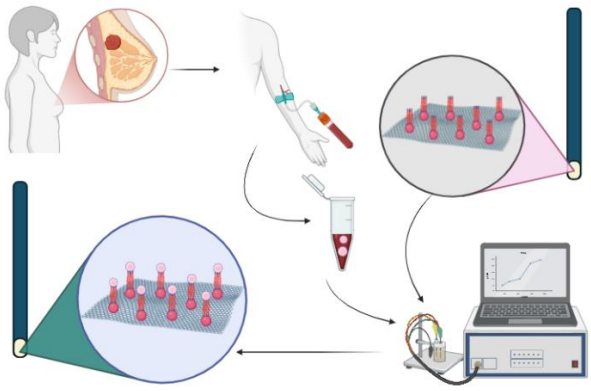

Figure 2. Applications of electrochemical methods used in HER2 determination in biological fluids.

3. Conclusion

Studies covered in the present review point out that electrochemical immunosensors are good alternatives to current methodologies actively used in the clinic, for the determination of HER2 levels. They offer particular advantages as detailed here, showing promise for their potential for broader use at the clinic, especially in settings with lower resources and without trained personnel. Further research is clearly needed to improve these sensors to enable their applicability at the clinic at a wider scale and to enhance their analytical performance which will make them first option to consider in the analysis of HER levels in body fluids (Figure 2). We believe that this article will raise significant awareness for the production of rapid, economical, simple and stable biosensors suitable for on-site analysis for HER2 determination.

Authors' contributions:

All authors have equal contributions.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of Ethical Standards

The author(s) of this article declare that the materials and methods used in this study do not require ethical committee permission and/or legal-special permission.

Conflict of Interest

There is no conflict of interest in this study.

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