Current Topics in Electrochemistry
Vol. 22, 2020

Review

Electrochemical immunosensor to detect hepatitis B antigen (HBsAg) and hepatitis B e antigen (HBeAg) as biomarkers of hepatitis B infection: A review

Ghina Nur Fadhilah, Muhammad Yusuf and Yeni Wahyuni Hartati*

Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Padjadjaran, Indonesia.

ABSTRACT

Immunosensors are biosensors based on the interaction between antibodies and antigens on the transducer surface. The use of immunosensors is a promising method for detecting pathogens, including viral pathogens. This is because antibodies are natural receptors in binding harmful antigens to an organism so that they have high selectivity and natural binding efficiency. Hepatitis B infection is an infectious disease caused by the hepatitis B virus (HBV). This review focuses on the use of electrochemical immunosensors, both labeled and unlabeled, reported in the last three years for the detection of hepatitis B antigen (HBsAg) and hepatitis B e antigen (HBeAg) as biomarkers of hepatitis B viruses' infection. The principle of detection and the limit of detection of the developed electrochemical immunosensors are reported in this review.

KEYWORDS: hepatitis B, HBsAg, HBeAg, electrochemical immunosensor.

INTRODUCTION

Viruses are pathogens with a length of 200-1,000 nm and a diameter of 20-300 nm that can damage cells, tissues, organs and can cause death in living things [1]. Viruses have defense mechanisms that can change rapidly, and which can adapt to destroying and manipulating the immunity of the host cell [2]. Viral infections cause thousands of deaths worldwide every year [2, 3]. One of the well-known viral pathogens is the hepatitis B virus (HBV). HBV infection has become a serious health problem worldwide where more than 350 million people are infected and at least 1 million people die every year [4].

Symptoms that appear in this disease include inflammation of the liver, vomiting, and the body turning yellow. HBV can become chronic due to a persistent viral reservoir that can cause severe complications such as cirrhosis of the liver and liver cancer, as well as hepatocellular carcinoma [5].

Serological markers in HBV infection consist of anti-HBc IgM and IgG, HBeAg, anti-HBe, and HBsAg [6]. HBsAg or hepatitis B antigen is a serological marker for HBV. HBsAg will appear in the blood serum between 1-10 weeks after a person is exposed to HBV [7]. The presence of HBeAg or hepatitis B e antigen indicates active viral replication. HBeAg is an important biomarker for detecting chronic hepatitis B infection where HBeAg will appear at the beginning of chronic hepatitis B infection [8].

Methods of diagnosis of HBV infection are urgently needed to minimize infectious complications and mortality. Enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), rapid diagnostic tests (RDTs) immunochromatography-based, and electrochemiluminescence immunoassay are some of the methods that are widely used in HBV diagnostics. However, the ELISA method is relatively complicated and electrochemiluminescence immunoassay requires a long time and reliable

*Corresponding author: yeni.w.hartati@unpad.ac.id

analysis. Meanwhile, RDT has limitations in terms of its qualitative analysis [9, 10].

An amplification-based method such as PCR allows quantitative analysis, is highly sensitive and efficient at amplifying target areas in the viral genome. Therefore, the PCR method is the main method to detect HBV viruses. PCR method has a range limit of detection (LOD) to detect HBV around 5 IU/ mL - 190 IU/mL [11-15]. One IU of hepatitis B antigen is comparable to 0.88 ± 0.20 ng [16]. However, PCR is prone to contamination, if you use an inappropriate primer, the results obtained can be misleading, and PCR analysis takes several hours [3]. Therefore, an alternative HBV diagnostic method that is fast, effective, low cost, portable and has good specificity and sensitivity is needed.

Biosensors are analytical devices that are used for the detection of a chemical substance incorporating a biological material as the recognition molecule, which is integrated within a physicochemical detector. Biosensors consist of a biological element that can sense the analyte and a transducer that can convert the recognition event into detectable parameters [17]. Immunosensors are biosensors based on the interaction between antibodies and antigens on the detection system. The use of immunosensors is a promising method for detecting pathogens, including viral pathogens because the antibodies are natural receptors that bind to harmful antigens in an organism with high affinity, selectivity, and efficiency [18].

Immunosensors can be classified into optical immunosensors, piezoelectric immunosensors, and electrochemical immunosensors. The electrochemical immunosensor is an analytical tool that combines the specific interactions between antibodies and antigens with an electrochemical reaction on the electrode surface [19]. Electrochemical immunosensors have many advantages including portability, low cost, fast detection time, and high sensitivity. Furthermore, electrochemical immunosensors have been extensively developed for use in cancer screening, virus measurement, and diagnosis [20, 21].

This review describes electrochemical immunosensors reported in the last three years, the application of electrochemical immunosensors to detect HBsAg, and HBeAg as biomarkers of HBV, and compares the limit of detection (LOD) for each method in detecting HBV.

Hepatitis B Virus (HBV)

Hepatitis B Virus (HBV) infection has become a serious health problem worldwide where more than 350 million people are infected and at least 1 million people die every year [4]. Chronic HBV infection or chronic hepatitis B (CHB) can cause chronic liver disease, and hepatocellular carcinoma (HCC) [5].

HBV belongs to the hepadnaviridiae family that replicates *via* reverse transcription of RNA intermediates. Serological markers in HBV infection consist of HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc IgM and IgG [6]. HBsAg and HBeAg have received attention as biomarkers for controlling HBV infection [7].

HBsAg or hepatitis B antigen is a serological marker for HBV. After a person is exposed to HBV, HBsAg will appear in the blood serum between 1-10 weeks. The presence of HBsAg for more than 6 months indicates that HBV infection is in a chronic stage [6]. HBsAg can be a quantitative risk factor for hepatocellular carcinoma (HCC) and chronic liver disease [7]. The presence of HBeAg or hepatitis B e antigen indicates active viral replication. HBeAg is an important biomarker for detecting chronic hepatitis B infection where HBeAg will appear at the beginning of chronic hepatitis B infection [8].

Antibody

An antibody is a glycoprotein that is shaped like the letter "Y" and secreted by B lymphocytes called plasma cells [22]. The antibody molecule has four polypeptide chains, out of which two are light chains (L) and two are heavy chains (H) as can be seen in Figure 1. These two chains are connected by disulfide bridges and non-covalent bonds [22, 23]. Heavy chains and light chains each have variable regions (V) and constant regions (C). The variable region is composed of 110-130 amino acids, and plays a role in providing specific binding sites with antigens, and the function of constant region is to maintain the stability of the antibody structures. Each heavy chain has one variable region (VH), and three constant regions (CH1, CH2, CH3) as shown in Figure 1 (darker shade). Each light chain has one variable region (VL), and a constant region (CL) as shown in lighter color in Figure 1 [23-25].

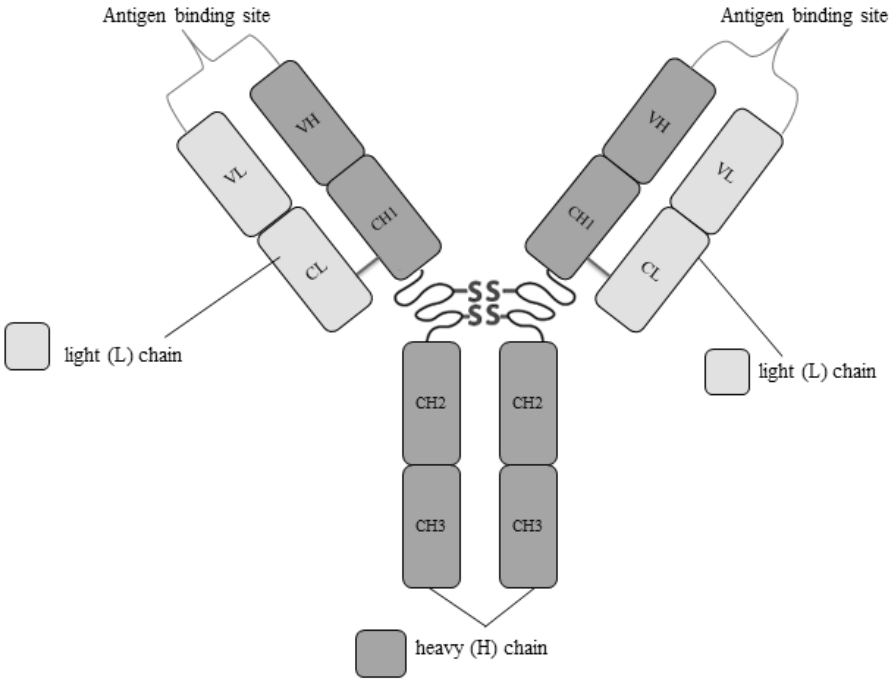

Figure 1. Structure of antibody consisting of regions of the heavy chain (VH-CH1-CH2-CH3) and light chain (VL-CL).

Immunosensor

The basic principle of the biosensor is to detect known molecules and convert the interaction of bioelement with the analyte into signals that are read by the transducer. In some biosensors, some components are immobilized on a solid surface (generally the sensor chip), and other components in the solution can be detected [19, 26]. Bioelements that are commonly used in biosensors include antibodies, nucleic acids, hormones, and enzymes [27], and the immunosensor involves the antibodies that immobilize on the transducer surface.

In the electrochemical immunosensor system, two things need to be considered. The first is the immobilizing technique of the antibodies on the electrode surface, and the second is the strengthening of the electrochemical signal from the immunosensor [8, 28, 29]. The orientation of the immobilized antibody on the electrode surface strongly influences the sensitivity of the immunosensor [30]. Based on the signal generated, electrochemical immunosensors are classified into potentiometric, amperometric, conductometric, and impedance electrochemical immunosensors [31]. The simplicity and sensitivity of electrochemical immunosensors have driven the development of this tool for various analytical purposes [18, 19].

The immunosensor detection mechanism can be carried out *via* a labeled and label-free reporter compound. A label-free or nonlabeled electrochemical immunosensor is designed so that the antibody immobilized on the sensor surface binds directly to the antigen, and makes a change in the electrical responses. In contrast, labeled electrochemical immunosensor use a marker to help generate a signal [31, 32]. Table 1 shows the electrochemical immunosensors that were reported in the last three years for the detection of HBV.

Label-free electrochemical immunosensor for the detection of HBsAg and HBeAg

In a label-free immunosensor, the antibody is immobilized on the surface of transducer, and when binding to the antigen, provides a linear relationship between the concentrations of the antigen and the resulting electrical signal [31].

A label-free electrochemical immunosensor has been developed using graphene oxide (GO)/Fe_3O_4/Prussian Blue (PB) nanocomposite for detecting the HBsAg. GO/Fe_3O_4/PB and gold nanoparticles (AuNP) were

Table 1. The electrochemical immunosensors reported in the last three years for the detection of HBsAg and HBeAg as biomarkers of Hepatitis B viruses' infection.

No	Detection target	Electrochemical measurement	LOD	Reference
1	HBsAg	CV, DPV and EIS	0.166 pg/mL	[33]
2	HBsAg	DPV	0.86 ng/mL	[34]
3	HBsAg	DPV and CV	10.2 fg/mL	[35]
4	HBsAg	CV and EIS	166 fg/mL	[36]
5	HBsAg	DPV	6.7 fg/mL	[37]
6	HBsAg	EIS	0.15 pg/mL	[38]
7	HBeAg	CV and EIS	20 fg/mL	[39]
8	HBeAg	CV, DPV and EIS	0.064 pg/mL	[8]
9	HBeAg	EIS	26 fg/mL	[40]

used to coat screen-printed electrodes and were reported to increase the sensitivity of the sensor. The hepatitis B antibody (HBsAb) was immobilized onto the modified surface; meanwhile, the PB was used as a probe. The decrease in the peak PB current is proportional to the HBsAg concentration captured on the immunosensor. The electrical signal generated from the interaction between HBsAb and HBsAg is measured using cyclic voltammetry (CV), differential pulse voltammetry (DPV), and electrochemical impedance spectroscopy (EIS) techniques. The developed immunosensor can detect HBsAg in a concentration range of 0.5 pg/mL to 200 ng/mL with an LOD of 0.166 pg/mL[33].

The HBsAgs have also been detected using a screen-printed carbon electrode (SPCE) modified with carbon nanotubes-gold nanoparticles (AuNPs-CNT), and silver nanoparticles (AgNP). However, SPCE has a disadvantage, namely the working electrode surface is narrow such that the electrode becomes less sensitive. To overcome this, the electrodes can be modified to increase their sensitivity. AuNP is widely used in SPCE modification, wherein AuNP can immobilize proteins through covalent bonds between Au atoms with amine and cysteine groups on proteins. AgNPs are used as probes in HBsAg detection using DPV. AgNPs are used because of their strong conductivity, good biocompatibility, and electrochemical reactivity. The reported immunosensor revealed the linear concentration range of HBsAg of 1-40 ng/mL, and the LOD was 0.86 ng/mL [34].

A label-free electrochemical immunosensor to detect HBsAg using the nano enzyme PtPd nanocubes@MoS2 (PtPd NCs@MoS_2) to amplify the electrochemical signal has been developed. PtPd NCs@MoS_2 is used because it has excellent peroxidase activity and conductivity, a large surface area, and can amplify certain signals. The electrochemical measurement was conducted using CV and DPV. The developed immunosensor resulted an LOD of 10.2 fg/mL and can detect HBsAg in the concentration range of 32 fg/mL to 100 ng/mL [35].

A composite PdCu TPs/PG has been developed as a catalyst to increase the electrochemical signal in the immunosensor for the detection of HBeAg. PdCu TPs/PG showed excellent Michaelis-Menten kinetic catalytic parameters, conductivity, and peroxidase properties. PdCu TPs/PG is used to catalyze the electrochemical active-matrix H_2O_2 and amplify the resulting signal currents for HBeAg detection. The electrochemical measurements were carried out by CV and EIS with a redox system of $K_3[Fe(CN)_6]$ solution in 0.1 M KCl. The developed immunosensor could detect HBeAg in the concentration range of 60 fg/mL to 100 ng/mL with LOD of 20 fg/mL [39].

Labeled electrochemical immunosensor for the detection of HBsAg and HBeAg

A labeled electrochemical immunosensor uses markers in the form of enzymes, fluorescence, or redox probes to help generate signals. Labels commonly used in labeled immunosensors include

the HRP enzyme, fluorophores such as fluorescein, and luminol that exhibits chemiluminescence [32].

The markers are usually redox reporter compounds that can generate an electrical signal when antibodies bind to the target molecule. In sandwich-based immunosensors, a primary antibody (Ab_1) was immobilized on the electrode surface followed by secondary antibody (Ab_2) which had been labeled. Secondary antibodies that have been labeled will bind to the binding site on the antigen (Ag). The resulting signal generally comes from the catalytic reaction of the enzyme molecule which is used as a label on the antibodies used to detect the analyte. The resulting product contains electrical changes detected by electrodes [42]. The principle of labeled immunosensors can be seen in Figure 2.

A sandwich-type electrochemical immunosensor based on RhPt NDs/NH_2-GS and Au NPs/PPy Ns to detect HBsAg has been developed. Polypyrrole nanosheet (Au NPs/PPy NS) is used as a platform; meanwhile, functionalized graphene (RhPt NDs/ NH_2GS) was used as labels RhPt NDS/NH_2GS as a label has high catalytic properties for the reduction of H_2O_2 to amplify the signal immunosensor. Au NPs/PPy NS can increase electron transfer and provide a good microenvironment to immobilized antibodies effectively thereby increasing the sensitivity of the immunosensor. The electrochemical signal was measured using CV and EIS. The developed immunosensor can detect HBsAg in the concentration range of 0.0005 ng/mL to 10 ng/mL with an LOD of 166 fg/mL [36].

A sandwich-type labeled electrochemical immunosensor based on gold with m-Au@Pd@Pt to detect HBsAg has been developed. Gold nanorods (AuNRs) were used as the substrate material and Au@Pd@Pt (m-Au@Pd@Pt) core-shell mesoporous nanospheres were used as labels for antibodies (Ab_2, Ab_2 labels) to detect HBsAg surfaces. Au NRs have good conductivity and biocompatibility which can accelerate electron transfer at the electrode interface and increase the charge capacity of capture antibodies. The DPV technique was carried out for measuring the electrical signal. The LOD was found to be 6.7 fg/mL with the linear range concentration of 20 fg/mL to 200 ng/mL using the developed immunosensors [37].

An electrochemical immunosensor labeled with molybdenum disulfide@cuprous oxide nanohybrid (MoS_2@Cu_2O-Pt) to detect HBsAg has been developed. Compared with single metal oxides, the nanocomposites with a hybrid structure can optimize the interface contact better. The EIS data showed the linear correlation between the impedance and

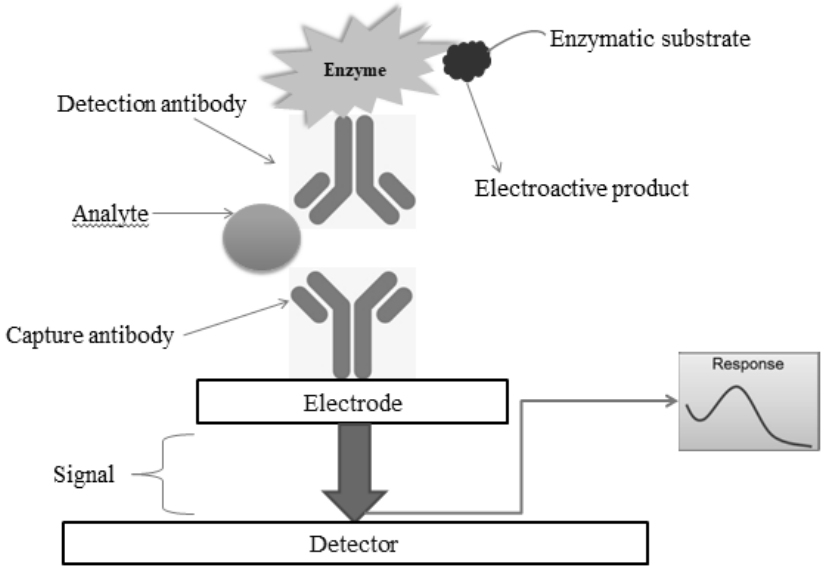

Figure 2. The generation of electrochemical signal in a labeled immunosensor. The resulting signal generally comes from the catalytic reaction of the molecule which is used as a label on the antibodies to detect the analyte. The resulting product contains electrical changes detected by electrodes.

the concentration of HBsAg in a range of 0.5 pg/mL to 200 ng/mL with LOD of 0.15 pg/mL [38].

A labeled electrochemical immunosensor to detect the hepatitis B e antigen (HBeAg) has been developed. Electrochemical signal amplification is crucial for electrochemical immunosensors to improve detection performance. The developed immunosensor uses horseradish peroxidase (HRP) and catalysts gold nanoporous (NPG) to increase the signal where HRP catalyzes catechols and provides a signal label on secondary antibodies. NPG has electro-catalytic activation and hence can increase the electrochemical signal generated by HRP. The electrochemical signal was measured using CV, DPV, and EIS. The developed immunosensors can detect HBeAg at a concentration of 1 pg/mL to 1 ng/mL with LOD of 0.064 pg/mL [8].

A sandwich-type labeled electrochemical immunosensor to detect HBeAg using a glassy carbon electrode (GCE) has been developed. The GCE electrode is modified with AuNPs that have been functionalized with graphene oxide (p-GO@Au). This modification aims to improve electron transfer and increase the sensitivity of the immunosensor. Molybdenum disulfide functionalized with multiwalled carbon nanotubes (Au@Pd/MoS_2@MWCNTs) was used as a label on secondary antibodies (Ab_2). Au@Pd/MoS_2@MWCNTs show good catalytic stability and efficiency and hence are used as labels in Ab_2. The EIS measurement of the developed immunosensors showed the good linearity of HBeAg at a concentration of 0.1 pg/mL to 500 pg/mL with LOD of 26 fg/mL [40].

CONCLUSION

Electrochemical immunosensors have been developed as alternative diagnostic methods for detecting hepatitis B antigen (HBsAg) and hepatitis B e antigen (HBeAg) as biomarkers of hepatitis B virus infection. The LOD value on the immunosensor was smaller than the LOD value in the PCR method. While the LOD range on the PCR method in detecting HBV is around 5 IU/mL - 190 IU/mL with one IU of hepatitis B antigen equal to 0.88 ± 0.20 ng, the LOD value on the electrochemical immunosensor collected varied, in the range of 6.7 fg/mL - 0.166 pg/mL. So, it can be concluded that the use of electrochemical immunosensors can be a prospective method or a promising supporting method for detecting hepatitis B virus infection.

CONFLICT OF INTEREST STATEMENT

Nothing to declare.

REFERENCES

1. Chen, L. and Liang, J. 2020, Mater. Sci. Eng. C, 112, 110924.
2. Saylan, Y., Erdem, Ö., Ünal, S. and Denizli, A. 2019, Biosensors, 9, 65.
3. Ambartsumyan, O., Gribanyov, D., Kukushkin, V., Kopylov, A. and Zavyalova, E. 2020, Int. J. Mol. Sci., 21, 3373.
4. Wang, Z., Li, Y., Liang, W., Zheng, J., Li, S., Hu, C. and Chen, A. 2018, Sci. Rep., 8, 3837.
5. Matsunaga, S., Jeremiah, S.S., Miyakawa, K., Kurotaki, D., Shizukuishi, S., Watashi, K., Nishitsuji, H., Kimura, H., Tamura, T., Shimotohno, K., Wakita, T. and Ryo, A. 2020, iScience., 23(3), 100867.
6. Song, J. E. and Kim, D. Y. 2016, Ann. Transl. Med., 4(18), 338.
7. Downs, L. O., Smith, D. A., Lumley, S. F., Patel, M., Mcnaughton, A. L., Mokaya, J., Channon, K., Davies, J., Barnes, E., Jeffery, K. and Matthews, C. 2019, Am. Soc. Microbiol., 10(3), 1.
8. Zhang, Y., Gao, Y., Zhang, X., Wang, H., Xia, T., Bian, C., Liang, S., Tang, X. and Wang, X. 2019, Sensors Actuators, B Chem., 284, 296.
9. Cabral, D. G. A., Lima, E. C. S., Moura, P. and Dutra, R. F. 2016, Talanta., 148, 209.
10. Hartati, Y. W., Gaffar, S., Alfiani, D., Pratomo, U., Sofiatin, Y. and Subroto, T. 2020, Sens. Bio-Sensing Res., 29, 100343.
11. Solange, N. E., Alphonsine, M., Moussa, D., Ngolo, C., Syndou, M., Aboubacar, S., Serge, A., Hortense, F., Mireille, D. 2014, J. Microbiol. Res. Rev., 2(4), 34.
12. Wu, B., Xiao, F., Li, P., Du, Y., Lin, J., Ming, K., Chen, B., Lei, X., Xu, B. and Liu, D. 2017, PLoS One, 12(2), 1.
13. Liu, C., Chang, L., Jia, T., Guo, F., Zhang, L., Ji, H., Zhao, J. and Wang, L. 2017, Virol. J., 14(94).

14. Sitnik, R., Paes, Â., Mangueira, C. P. and Pinho, J. R. R. 2010, Rev. Inst. Med. Trop. Sao Paulo., 52, 119.
15. Zauli, D. A. G., Menezes, C. L. P. de., Oliveira, C. L. de., Mateo, E. C. C. and Ferreira, A. C. de S. 2016, Brazilian J. Microbiol., 47, 987.
16. Chudy, M., Scheiblauer, H., Hanschmann, K. M., Kress, J., Nick, S., Wend, U., Shuttler, C., Nubling, C. M. and Gerlich, W. H. 2013, J. Clin. Virol., 58(1), 47.
17. Metkar, S. J. and Girigoswami, K. 2019, Biocatal. Agric. Biotechnol., 17, 271.
18. Jarocka, U., Sawicka, R., Góra-Sochacka, A., Sirko, A., Dehaen, W., Radecki, J. and Radecka, H. 2016, Sensors Actuators, B Chem., 228, 25.
19. Karunakaran, C., Rajkumar, R. and Bhargava, K. 2015, Introduction to Biosensors, Elsevier Inc.
20. Baradoke, A., Jose, B., Pauliukaite, R. and Forster, R. J. 2019, Electrochim. Acta., 306, 299.
21. Chan, K. F., Lim, H. N., Shams, N., Jayabal, S., Pandikumar, A. and Huang, N. M. 2016, Mater. Sci. Eng. C, 58, 666.
22. Lipman, N. S., Jackson, L. R., Trudel, L. J. and Weis-Garcia, F. 2005, ILAR J., 46, 258.
23. Zeng, X. Z., Shen. and Mernaugh, R. 2012, Anal. Bioanalitical Chem., 402, 3027.
24. Conroy, P. J. S., Hearty., Leonard, P. and O'Kennedy, R. J. 2009, Semin. Cell Dev. Biol., 20, 10.
25. Ahamadi-Fesharaki, R., Fateh, A., Vaziri, F., Solgi, G., Siadat, S. D., Mahboudi, F. and Rahimi-Jamnani, F. 2019, Mol. Ther. - Oncolytics., 14, 38.
26. Fakhrabadi, N. H., Rohani, A. A. and Farahbakhsh, A. 2015, Adv. Mater. Researach, 496, 17.
27. Monošík, R., Streďanský, M. and Šturdík, E. 2012, Acta Chim. Slovaca., 5, 109.
28. Felix, F. S. and Angnes, L. 2018, Biosens. Bioelectron., 102, 470-478.
29. Hartati, Y., Satriana, N., Gaffar, S., Mulyana, J., Wyantuti, S. and Sofiatin, Y. 2020, ICONISTECH.
30. Hartati, Y. W., Daniswara, M. H., Nurmalasari, R., Gaffar, S. and Subroto, T. 2018, Molekul., 13, 1.
31. Banga, I., Tyagi, R., Shahdeo, D. and Gandhi, S. 2019, Biosensors and their application for the detection of avian influenza virus. Elsevier Inc.
32. Karunakaran, C., Pandiaraj, M. and Santharaman, P. 2015, Immunosensors, Elsevier Inc., 205.
33. Wei, S., Xiao, H., Cao, L. and Chen, Z. 2020, Biosensors., 10, 1.
34. Upan, J., Banet, P., Aubert, P. H., Ounnunkad, K. and Jakmunee, J. 2020, Electrochim. Acta., 349, 136335.
35. Tan, Z., Dong, H., Liu, Q., Liu, H., Zhao, P., Wang, P., Li, Y., Zhang, D., Zhao, Z. and Dong, Y. 2019, Biosens. Bioelectron., 142, 111556.
36. Pei, F., Wang, P., Ma, E., Yang, Q., Yu, H., Gao, C., Li, Y., Liu, Q. and Dong, Y. 2019, Bioelectrochemistry., 126, 92.
37. Li, M., Wang, P., Pei, F.,Yu, H., Chen, P., Dong, Y., Li, Y., Liu, Q. and Li, D. 2018, J. Electroanal. Chem., 809, 14.
38. Li, F., Li, Y., Feng, J., Gao, Z., Lv, H., Ren, X. and Wei, Q. 2018, Biosens. Bioelectron., 100, 512.
39. Tan, Z., Cao, L., He, X., Dong, H., Liu, Q., Zhao, P., Li, Y., Zhang, D. and Ma, W. 2020, Bioelectrochemistry, 133, 107461.
40. Gao, Z., Li, Y., Zhang, X.,. Feng, J., Kong, L., Wang, P., Chen, Z., Dong, Y. and Wei, Q. 2018, Biosens. Bioelectron., 102, 189.
41. Florea, A., Melinte, G., Simon, I. and Cristea, C. 2018, Sensors, 9, 38.
42. Cho, I. H., Lee, J., Kim, J., Kang, M. S., Paik, J. K., Ku, S., Cho, H. M., Irudayaraj, J. and Kim, D. H. 2018, Sensors, 18, 207.