Eclia Test- Review

G.Nivedhita[1], M.P.Brundha[2]

[1]Research Associate, Dental Research Cell, Department of General Pathology, [2]Associate Professor, Department of General Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

Abstract

Chemiluminescent immunoassay is a modification of standard enzyme immunoassay which is a biochemical technique that has evolved to be used in recent times. Immunoassay is a diagnostic biochemical test used to quantify an analyte or a specific substance from blood or other body fluids. Electrochemiluminescence immunoassay (ECLIA) is a new developing method to determine antibodies in human being's blood sample.this has evolved from various other immunoassay methods that were previously used for detection of enzyme/antibody etc. The test takes place following three principles. Electrochemiluminescence immunoassay testing has been used to detect many markers such as typhoid, cardiac, tumor markers and various infectious diseases in in-vitro analysis. It is also frequently used in the detection of recombinant specific treponemal antigens. Research articles were searched from search engines such as MeSH, pubMed, Core, Google Scholar, Cochrane etc. the time period considere was from 2000 - 2020 which is 20 years. Articles were studied and data were collected under different headings. In Spite of many advantages of immunoassays, they have some limitations. Immunoassays rely primarily on activity between analyte and the natural antibody, they may have more intrinsic imprecision than other methods used in medicine analysis. Biological markers might produce false positive results and usage of viral markers is still not found for the process of electrochemiluminescence immunoassay test. Late night salivary cortisol concentration, an indicator of cushing's syndrome, is detected using this test. This research provides in-depth knowledge and awareness of electrochemiluminescence immunoassay tests and paves the way for better diagnostic methods for the future.

Keywords: *Electrochemiluminescence, Antigen/ antibody detection, Light night salivary cortisol, Diagnostic test.*

Introduction

Chemiluminescent immunoassay is a modification of standard enzyme immunoassay which is a biochemical technique that has evolved to be used in recent times. It is often used as a diagnostic tool in medicine[1].It is expressed by a luminescence molecule which happens by the emission of visible or near to visible radiation that occurs due to transmission of electrons from excited state to ground state. The potential energy that is an indication of detection of an indicator[2].

Immunoassay is a diagnostic biochemical test used to quantify an analyte or a specific substance from blood or other body fluids. The molecule observed by the immunoassay is frequently referred[3] to as the analyte and in some instances proteins, although it may exist different sorts of atoms, of different types and sizes, as long as the specific antibody has sufficient attributes for the assay produced. Analyses in natural liquids e.g. serum or excrement are often assessed using immunoassays for medical and investigation purposes[4].

Corresponding Author:
M.P. Brundha,
Associate Professor, Department of General Pathology
Saveetha Dental College and Hospitals,
Saveetha Institute of Medical and Technical Sciences,
Saveetha University. 162, Poonamalle High Road,
Chennai - 600077, Tamil Nadu, India.
Mail ID: brundha.sdc@saveetha.com
Phone Number:+91 9884421482

Electro chemiluminescence immuno assay is a new developing method to determine antibodies in human being's blood sample.this has evolved from various other immunoassay methods that were previously used for detection of enzyme/antibody etc[5] Determination of antibody is done by chemical reactivity with materials like ruthenium, osmium etc., that are coated on monoclonal antibodies and on reaction produce luminescent molecules hence the name. This production of luminescent molecules makes determination uncomplicated[6].

The test takes place following three principles: Competitive principles for example small analytes, Sandwich principle for large analytes, Bridging principle for dilution of antibodies in the sample. All these three principles put together makes this test sensitive and less time consuming [7]. Enhances like phenolic and its derivatives are used to ameliorate the sensitivity of the test to detect antibodies quicker. Every test follows a sequential procedure to express the result/analysis. Like wise electro chemiluminescence immuno assay also follows a fully automated procedure that reduces time and any errors possible [8].

The various advantages of electrochemiluminescence immunoassay test is that it is an extremely sensitive and time thrifty method to determine the antibody in the blood, which makes it a very potential test for many disease diagnosis. It is also automated so there are minimal or no errors possible while doing this test[9]. The chances of false positive and false negative results are obvious. Just like all any expediency having a negative aspect, viral markers[10] and biological markers are still not usable in this method[11]. Enhancers that are used to improve the chemi-luminescent reaction are enzyme protectors which increase the time of exposure of light without much reduction in the intensity[12].

Electrochemiluminescence immunoassay testing has been used to detect many markers such as typhoid, cardiac, tumor markers and various infectious diseases in in-vitro analysis. It is also frequently used in the detection of recombinant specific treponemal and some bacterial[13,14] antigens. It is also helpful in detecting the prevalence of congenital hyperthyroidism.In addition the working dynamic range is wider compared to previous immuno assay methods. The relationship between the intensity of luminescence produced and the concentration of substance measured is one-dimensional[15].

The importance and availability of such accurate results producing tests have to be known world wide which is why research on this test providing in-depth knowledge and awareness of the same paves path for better diagnostic methods for future.Research articles were searched from search engines such as MeSH, pubMed, Core, Google Scholar, Cochrane etc. the time period considere was from 2000 - 2020 which is 20 years. Inclusion criteria for articles were electrochemiluminescence immunoassay test application, electrochemiluminescence immunoassay test for detection of various pathogenesis proteins and comparative studies between different immuno assay. Exclusion criterion was presence of other systemic diseases that causes complications in testing procedures. Articles were studied with standard quality assessment tool[16] and data were collected under different headings such as electrochemiluminescence immunoassay sampling and its collection, Processing of sample, Analytics from Saliva, Advantages and Disadvantages. All the data were analysed thoroughly to conclude with the processing and analysis of electrochemiluminescence immunoassay test to provide a proper article on its working principle and give more evidence.

ELECTROCHEMILUMINESCENCE IMMUNOASSAY - ECLIA

Electrochemiluminescence Immunoassay Sampling And Its Collection

Electrochemiluminescence detection is a combination of sensitivity, dynamic range, and convenience that is not matched by any other diagnostic method[17]. It is a biological assay involving multi array technology and electrochemiluminescence which increases the speed and density of information of biological assays. The procedure requires less time and effort[18]

Electrochemiluminescence immunoassay test is done on dried blood spots to detect the antibodies. Blood samples are collected in conventional methods and stored in plain vacutainers[19]. Serum and plasma are separated by centrifuging the blood for 20 minutes and then transferred to analyse Plasma collected with

anticoagulants and are centrifuged for 15 minutes and transferred to analyse. Anticoagulants used are sodium citrate, ethylenediaminetetraacetic acid (EDTA), heparin which are some commonly used blood thinning agents during sample collection. Sodium citrate chelates the free Calcium ions (Ca^{2+}), and inhibits the coagulation of blood. Though it has the ability to do so they have weak anticoagulative effects and poor sensitivity[20]. Ethylenediaminetetraacetic acid (EDTA) also chelates the free Calcium ions (Ca^{2+}), and inhibits coagulation. It doesn't affect red blood cells but affects platelet aggregation. Cell preservation is optimum in Electrochemiluminescence immunoassay. High concentrations of ethylenediaminetetraacetic acid (EDTA) are hypertonic in comparison to red blood cells therefore high caution is required during collection of small amounts of blood samples as the red blood cells would shrink Heparin so far has been the best anticoagulant used for blood sample collection and storage. Heparin inhibits antithrombin III. It is a strong anti coagulant[21]. It is stable in high temperatures also. Heparin is hard to hemolyse and causes leukocytes aggregation. After blood samples are collected they are analysed for antibodies/ antigens[22].

Processing of Samples

After collection of samples and separating them, the sample is firstly incubated with specific anti T3 antibody labelled with ruthenium complex which is also known as biotinylated T3, along with Streptavidin coated paramagnetic microparticle[23] Free binding sites of the labelled antibody are occupied by the formation of antigen - hapten complex and this whole complex is bound by micro-particles of biotin and streptavidin[24]. After the second incubation, the immune complexes are transferred to a measuring cell where the complexes are magnetically trapped on a working electrode[25]. The unbound reagent and sample are washed away with a buffer. In the reaction the chemiluminescent reaction is electrically estimated to produce light. The amount of light provided by the reaction is indirectly proportional to the concentration of antigen/ antibody present in the sample tested[26].

Electrochemiluminescence immunoassay is Roche's technology for detection by immunoassay[27]. Based on the combination of this technology and well designed specific and sensitive immunoassay electrochemiluminescence immunoassay test device provides reliable results. The use of ruthenium - complex and tripropylamine has led to the development of Electrochemiluminescence immunoassay. The reaction for detection of the reaction complex is stimulated by voltage applied to the sample solution which also facilitates for the precise control over the reaction. Electrochemiluminescence immunoassay technology follows many immunoassay principles while providing superior performance and better sensitive results[28].

Analytics from Saliva

Saliva test or Salivaomics is a diagnostic procedure that involves biochemical laboratory analysis of saliva which is an important biological fluid that can be used for steroid assay and identification of various hormones and markers of endocrine, immunologic, inflammatory, infectious and other types of conditions. It is very useful for assaying steroid hormones like cortisol, genetic material like ribonucleic acid (RNA), proteins such as enzymes and antibiotics and a variety of other substances including natural metabolitesTesting of saliva is used for the screening of various conditions and diseases such as Cushing's syndrome, anovulation, human immunodeficiency virus (HIV), cancer, etc[29] it is one of the least invasive tests. They give the most reliable results. It is the best method for immediate detection of any condition as the oral fluid is well connected to the bloodstream. The proponents of saliva testing are that there is an ease in collection of sample, safety, non-invasiveness, affordability, accuracy and capacity to circumvent venipuncture. Collection of multiple samples [30]can also be readily obtained. It is also useful for performing chronobiological assessment for long duration. Collection of passive drool facilitates large sample collection. It has the ability to freeze and the left over specimen can be used for future purposes.

Late night salivary cortisol concentration is detected using this test. Late night salivary cortisol is an indicator of cushing's syndrome. Therefore electrochemiluminescence immunoassay test is preferred for bio-screening. Late night salivary cortisol has an effect on endogenous hypercortisolism which in turn leads to adrenal incidentalomas[31] When the salivary cortisol during the screening is very low, synthetic

glucocorticoids are added which cross reacts to give results for immune assay.When cortisone - cortisol ratio remains constant it is indicative of contamination by topical hydrocortisone. Late night salivary cortisol measurements every 6 months are essential for absence or recurrence of symptoms. Electrochemiluminescence immunoassay test is a wide spread, cost effective screening test for Cushing's syndrome. It is also more advantageous being fully automated and less time consuming[32].

Advantages

Electrochemiluminescence immunoassay test is highly specific in recognition of antigens and antibodies and is used for detection of aflatoxin. Inexpensive practical screening is possible. It is a highly sensitive portable device. As there is absence of background optical signal there is an ease in control and the changing of electrode potential can be done manually to detect even ultra trace levels of antibodies. It has narrow emission bands which makes it highly sensitive. The band range of absorption is extremely wide. It has high resistance to photo bleaching. Nano crystals are added as semiconductors that increase sensitivity and rapid analysis of samples is possible.Semiconductors that are added are nanomaterials containing Cadmium sulphide (CdS), Cadmium selenide (CdSe), Cadmium telluride (CdTe) etc. addition of these semiconductors facilitate label free biosensing by low density lipoproteins. Magnetic nanoparticles[33] increase signal and sensitivity of immune devices. The simple precept for Electrochemiluminescence is that the luminescence substrate and the factor in the response lose electrons on the floor of an electrode, so as to be oxidized[34]. The electron donor loses a hydrogen ion (H^+) to turn out to be a sturdy lowering agent, which reduces the luminescence substrate to the excited state, and then the luminescence substrate releases photons to return to the floor state[34,35]. This system is carried out persistently on the floor of the electrode, and photons are continuously launched to hold the substrate awareness constant. The detection scope of electrochemiluminescence immunoassay of this method is wider than that of Enzyme linked immunosorbent assay (ELISA).

Electrochemiluminescence or electrogenerated chemiluminescence is a kind of luminescence produced during electrochemical reactions between analytes and antigen/ antibody complexes in the reacting solutions. Intermediates that are produced undergo exthothermic reaction to produce an excited state that luminates upon relaxation to a lower - level state. This luminance of the emitted photon correlates to the energy gap between the excited and relaxed state of the molecules[36]. The advantages of immunoassays are their ability to concurrently check for multiple antigen/antibodies, give speedy results and take proper usage of confirmatory testing. However, immunoassays may cross-react with different antigen/antibodies and change in ability and specificity. Therefore, unexpected immunoassay effects should be translated with caution and verified by confirmatory tests[37].

Disadvantages

Though this test is extremely sensitive and helpful there are few disadvantages as well. Peroxydisulfate solution with dissolved oxygen makes it difficult for antibodies to bind to ruthenium etc. and in relatively scarce quantities makes it difficult to separate[38]. Convenient method is by immobilizing antibody by single electrode to greatest extent by its difficulty to graft as it deactivates/degenerates the antibody. High concentration of substance reduces the signaling capability as less enzymes are labelled with antigen and remaining settle in the bottom of the microtiter plate. When there is no catalysis of substrate there is no signal produced. High levels of ruthenium and other substances in multiple sites tend to cause loss of biological activity of molecules[39].

In Spite of many advantages of immunoassays, they have some limitations. Immunoassays rely primarily on activity between analyte and the natural antibody, they may have more intrinsic imprecision than other methods used in medicine analysis[40]. The specificity of immunoassays is based on the protein directed to the analyte, yet some are not extremely specific/selective. Due to the ruthenium complex coating done for electrochemiluminescence immunoassay it is extremely selective and gives better and accurate results. This shows a good innovative [41]development in immunoassay generations.

Jie, Guifen, et al 2009 has stated that immuno sensor is better with biotinylated monoclonal

antibody labelled with ruthenium complex than plain antibodies[42]. Yao, Wu, et al 2008 has mentioned that electrochemiluminescence immunoassay tests are used for various diagnostic purposes such as monitoring the function of hormones, occurrence of any tumor markers, prevalence of infectious diseases by certain pathogens etc.[43] Gagnon, N, et al 2018 has tested various principles that are followed by the test.[44] Raff, H 2013 found that the particles like ruthenium, osmium etc are attached to agglutination, and the reporter molecules conjugate with target molecules. Non target molecules bind to antibodies.[45]

CONCLUSION

Electrochemiluminescence immunoassay is a widely used diagnostic method to find pathogens from body fluid with the help of antigen - antibody complex and production of luminescence based on the reaction. It is also cost effective and consumes very less time. Simple errors are avoided as it is an automated process. It has high specificity compared to other immunoassays. It is helpful in detection of various markers like tumor marker, infectious pathogens etc. Semiconductors increase the sensitivity of the device. It has a wider detection scope compared to Enzyme linked immunosorbent assay (ELISA). This study has reviewed various uses and applications and unique features of electrochemiluminescence immunoassay test and provides in-depth knowledge in understanding the method of electrochemiluminescence immunoassay test and given better knowledge and open up any advancements and new ideas for further improvement in the diagnostic method.

Acknowledgements : None.

Conflict of Interests : The authors declared that there is no conflict of interest.

Source of Funding : Self

Ethical Clearance: Not Required

References

1. Gan N, Zhou J, Xiong P, Hu F, Cao Y, Li T, et al. An ultrasensitive electrochemiluminescent immunoassay for aflatoxin M1 in milk, based on extraction by magnetic graphene and detection by antibody-labeled CdTe quantumn dots-carbon nanotubes nanocomposite. Toxins . 2013 Apr 29;5(5):865–83.
2. Zhou J, Liang Y, Zhang J, Cui L. The analyzation and clinical evaluation of ECLIA and CMIA in the detection of Treponema pallidum. Medicine . 2017 Jun;96(24):e7139.
3. Deutschbein T, Broecker-Preuss M, Flitsch J, Jaeger A, Althoff R, Walz MK, et al. Salivary cortisol as a diagnostic tool for Cushing's syndrome and adrenal insufficiency: improved screening by an automatic immunoassay. Eur J Endocrinol. 2012 Apr;166(4):613–8.
4. Hannah R, Ramani P, Brundha MP, Herald. J. Sherlin, Ranjith G, Ramasubramanian A, et al. Liquid Paraffin as a Rehydrant for Air Dried Buccal Smear [Internet]. Vol. 12, Research Journal of Pharmacy and Technology. 2019. p. 1197. Available from: http://dx.doi.org/10.5958/0974-360x.2019.00199.9
5. Shreya S, Brundha MP. Alteration of Haemoglobin Value in Relation to Age, Sex and Dental Diseases-A Retrospective Correlation Study [Internet]. Vol. 10, Research Journal of Pharmacy and Technology. 2017. p. 1363. Available from: http://dx.doi.org/10.5958/0974-360x.2017.00241.4
6. Prasad N, Jabbar PK, Jayakumari C, John M, Haridasan RK, Nair ATS, et al. Late Night Salivary Cortisol in Healthy Community Dwelling Asian Indians Assessed by Second Generation Eclia. J Clin Endocrinol Metab [Internet]. 2020 May 19; Available from: http://dx.doi.org/10.1210/clinem/dgaa269
7. Swetha S, Brundha MP. Analysis of knowledge about the hospital warning symbols among the postgraduate dental students-A comparative study [Internet]. Vol. 10, Research Journal of Pharmacy and Technology. 2017. p. 975. Available from: http://dx.doi.org/10.5958/0974-360x.2017.00177.9
8. Shah D, Chang C-D, Cheng K, Wachter A, Stewart J. COMBINED HIV ANTIGEN AND ANTIBODY ASSAY ON A FULLY AUTOMATED CHEMILUMINESCENCE BASED ANALYZER [Internet]. Bioluminescence and Chemiluminescence. 2001. Available from: http://dx.doi.org/10.1142/9789812811158_0089

9. Capozzi A, Lococo E, Grasso M, Longo A, Garofalo T, Misasi R, et al. Detection of antiphospholipid antibodies by automated chemiluminescence assay [Internet]. Vol. 379, Journal of Immunological Methods. 2012. p. 48–52. Available from: http://dx.doi.org/10.1016/j.jim.2012.02.020
10. Ferdioz J, Brundha MP. Awareness of stye. International Journal of Pharmaceutical Sciences Review and Research. 2016 Jan 1;40(1):30–2.
11. Timothy CN, Samyuktha PS, Brundha MP. Dental pulp Stem Cells in Regenerative Medicine – A Literature Review. Research Journal of Pharmacy and Technology. 2019;12(8):4052–6.
12. Chen R, Wang J, Liu Z. [Preliminary establishment of an electrochemiluminescence immunoassay method for detection of influenza virus B]. Sheng Wu Yi Xue Gong Cheng Xue Za Zhi. 2013 Oct;30(5):968–71.
13. Harsha L, Brundha MP. Prevalence of dental developmental anomalies among men and women and its psychological effect in a given population. Journal of Pharmaceutical Science and Research. 2017;9(6):869–73.
14. Brundha MP. A Comparative Study-The Role of Skin and Nerve Biopsy in Hansen's Disease. Journal of Pharmaceutical science and Research. 2015;7(10):837.
15. Kalaiselvi R, Brundha MP. Prevalence of hysterectomy in South Indian population [Internet]. Vol. 9, Research Journal of Pharmacy and Technology. 2016. p. 1941. Available from: http://dx.doi.org/10.5958/0974-360x.2016.00398.x
16. Health Evidence - Quality Assessment Tool [Internet]. 2016 [cited 2020 Jun 17]. Available from: https://www.healthevidence.org/documents/our-appraisal-tools/QA_Tool&Dictionary_10Nov16.pdf
17. Doi SAR, Clark J, Russell AW. Concordance of the late night salivary cortisol in patients with Cushing's syndrome and elevated urine-free cortisol. Endocrine. 2013 Apr 1;43(2):327–33.
18. Wellinghausen N, Dietenberger H. Evaluation of two automated chemiluminescence immunoassays, the LIAISON Treponema Screen and the ARCHITECT Syphilis TP, and the Treponema pallidum particle agglutination test for laboratory diagnosis of syphilis. Clin Chem Lab Med. 2011 Aug;49(8):1375–7.
19. Brundha MP, Pathmashri VP. Quantitative Changes of Red Blood cells in Cancer Patients under Palliative Radiotherapy-A Retrospective Study. Research Journal of Pharmacy and Technology. 2019;12(2):687–92.
20. Prashaanthi N, Brundha MP. A Comparative Study between Popplet Notes and Conventional Notes for Learning Pathology [Internet]. Vol. 11, Research Journal of Pharmacy and Technology. 2018. p. 175. Available from: http://dx.doi.org/10.5958/0974-360x.2018.00032.x
21. Preethikaa S, Brundha MP. Awareness of diabetes mellitus among general population. Research Journal of Pharmacy and Technology. 2018;11(5):1825–9.
22. Padmapriya B, Sangeetha MS, Nandhini G, Devi TT. Detection of Malarial Parasites using Image Processing Techniques from Blood Smear Slides. Research Journal of Pharmacy and Technology. 2018;11(10):4401–6.
23. Doddagowda SM, Shashidhar HA, Prasad CSBR. Leishman-Giemsa Cocktail - Is it an Effective Stain for Air Dried Cytology Smears. J Clin Diagn Res. 2017 Mar;11(3):EC16–8.
24. Kavitha V, Srinivas B. Platelet Count Estimation by Peripheral Smear Method and Automated Method in Pregnant Women. Asian Journal of Pharmaceutical Analysis. 2015;5(3):139–41.
25. Yin X-B, Qi B, Sun X, Yang X, Wang E. 4-(Dimethylamino)butyric acid labeling for electrochemiluminescence detection of biological substances by increasing sensitivity with gold nanoparticle amplification. Anal Chem. 2005 Jun 1;77(11):3525–30.
26. Darwish IA. Immunoassay Methods and their Applications in Pharmaceutical Analysis: Basic Methodology and Recent Advances. Int J Biomed Sci. 2006 Sep;2(3):217–35.
27. Ravichandran H, Brundha MP. Awareness about personal protective equipments in hospital workers (sweepers and cleaners). International Journal of Pharmaceutical Sciences Review and Research.

2016;40(1):28–9.

28. Mo X, Jin Y, Yang Y, Hu W, Gu W. Evaluation of a new chemiluminescence immunoassay for diagnosis of syphilis. Eur J Med Res. 2010 Feb 26;15(2):66–9.
29. Balaji S, Brundha MP, Path DNB. Awareness of About Breast Cancer among Dental Surgeons. Res J Pharm Biol Chem Sci. 2016;8(8):797.
30. Kumar MDA, Ashok Kumar MD, Brundha MP. Awareness about nocturia-A questionnaire survey [Internet]. Vol. 9, Research Journal of Pharmacy and Technology. 2016. p. 1707. Available from: http://dx.doi.org/10.5958/0974-360x.2016.00344.9
31. Saivignesh S, Brundha MP. Myeloid sarcoma. International Journal of Clinicopathological Correlation. 2019 Jul 1;3(2):41.
32. Green DA, Zucker J, Westblade LF, Whittier S, Rennert H, Velu P, et al. Clinical Performance of SARS-CoV-2 Molecular Testing. J Clin Microbiol [Internet]. 2020 Jun 8; Available from: http://dx.doi.org/10.1128/JCM.00995-20
33. Abhyankar V, Abidi AH. Multiplexing of Immune Markers via Electrochemiluminescence Immunoassays for Systems Biology. Methods Mol Biol. 2020;2131:349–64.
34. Zhang B, Ma W, Li F, Gao W, Zhao Q, Peng W, et al. Fluorescence quenching-based signal amplification on immunochromatography test strips for dual-mode sensing of two biomarkers of breast cancer. Nanoscale. 2017 Dec 7;9(47):18711–22.
35. Ulrich J, Päge I, Luley C, Gollnick H. Die Bestimmung von S100B mittels eines neuen Elektrochemilumineszenz-Immunoassays (ECLIA) [Internet]. Vol. 30, Aktuelle Dermatologie. 2004. Available from: http://dx.doi.org/10.1055/s-2004-832540
36. Shenoy PB, Brundha MP. Awareness of polycystic ovarian disease among females of age group 18-30 years. Journal of Pharmaceutical Science and Research. 2016; 8(8):813–6.
37. Sun X, Li B, Tian C, Yu F, Zhou N, Zhan Y, et al. Rotational paper-based electrochemiluminescence immunodevices for sensitive and multiplexed detection of cancer biomarkers. Anal Chim Acta. 2018 May 12;1007:33–9.
38. Paniel N, Radoi A, Marty J-L. Development of an electrochemical biosensor for the detection of aflatoxin M1 in milk. Sensors . 2010;10(10):9439–48.
39. Jiang W, Men S, Wen X, Yuan X, Pu D, Liu X, et al. A preliminary study for the establishment of a reference interval for vitamin B12 in China after performance verification of a second-generation ECLIA kit. J Clin Lab Anal. 2020 May;34(5):e23165.
40. Castro R, Prieto ES, Santo I, Azevedo J, Exposto F da L. Evaluation of an enzyme immunoassay technique for detection of antibodies against Treponema pallidum. J Clin Microbiol. 2003 Jan;41(1):250–3.
41. Mp B, Brundha MP, Nallaswamy D. Hide and seek in pathology- A research on game-based histopathology learning [Internet]. Vol. 10, International Journal of Research in Pharmaceutical Sciences. 2019. p. 1410–4. Available from: http://dx.doi.org/10.26452/ijrps.v10i2.606
42. Jie G, Li L, Chen C, Xuan J, Zhu J-J. Enhanced electrochemiluminescence of CdSe quantum dots composited with CNTs and PDDA for sensitive immunoassay. Biosens Bioelectron. 2009 Jul 15;24(11):3352–8.
43. Yao W, Wang L, Wang H, Zhang X. Cathodic electrochemiluminescence behavior of norfloxacin/peroxydisulfate system in purely aqueous solution. Electrochim Acta. 2008 Dec 30;54(2):733–7.
44. Gagnon N, Fréchette I, Mallet P-L, Dubé J, Houde G, Fink GD. Establishment of reference intervals for the salivary cortisol circadian cycle, by electrochemiluminescence (ECLIA), in healthy adults. Clin Biochem. 2018 Apr;54:56–60.
45. Raff H. Update on late-night salivary cortisol for the diagnosis of Cushing's syndrome: methodological considerations. Endocrine. 2013 Oct;44(2):346–9.