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Design, Synthesis and Evaluation of a Prostate-Specific Antigen Rapid Test Strip

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ABSTRACT

Background and Objective: Early detection of all cancers, including prostate cancer, is considerably helpful in their treatment. In addition to clinical examination of the prostate gland, prostate-specific antigen (PSA) is also used to diagnose prostate cancer. Methods such as ELISA, PCR, radioimmunoassay, etc. have been used to detect PSA, which, in addition to being expensive, require an experienced technician, complex equipment, and a lot of time. The aim of this study is to design and produce a rapid test for PSA measurement in Iran, contribute to its production, reduce imports and prevent capital outflow.

Methods: In this experimental study, colloidal gold nanoparticles were covalently conjugated with monoclonal antibodies, and different components of the diagnostic test strip were examined in terms of type and pore size, and different parts of the test strip were assembled and the antibodies were loaded onto the substrate. Serum samples from healthy subjects and patients with different serum dilutions ranging from 1 to 9 ng/mL for prostate-specific antigen were obtained from a diagnostic laboratory which had previously been measured with VIDAS. Then, the sera were measured with the designed test strip. Finally, the test strip was examined for accuracy and precision.

Findings: In measuring different serum dilutions of prostate-specific antigen from 1 to 9 ng/mL, the test strip designed in this study showed similar results to the commercial kit, demonstrating the accuracy and sensitivity of the test strip designed in this study.

Conclusion: A rapid prostate cancer detection test strip based on PSA measurement was designed and prototyped.

Keywords: *Prostate Cancer, Gold Nanoparticles, Prostate-Specific Antigen, Lateral Flow Immunoassay.*

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Introduction

Prostate-specific antigen (PSA) is a serine protease produced by epithelial cells of the prostate and its main function is liquefaction. PSA is currently a biomarker for the diagnosis and screening of prostate cancer and is the first cancer biomarker approved by the FDA. Serum PSA levels and isoforms in men allow the distinction between carcinoma and benign inflammatory disease of the prostate. Initially, it was thought that PSA was produced only by the prostate and was therefore a protein expressed exclusively in men. However, PSA has been reported to be a protein expressed by several non-prostatic tissues not only in men but also in women. Some investigators also report that in women, the expression of this protein is highly correlated with breast and colorectal cancer and therefore could serve as a potential biomarker for the early detection, diagnosis, and prognosis of these cancers in women (1).

The first report on the properties of antigens in the prostate was made by the American urologist, Flocks, in 1960 (2, 3). In 1966, Hara, a Japanese forensic scientist, reported a protein and named it "gamma-seminoprotein." He suggested its possible value as forensic evidence in rape cases (4). In 1970 and 1973, Li et al. (5, 6) reported antigens in human semen, one of which was later found to have the same amino acid sequence as PSA. In 1970, Ablin et al. reported finding two antigens specific to the human prostate, one of which was distinct from acid phosphatase (7, 8).

Prostate cancer is the second most common cancer in men worldwide (9). Prostate cancer is generally asymptomatic until it reaches an advanced stage. Therefore, screening for early detection is of great importance to reduce the risk of death from prostate cancer. Routine screening for prostate cancer relies on measuring the level of prostate-specific antigen in the blood and digital rectal examination (10). Men with a positive PSA test result (serum PSA above 4 ng/ml) usually undergo transrectal ultrasound-guided prostate biopsy, which is the gold standard for diagnosing PCa (11). Only with early and timely detection can we hope that the cancer is confined to the prostate gland, does not metastasize, and is completely curable (12, 13). Based on the research, the use of the prostate-specific antigen (PSA) tumor marker test in conjunction with clinical symptoms of the prostate is recommended for the diagnosis of prostate cancer. PSA has been shown to be the most reliable tumor marker for detecting prostate cancer in its early stages and monitoring disease recurrence after treatment (14).

Nowadays, various classical methods such as enzyme immunoassay, polymerase chain reaction, labeled immunoassay and electrochemical immunosensors are used for the detection of PSA biomarker. However, these methods are expensive and difficult, and the need for complex instruments and the long period can be mentioned as their disadvantages (15, 16). Measurement of PSA levels is widely used to identify men with a high risk of prostate cancer. PSA testing is currently performed in central laboratories, which is accompanied by a long period between blood collection and PSA results. Accordingly, the development of simple and accessible techniques for early detection and treatment of PSA in these patients is highly significant. In recent years, optical immunochromatographic or lateral flow assay techniques have been widely used for the rapid detection of biologically active compounds and diagnosis of diseases. One of the advantages of these methods is very simple detection, low cost, and short laboratory turnaround time (17-19).

Another advantage of this method is that it does not require expensive equipment or a trained technician, and anyone can easily perform it at home (20). The advantages and disadvantages of the lateral flow immunoassay method are reviewed in Table 1 (21). The currently available serum or plasma-based immunoassays are time-consuming and require complex technical equipment. Therefore, various strip tests have been developed for the qualitative and semi-quantitative determination of PSA based on serum or whole-blood immunochromatographic measurements (21, 22).

The rapid tests available in Iran for measuring this antigen are of foreign brands and are imported; therefore, the aim of this study is to design and produce a rapid test for measuring prostate-specific antigen domestically and to contribute to its production, reduce imports and prevent capital outflow.

Table 1. Advantages and disadvantages of the lateral flow immunoassay method

Advantages	Disadvantages
Single-step process with no washing step.	Single-step
Fast, low cost and small sample size	Inappropriate sample volume affects measurement accuracy.
Qualitative or semi-quantitative result	Limitations in sample size limit the sensitivity of the test.
It is not possible to enhance the response with an enzymatic reaction.	Good antibody preparation is essential.
Simple test method	The time to analyze the sample depends on its viscosity.
Proteins, haptens, nucleic acids, and amplicons can be identified.	Possibility of clogged pore due to sample components
Pretreatment is not required for liquid samples.	Pretreatment is required for non-liquid samples.

Methods

This applied experimental research was approved by the Ethics Committee of Babol University of Medical Sciences with the code IR.MUBABOL.REC.1400.135.

Preparation of chemicals: Gold nanoparticles (AuNP) (Iranian Nanomaterials Company, Iran), human anti-PSA antibody (Zist Fanavaran Company, Iran), polyclonal antibody (Zist Fanavaran Company, Iran), nitrocellulose membrane and cellulose membrane (ATR-MED Company, Iran) were prepared.

Preparation of 1 mM Phosphate Buffer Saline (PBS) solution: 3.2gr Nacl+0.08gr KCL+0.868gr Na2HPO4.7H2O+0.103gr KH2PO4 reached 40 cc with distilled water and then its pH was adjusted to 7.4.

Preparation of sample pad buffer: A 10cc solution (Shafamol Company, Iran) was prepared and used to treat the sample pad.

Preparation of nitrocellulose bed treatment buffer: A 25cc solution (Shafamol Company, Iran) was prepared and used to treat the bed.

Sample collection: Serum samples from healthy individuals and patients with prostate malignancy, as well as positive ELISA control and PSA autoanalyzer calibration solution, were obtained from Ibn Sina Medical Diagnostic Laboratory and Razi Pathobiology and Genetic Lab in Babol.

Preparation of AuNp-Anti PSA Antibody Conjugates: For this purpose, 20-25 nm nanoparticles were mixed with ethylene glycol (Sigma, Germany) and incubated for 2 hours at room temperature in the dark. At this stage, EDC solution (Shafamol Company, Iran) was added to the nanoparticles and ethylene glycol solution and then NHS solution (Shafamol Company, Iran) was added to the mixture. Then, it was incubated for 30 minutes at room temperature with gentle shaking and after incubation, centrifuged at 3600 rpm and the supernatant was discarded. Anti-PSA antibody was added to the final precipitate after dissolving in PBS and incubated for 3 hours at room temperature (21, 23).

Covalent bonding is typically achieved through carbodiimide cross-linking chemistry using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride/N-hydroxysuccinimide (EDC/NHS). This method has been previously used to efficiently attach primary amines on antibodies to carboxyl-terminated AuNPs. Primary amines on an antibody molecule act as identification sites that can detect and bind antigens (24).

Sample pad preparation: The sample pad was cut to 0.4×1 cm, treated with a sample pad buffer, and then dried at room temperature (14).

Conjugate pad preparation: The conjugate pad was cut to 0.4 cm wide and 1 cm long. The conjugate solution was poured onto the substrate and allowed to dry at room temperature (25).

Preparation of nitrocellulose membrane for lateral flow: The nitrocellulose substrate was cut to dimensions of 0.4×2.5 cm and two areas for test and control lines were determined on it.

Preparation of test and control areas: After preparing the nitrocellulose substrate, monoclonal and polyclonal antibodies were loaded onto the test and control lines (25).

Assembly (assembly of substrates): The sample pad, conjugate pad, and finally, the nitrocellulose substrate were assembled (25).

Lateral flow immunoassay process: 100 µL of serum sample was slowly poured onto the sample pad to move along the test strip. The solution was transferred from the sample pad to the conjugate pad and the conjugate solution was washed from the conjugate pad and carried with it to the nitrocellulose substrate. In that area, the reaction was performed in the test and control line.

Lateral flow immunoassay strip test with clinical samples: Serum samples from patients with prostate cancer and healthy subjects for this cancer, previously measured by VIDAS immunoanalyzer, were evaluated by the test strip designed and synthesized in this study.

Test validation: For this purpose, the test strip designed in this study was compared with the ABON Rapid Test Strip, and several serum samples with different concentrations were measured by both tests.

Results

Preparation of AuNp-Anti PSA conjugates: As a result of covalent conjugation, a stable solution without precipitation and with the same ruby red color was formed, and the antibodies were bound to the nanoparticles with the correct orientation (26, 27) (Figure 1).

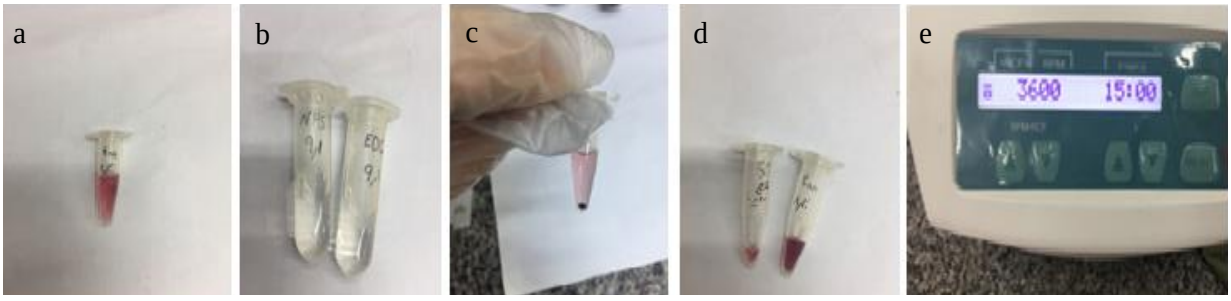

Figure 1. A view of the implementation of the covalent conjugation method. a: A view of the color of the colloidal solution of gold nanoparticles, b: Freshly prepared solutions of EDC and NHS powders, c: A view of the precipitate formed from nanoparticles after centrifugation, d: Precipitation of nanoparticles dissolved in PBS, e: A view of the appropriate time and distance for the formation of precipitate in the colloidal solution of gold nanoparticles

Assembly: The best mode of sample movement along the test strip was when different parts of the test strip were selected with the same length and width ratio as mentioned in the working method and were assembled in the same ratio and order (Figure 2).

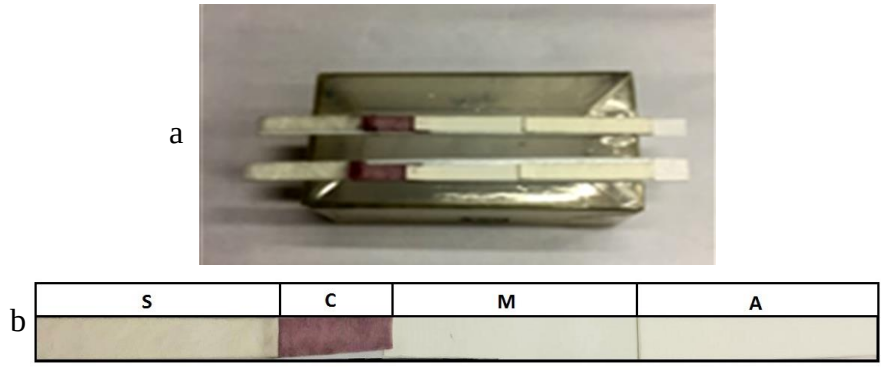

Figure 2. a: General view of the assembled test strip, b. Detail view of the assembled test strip: S: sample pad, C: conjugate pad, M: nitrocellulose substrate, A: absorbent pad

LFIA strip assay with clinical samples: The lateral flow assay produced a darker and clearer band in the test line as PSA level increased in serum samples, and the control line was formed in all assays (Figure 3).

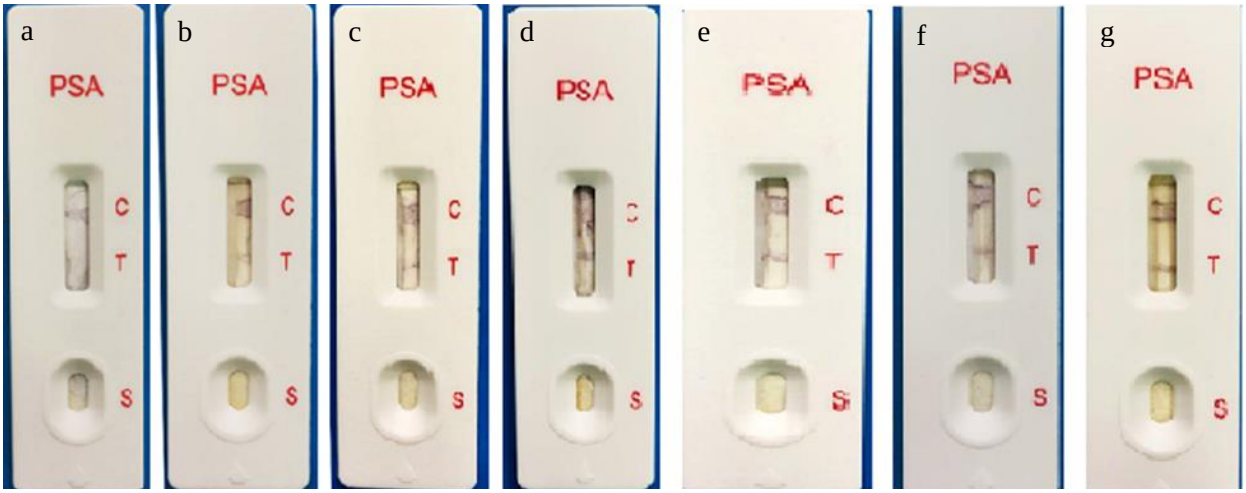

Figure 3. Measurement of serum samples with the test strip designed in this study. a: Measurement of serum sample with a concentration of 1 ng/ml, b: Measurement of serum sample with a concentration of 2 ng/ml, c: Measurement of serum sample with a concentration of 3 ng/ml, d: Measurement of serum sample with a concentration of 5 ng/ml, e: Measurement of serum sample with a concentration of 7 ng/ml, f: Measurement of serum sample with a concentration of 8 ng/ml, g: Measurement of serum sample with a concentration of 9 ng/ml

When measuring different serum dilutions of prostate-specific antigen from 1 to 9 ng/mL, the test strip designed in this study showed similar results to the commercial kit, demonstrating the accuracy and sensitivity of the test strip designed in this study (Figure 4).

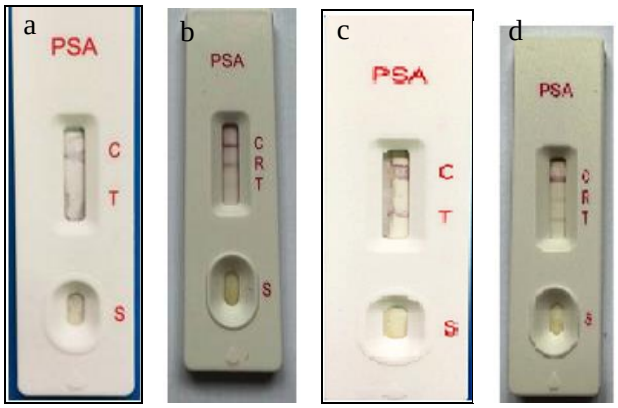

Figure 4. Comparison of measurements by the test strip designed in this study and the ABON kit. a: Serum measurement with a PSA level of 1ng/ml in test strip designed in this study, b: Serum measurement with a PSA level of 1ng/ml in ABON kit, c: Serum measurement with a PSA level of 28ng/ml in test strip designed in this study, d: Serum measurement with a PSA level of 28ng/ml in ABON kit.

Note: The ABON kit is a semi-quantitative kit and the kit designed in this design is a qualitative kit. Serum with a PSA level of 1ng/ml is considered a negative sample. Serum with a PSA level of 28ng/ml is considered a positive sample.

Discussion

In this study, we achieved an optimal method for conjugating gold nanoparticles with anti-PSA antibody and designed a test strip based on the lateral flow immunoassay technique that has the ability to qualitatively measure prostate-specific antigen in serum.

Prostate cancer is one of the most common cancers and the third leading cause of cancer death in men. Therefore, early, definitive, and sensitive diagnosis of this disease is essential for timely initiation of treatment. Previous studies have shown that PSA is the most reliable tumor marker for early detection of prostate cancer and monitoring disease recurrence after treatment (28). A simple, qualitative, one-step test (PSA Rapid Screen) has been evaluated to increase uptake and reduce the cost of prostate cancer screening programs. The PSA Rapid Screen test is a lateral flow chromatographic immunoassay that provides positive or negative results for PSA levels >4 ng/ml (29, 30).

Point-of-care (POC) testing is a diagnostic method in clinical and environmental analysis and food safety that provides rapid results in shorter times compared to centralized laboratories. Lateral flow assay (LFA)-based (POC) devices are one of the fastest growing strategies for qualitative and quantitative analysis. Favorable substrate interactions must be developed for the use of antibodies in biosensors (18).

Antibody molecules can be attached to surface-functionalized AuNPs through chemisorption and various functionalities such as cysteine or amine groups or electrostatic interactions using ionic interactions or hydrophobic targeting. Antibody-bound AuNPs must be stable under a wide range of wet chemistry conditions (such as pH, salt concentration, and type or temperature). Therefore, covalent attachment is preferred. In direct competition, strong amide bonds through pegylated carboxyl groups around AuNPs have enhanced directional functionalization with increased accessibility to the functional site (31, 32).

The range of orientations and packing density resulting from crosslinking nanoparticles to antibodies at the primary amine can potentially lead to antibodies with poor binding to their target antigens. Despite significant drawbacks, such as unfavorable antibody orientation, EDC/NHS conjugation has the distinct advantage of providing a stable and consistent covalent bond (33). The EDC/NHS covalent conjugation method was also used in this study.

The PSA test has been widely used in recent years. Although mass screening for prostate cancer is one of the most controversial topics in oncology, two large, high-quality clinical trials have been conducted to evaluate PSA screening. A study by Grönberg et al. showed that prostate cancer mortality rates decreased by 44% among men aged 55 to 69 years based on 14-year screening (11). Ashida et al. reported a 21% reduction in prostate cancer mortality rate based on screening over 13 years of follow-up (34).

An important issue in PSA screening is the possibility of detecting asymptomatic prostate cancer that never leads to death (overdiagnosis) and then treating this prostate cancer (overtreatment). Other issues include cost and convenience, which are possible reasons for the low PSA screening rate in Japan. The one-step PSA test is provided free of charge (35).

Based on the results of the evaluations in this study, we were able to design and produce a test strip for measuring PSA in the serum of individuals based on the lateral flow assay with markers made of gold nanoparticles and their reaction visible to the naked eye.

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References

1.Pérez-Ibave DC, Burciaga-Flores CH, Elizondo-Riojas MÁ. Prostate-specific antigen (PSA) as a possible biomarker in non-prostatic cancer: A review. Cancer Epidemiol. 2018;54:48-55.
2.Flocks RH, Bandhaur K, Patel C, Begley BJ. Studies on spermagglutinating antibodies in antihuman prostate sera. J Urol. 1962;87:475-8.
3.Flocks RH, Urich VC, Patel CA, Opitz JM. Studies on the antigenic properties of prostatic tissue. I. J Urol. 1960;84:134-43.
4.Hara M, Inoue T, Koyanagi Y, Goto J, Yamazaki H, Fukuyama T. Preparation and immunoelectrophoretic assessment of antisera to human seminal plasma. Nippon Hoigaku Zasshi. 1966;20:356.
5.Li TS, Behrman SJ. The sperm- and seminal plasma-specific antigens of human semen. Fertil Steril. 1970;21(7):565-73.
6.Li TS, Beling CG. Isolation and characterization of two specific antigens of human seminal plasma. Fertil Steril. 1973;24(2):134-44.
7.Ablin RJ, Soanes WA, Bronson P, Witebsky E. Precipitating antigens of the normal human prostate. J Reprod Fertil. 1970;22(3):573-4.
8.Ablin RJ, Bronson P, Soanes WA, Witebsky E. Tissue- and species-specific antigens of normal human prostatic tissue. J Immunol. 1970;104(6):1329-39.
9.Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JW, Comber H, et al. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. Eur J Cancer. 2013;49(6):1374-403.
10.Ahmed HU, El-Shater Bosaily A, Brown LC, Gabe R, Kaplan R, Parmar MK, et al. Diagnostic accuracy of multi-parametric MRI and TRUS biopsy in prostate cancer (PROMIS): a paired validating confirmatory study. Lancet. 2017;389(10071):815-22.
11.Grönberg H, Adolfsson J, Aly M, Nordström T, Wiklund P, Brandberg Y, et al. Prostate cancer screening in men aged 50-69 years (STHLM3): a prospective population-based diagnostic study. Lancet Oncol. 2015;16(16):1667-76.
12.Litwin MS, Tan HJ. The Diagnosis and Treatment of Prostate Cancer: A Review. JAMA. 2017;317(24):2532-42.
13.Hayes JH, Barry MJ. Screening for prostate cancer with the prostate-specific antigen test: a review of current evidence. JAMA. 2014;311(11):1143-9.
14.Barnett JM, Wraith P, Kiely J, Persad R, Hurley K, Hawkins P, et al. An inexpensive, fast and sensitive quantitative lateral flow magneto-immunoassay for total prostate specific antigen. Biosensors (Basel). 2014;4(3):204-20.
15.Garg S, Sachdeva A, Peeters M, McClements J. Point-of-Care Prostate Specific Antigen Testing: Examining Translational Progress toward Clinical Implementation. ACS Sens. 2023;8(10):3643-58.
16.Van Poppel H, Albreht T, Basu P, Hogenhout R, Collen S, Roobol M. Serum PSA-based early detection of prostate cancer in Europe and globally: past, present and future. Nat Rev Urol. 2022;19(9):562-72.
17.Farshchi F, Hasanzadeh M. Nanomaterial based aptasensing of prostate specific antigen (PSA): Recent progress and challenges in efficient diagnosis of prostate cancer using biomedicine. Biomed Pharmacother. 2020;132:110878.
18.Maj-Hes A, Sevcenco S, Szarvas T, Kramer G. Claros System: A Rapid Microfluidics-Based Point-of-Care System for Quantitative Prostate Specific Antigen Analysis from Finger-Stick Blood. Adv Ther. 2019;36(4):916-22.
19.Wu CC, Lin HY, Wang CP, Lu LF, Yu TH, Hung WC, et al. Evaluation of a rapid quantitative determination method of PSA concentration with gold immunochromatographic strips. BMC Urol. 2015;15:109.
20.Fernández-Sánchez C, McNeil CJ, Rawson K, Nilsson O, Leung HY, Gnanapragasam V. One-step immunostrip test for the simultaneous detection of free and total prostate specific antigen in serum. J Immunol Methods. 2005;307(1-2):1-12.

21.Koczula KM, Gallotta A. Lateral flow assays. Essays Biochem. 2016;60(1):111-20.
22.Wang W, Fan B, Zhang X, Guo R, Zhao Y, Zhou J, et al. Development of a colloidal gold immunochromatographic assay strip using monoclonal antibody for rapid detection of porcine deltacoronavirus. Front Microbiol. 2023;13:1074513.
23.Rodríguez MO, Covián LB, García AC, Blanco-López MC. Silver and gold enhancement methods for lateral flow immunoassays. Talanta. 2016;148:272-8.
24.Mustafaoglu N, Kiziltepe T, Bilgicer B. Site-specific conjugation of an antibody on a gold nanoparticle surface for one-step diagnosis of prostate specific antigen with dynamic light scattering. Nanoscale. 2017;9(25):8684-94.
25.Sajid M, Kawde AN, Daud M. Designs, formats and applications of lateral flow assay: A literature review. J Saudi Chem Soc. 2015;19(6):689-705.
26.Dykman LA, Khlebtsov NG. Gold nanoparticles in biology and medicine: recent advances and prospects. Acta Naturae. 2011;3(2):34-55.
27.Makhsin SR, Teoh PL, Razak KA. Synthesis of Gold Nanoparticles and Its Conjugation Strategies to Biomolecules for Biomedical Applications. Rev Adv Sci Eng. 2015;4(1):3-21.
28.Wang L, Lu B, He M, Wang Y, Wang Z, Du L. Prostate Cancer Incidence and Mortality: Global Status and Temporal Trends in 89 Countries From 2000 to 2019. Front Public Health. 2022;10:811044.
29.Miano R, Mele GO, Germani S, Bove P, Sansalone S, Pugliese PF, et al. Evaluation of a new, rapid, qualitative, one-step PSA Test for prostate cancer screening: the PSA RapidScreen test. Prostate Cancer Prostatic Dis. 2005;8(3):219-23.
30.Dok An C, Yoshiki T, Lee G, Okada Y. Evaluation of a rapid qualitative prostate specific antigen assay, the One Step PSA(TM) test. Cancer Lett. 2001;162(2):135-9.
31.Berg W, Linder C, Eschholz G, Link S, Schubert J. Possibility of improving the acceptance rateof early detection testing for prostate cancerwith a one-step test for prostate-specific antigen in whole blood. Urol Int. 1999;63(2):102-6.
32.Jazayeri MH, Amani H, Pourfatollah AA, Avan A, Ferns GA, Pazoki-Toroudi H. Enhanced detection sensitivity of prostate-specific antigen via PSA-conjugated gold nanoparticles based on localized surface plasmon resonance: GNP-coated anti-PSA/LSPR as a novel approach for the identification of prostate anomalies. Cancer Gene Ther. 2016;23(10):365-9.
33.Hori F, Harada Y, Kuretake T, Uno S. Impedance Analysis of Colloidal Gold Nanoparticles in Chromatography Paper for Quantitation of an Immunochromatographic Assay. Anal Sci. 2016;32(3):355-9.
34.Ashida S, Yamasaki I, Kawada C, Fukuhara H, Fukata S, Tamura K, et al. Evaluation of a rapid one-step PSA test for primary prostate cancer screening. BMC Urol. 2021;21(1):135.
35.Berg W, Linder C, Eschholz G, Schubert J. Pilot study of the practical relevance of a one-step test for prostate-specific antigen in capillary blood to improve the acceptance rate in the early detection program of prostate carcinoma. Int Urol Nephrol. 2001;32(3):381-8.