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Bio-Barcode Technology for Detection of *Staphylococcus aureus* Protein A Based on Gold and Iron Nanoparticles

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Abstract

S. aureus is one of important causes of disease, food poisoning in humans and animals. The generally methods for detection of *S. aureus* is time consuming. Therefore, a new method is necessary for rapid, sensitive and specific diagnosis of *S. aureus*. In the present study, two probes and a Bio-barcode DNA were designed for detection of *S. aureus* (Protein A). Firstly, magnetic nanoparticle (MNPs) and gold nanoparticle (AuNPs) were synthesized at 80 ˚C and 100 ˚C, respectively. The AuNPs and the MNPs were functionalized with probe1, Bio-barcode DNA and probe2, respectively. Target DNA was added into the nanomaterial's system containing bio-barcode DNA-AuNPs-probe1 and probe2-MNPs to formed bio-barcode DNA-AuNPs-probe1-target DNA-probe2-MNPs complex. The bio-barcode DNA-AuNPs-probe1-target DNA-probe2-MNPs complex was separated with magnetic field. Finally, the bio-barcode DNA was released from surface of complex using DTT (0.8 M) and there was isolated of nanoparticles by magnetic field and centrifuge. The fluorescence intensity of bio-barcode DNA was measured in different concentrations of *S. aureus* (10^1 to 10^8 CFU mL^{-1}) by fluorescence spectrophotometry. The results showed that standard curve was linearly from 10^2 to 10^7 CFU mL^{-1}. Limit of detection of bio-barcode assay for both PBS and real samples was 86 CFU mL^{-1}.

Keywords: *S. aureus*, bio-barcode DNA, MNPs, AuNPs.

Abbreviations

Dithiothreitol: DTT, Tetraethoxysilane: TEOS, Scanning Electron Microscopy: SEM, Gold Nanoparticles: AuNPs, Dynamic Light Scattering: DLS, 3-Aminopropyltriethoxysilane: APTES, Sulfo-succinimidyl-4-*N*-

maleimidomethyl cyclohexane-1-carboxylate: SMCC, Luria-Bertani Broth: LB-broth, Magnetic Nanoparticles: MNPs, Texas red: Tex.

1. Introduction

S. aureus is causes variety of more serious diseases in humans including: pneumonia, endocarditis and septicemia [1]. In many cases, *S. aureus* diseases are hospital-acquired. Actually there are secondary infections that the patients get from hospital [2]. Moreover, *S. aureus* is one of the most important causes of food poisoning in humans and animals. According to the USDAs economic research service, infectious food borne illness caused by *S. aureus* food poisoning is more than 180 thousand cases annually in the US [2]. Therefore, the sensitive, specific and rapid diagnosis of *S. aureus* is very important in food industry, medical detection and the best therapy for patients. [3].

There are numerous techniques for diagnosis of *S. aureus*. One of the common diagnosis methods is use of specific culture media that requires at least several days for detection [4-7]. There are inexpensive and sensitive, but this method is time consuming [1]. Additionally, the other diagnosis method is enzyme-linked immunosorbent assay (ELISA) with high sensitivity. One of the limitations of this technology is typically low limit of detection (10^4 CFU mL^{-1}) [8]. Techniques based on nucleic acid methods for example, polymerase chain reaction (PCR) have high sensitivity and high specificity for diagnosis of *S. aureus*, but these methods need skillful technicians and require complicated procedures [2]. Currently, the fluorescence probe DNA is frequently used for diagnosis of pathogenic bacteria but the fluorescence probe DNA assay can diagnose 10^2–10^7 CFU mL^{-1} of bacteria for example *E. coli* [10]. To improve, solve this problem and the limit of analysis, Fu et al were used the quantum dots (QDs) as a signal probe reporter for bacteria detection, but it had low sensitivity, specificity and could not detect lower than 3×10^2 CFU mL^{-1} of bacteria [11]. Today, the fluorescence barcode DNA based on magnetic nanoparticles (MNPs) was used for the detection of microorganism [4]. Also, this method is very sensitive and specific like PCR and real time PCR for detection of protein and nucleic acid [12]. One of problem other methods are isolated sample by centrifuge but, in this study the fluorescence bio-barcode DNA is very simple and rapidly separated from unreacted elements in reaction mixture by a magnet. Furthermore, the bio-barcode DNA strand released from the nanoparticles surfaces can be used as signal reporter to quantitatively detect the target DNA [13-14].

In the present study, the fluorescence bio-barcode technology for first time was used to diagnose *S. aureus* (protein A). Firstly; both MNPs and gold nanoparticles (AuNPs) were prepared. Then, the nanoparticles were conjugated with probes and bio-barcode DNA. The MNP-probe2 was used for separating of *S. aureus* target DNA. Bio-barcode DNA-AuNPs-probe1 was added to the reaction mixture. Then, MNP-probe2-target

DNA was hybridized with probe1-AuNP-bio-barcode DNA to formed MNP-probe2-target DNA-DNA probe1-AuNPs-bio-barcode DNA complex. The MNP-probe2-target DNA-DNA probe1-AuNPs-bio-barcode DNA complex was isolated from the reaction mixture by using magnetic field. Finally, the MNPs-probe2-target DNA-probe1-AuNPs-bio-barcode DNA complex was resolved in DTT solution (0.8 M) to release fluorescence barcode DNA from the surface of AuNPs. Fluorescence intensity of bio-barcode DNA was measured by fluorescence spectrophotometry. The reaction conditions including: pH, probes concentration, bio-barcode DNA concentration, target DNA concentration, MNPs and AuNPs concentrations were optimized. The nanomaterial's method can be detecting target DNA sequences (Protein A) in the stool, urine and blood culture samples. The use of nanomaterial's system was easily with high stability and reproducibility.

2. Materials and methods

2.1. Materials

Sodium citrate and $HAuCl_4 \cdot 3H_2O$ were purchased from Sigma Aldrich (St. Louis, MO, US). Probes and bio-barcode DNA were prepared and synthesized by Bioneer Company Korea. Luria Bertani Broth (LB broth), Ethyl acetate, Tetraethoxysilane (TEOS), Dithiothreitol (DTT), 3-aminopropyltriethoxysilane (APTES) and NaCl were prepared from Merck, Germany. Sulfo-NHS acetate (NHSS) and Sulfo-succinimidyl 4-Nmaleimidomethyl cyclohexane-1-carboxylate (SMCC) were prepared from Thermo, USA. The target DNA was extracted from *S. aureus* (ATCC29213) using a DNA purification kit, Roche, Germany.

2.2. Instruments

The concentration of target DNA was measured by UV-VIS Array Spectrophotometer Teif Sanj Pishro Pajohesh (Laboratory Industrial & Research System, Iran). The fluorescence intensity of barcode DNA was recorded by fluorescence spectrophotometry F7000, USA. The size, shape and the potential of nanoparticles were measured by DLS analyzer (Malvern, USA) and SEM (Evo18 ZIESS, Germany). The concentration of DNA extract was analyzed by UV-VIS spectrophotometry Nano drop 2000 C and gel agarose electrophoresis. For the homogenization of synthesized nanoparticles, Ultrasonic Homogenizer (Development of Ultrasonic Technology, Iran) was used.

2.3. Oligonucleotide design

Two probes and a fluorescence bio-barcode DNA were designed based on data DNA sequences of proteins A that the percent in the NCBI. The sequences of target DNA, fluorescence bio-barcode DNA and probes are showed below:

Probe1: 5´thiol-GCCTAACTTGAACGAAGAAC3´, Probe2: 5´CAGGCTTGTTGTTGTCTTCC-thiol3´, Bio-barcode DNA: 5́Tex-CATCCAAAGCCTTAAAGACG-thiol3´ and target DNA: GCCTAACTTG AACGAAGAAC AACGCAATGG TTTCATCCAA AGCTTAAAAG ATGACCCAAG TCAAAGTGCT AACCTTTTAG CAGAAGCTAA AAAGCTAAAT GATGCACAAG CACCAAAAGC TGACAACAAA TTCAACAAAG AACAACAAAA TGCTTTCTAT GAAATTTTAC ATTTACCTAA CTTAACTGAA GAACAACGTA ACGGCTTCAT CCAAAGCCTT AAAGACG. The sequence of fluorescence bio-barcode DNA was used as a fluorescent reporter in the detection of target DNA. Probes and barcode DNA were prepared by Bioneer Company, Korea and they were dispersed in D.D.W.

2.4. Bacterial strains

All of strains including: *P. aeruginosa* ATCC27853, *S. dysentery* PTCC 1188, *S. aureus* ATCC 29213, *K. pneumoniae* ATCC7881, *E. coli* ATCC 25922 and *S. epidermidis* ATCC12228 were prepared from Mousavi hospital, Zanjan, Iran (as standard controls). All of the bacteria were cultured in LB broth at 37 $^{\circ}$C. *S. aureus* cells were grown overnight until an OD_{600} of 0.5 was obtained. The desired concentrations of cultures were prepared by serially diluting with PBS buffer (100 mM). *S. aureus* was centrifuged at 3000×g, 4 $^{\circ}$C. Then, it was rinsed three times in a PBS at room temperature. For colony counting experiments, the samples were surface plated on LB agar plates and were incubated for 24-48 h at 37 $^{\circ}$C. The colonies growths on plates were counted in order to determine the number of Colony-Forming Units per Milliliter (CFU mL^{-1}). Finally, different concentrations of bacterial were prepared in PBS buffer from 10^1 to 10^9 CFU mL^{-1}. The bacterial DNA samples were extracted by a DNA purification kit (Roche, Germany) [9, 12].

2.5. Preparation of AuNPs

AuNPs were prepared using the chemical reduction method [12, 15, 21 and 28]. Briefly, the $HAuCl_4$ (10 mM) was solved in 100 mL distilled water. Subsequently, the reaction mixture was allowed to boil. Tri-sodium citrate solution (10 mL, 38.8 mM) was added into the reaction mixture and the reaction mixture was heated for 20 min with vigorous stirring. When the mixed solution color changed to deep red, the heater was turned off. The reaction mixture was allowed to cool under vigorous stirring at 25 $^{\circ}$C for about 3 h. UV–VIS spectrophotometry, a Scanning Electronic Microscope (SEM) and Dynamic Light Scattering (DLS) were used to determine the size, shape and the potential of synthesized AuNPs.

2.6. Preparation of MNPs

Fe_3O_4 nanoparticles (MNPs) were synthesized by the co-precipitation method with some modifications [12, 16, 17 and 28]. Briefly, 0.8 g $FeCl_2 \cdot 4H_2O$ and 1.6 g $FeCl_3 \cdot 6H_2O$ were dispersed in 10-15 mL of deionized water with vigorous mechanical stirring (700 rpm), at 80 °C. Then, 4 mL ethanol and 4 mL of propanol were added to the reaction mixture for 30-45 min to color of the reaction mixture changed to brown. Then, ammonium hydroxide (25%, 20-25 mL) was slowly added into the reaction mixture under stirring (700 rpm), at 80 °C for 2 h. Subsequently, 4 mL tetraethoxysilane (TEOS) was added to reaction mixture for 1h, 700 rpm at 80 °C. 3-aminopropyltriethoxysilane (APTES, 1 mL) was added to the reaction solution under stirring (700 rpm) at 80 °C for 3 h. The reaction between TEOS and APTES was done in the presence of ammonium hydroxide (25%). Finally, the MNPs were collected by magnetic field, and then washed three times with D.D.W. The MNPs were dispersed in 30 mL of sodium citrate solution (0.5 M) to prevent aggregation. Also, the MNPs were sonicated with ultrasonic homogenizer for 10 min. After MNPs separation, they were dispersed into 30 mL D.D.W.

2.7. Conjugation of AuNPs

The conjugation of AuNPs with probe was performed according procedures described by Liu et al [18]. Twenty microliters of each (100 nM) thiol-modified probe and bio-barcode DNA reduced of previous were added to 960 µL of AuNPs for 4-5 h. The bio-barcode DNA-AuNPs-probe1 complex was washed three times in phosphate buffer (PBS, 100 mM, pH 7.4) using centrifuged at 12000 g, for 15 min at 4 °C. The AuNPs conjugated were blocked in Sulfo-NHS acetate solution (10 mM) at 25 °C for 30 min. Finally, the bio-barcode DNA-AuNPs-probe1 complex was dispersed in NaCl (0.3 M, pH 7), and PBS solution (100 mM, pH 7.4) to increased stability of complex.

2.8. Conjugation of MNPs

For conjugation of MNPs with probe2, three hundred microgram of sulfo-SMCC was added to MNPs (5 mg, 1 mL) solve in connecting buffer containing 0.2 M NaCl, 0.1 M PBS buffer, pH 7.4 for 2-3h. The MNP-SMCC was separated by magnetic field. The MNPs-SMCC was washed by the connecting buffer for three times. The probe2 (100 nM) was added into connecting buffer containing MNP-SMCC (1 mL, 5 mg) to reaction performed for 5 h. The mechanism of reaction is schematically showed in Fig. 1B. The MNP-SMCC-probe2 complex was washed three times with connecting buffer. In order to block the unreacted Sulfo-SMCC with probe2 on the surface of MNP-SMCC, the MNP-SMCC-probe2 was dispersed in Sulfo-NHS Acetate (10 mM, 30 mL) for 6 h, at room temperature. The functionalized nanoparticles were separated with magnetic field and rinsed with storage buffer containing PBS buffer (100 mM), NaCl (0.2 M) and pH 7.4. The size, structure and potential of the MNPs synthesized were researched by SEM and DLS.

2.9. Diagnosis of Genomic DNA

Detection of genomic DNA was performed by mixing the MNP-probe2 complex; bio-barcode DNA-AuNP-probe1 complex and the target DNA in the 1.5 mL micro tube. Firstly, genomic DNA (50 µL) with different concentrations was extracted from *S. aureus* from 10^2 to10^9 CFU mL^{-1}. The target DNA was heated for 5 min at 95 ˚C until dsDNA change to ssDNA. The genomic DNA_s prepare were added to 700 µL of 5 mg mL^{-1} of the MNP-probe2 complex and 250 µL of bio-barcode DNA-AuNP-probe1 complex. The reaction was performed at 54 ˚C for 30 min and 150-200 rpm. The MNP-probe2-genomic DNA-probe1-AuNP-florescence barcode DNA complex was rinsed with PBS buffer for three times to separate the unreacted MNP-probe2, florescence barcode DNA-AuNP-probe1 and genomic DNA. Finally, the nanomaterial's system (MNP-probe2-genomic DNA-probe1-AuNP-florescence barcode DNA) was suspended in 1000 µL of DTT 0.8 M for 10 min, under stirring (700 rpm) at 65 ˚C to release bio-barcode DNA. The presences of MNPs and AuNPs in the environment were leads to reduce fluorescence intensity of the florescence barcode DNA. So to solve this problem, the MNPs and the AuNPs were quickly removed by a magnetic field of supernatant. The supernatant was contained florescence barcode DNA. The supernatant was analyzed using the fluorescence spectrophotometry (Fig.1).

Fig.1

2.10. Sensitivity and specificity

In order to determine optimal sensitivity of our method, different concentrations of *S. aureus* (3.8×10^9, 3.4×10^8, 3.5×10^7, 3.2×10^6, 3.5×10^5, 3.2×10^4, 2.3×10^3 and 2.8×10^2 CFU mL^{-1}) were analyzed by fluorescence spectrophotometry. Limit of detection (LOD) was determined by standard deviation. Concentrations of samples containing *S. aureus* were measured based on the standard curve. Additionally, specificity of nanomaterial's system was determined using bacterial strains including *P. aeruginosa* ATCC27853, *S. dysentery* PTCC 1188, *K. pneumoniae* ATCC7881, *E. coli* ATCC 25922 and *S. epidermidis* ATCC12228 and *S. aureus* ATCC 29213 as a negative and positive controls. All of the bacterial (target DNA) at concentration of 3.8×10^5 CFU mL^{-1} were added to the reaction mixture containing the nanomaterial's system that was mentioned in section 2.9 [19].

3. Results and discussion

3.1. Materials characterization

3.1.1. UV–Vis spectrophotometry

UV-Vis spectra for pure AuNPs is shown in Fig. 2; curve A. The result was showed that the maximum absorbance at 520 nm for AuNPs. Conjugation of AuNPs with probe1 and bio-barcode DNA were changed absorbance wavelength from 520 nm to 525 nm, which is shown in Fig. 2; curve B. Also, the genomic DNA was added to probe1-AuNP-florescence barcode DNA complex to form the genomic DNA-probe1-AuNP-florescence barcode DNA complex. The absorbance wavelength of genomic DNA-probe1-AuNP-florescence barcode DNA complex was changed from 525 nm to 560 nm (Fig. 2, curve C).

Fig.2

3.1.2. SEM

For take SEM microscopy image, all of the samples were lyophilized and dried. The SEM pictures of nanoparticles showed that the shape of nanoparticles was spherical for AuNPs, florescence barcode DNA-AuNP-probe1 and florescence barcode DNA-AuNPs-probe-genomic DNA. The shape of MNPs, MNP-probe2 and MNP-probe2-genomic DNA were spherically and semi-spherically observed. The sizes of AuNPs and MNPs were 8-15 nm and 45-53 nm, respectively (Fig. 3 A and E). The size of AuNPs and MNPs conjugation with probes and target DNA were enhanced from 15 nm to 23 nm and 50 nm to 60 nm, respectively (Fig. 3 C, G and J). Finally, the size of florescence barcode DNA-AuNPs-probe1-genomic DNA-probe2-MNP complex was 80 nm to 100 nm that the showed in Fig. 3 L.

3.1.3. DLS

DLS was used to determine the size, potential and reaction between probes and AuNPs or MNPs that is shown in Fig. 3. Firstly, the size and potential of MNPs and AuNPs were 60 nm, -15.6 mV and 8.0 nm, -26.7 mV, respectively (Fig. 3 B and F). After conjugation of fluorescence barcode DNA and probes with AuNPs and MNPs, the potential and size of fluorescence barcode DNA-AuNPs-probe1 and MNP-probe2 were enhanced from 8.0 nm to 16.5 nm, -26.7 mV to -30.0 mV and 60 nm to 70-75 nm, -15.6 mV to -21.0 mV, respectively (Fig. 3 D and H) which is due to phosphate groups of oligonucleotides. The DLS results showed binding of the probes on the surface of AuNPs and MNPs. The conjugation of target DNA with MNP-probe2 was increased the size of MNP-probe2 from 75 nm to 90-100 nm (Fig. 3 K). Finally, the size of MNP-probe2-target DNA-probe1-AuNPs-barcode DNA sandwich was 90-110 nm (Fig .3 M).

Fig.3

3.2. Optimization

In order to detect *S. aureus*, different indexes were optimized at 3.5×10^5 CFU mL^{-1}. Under optimization conditions which include; probes hybridization temperature, amount of MNPs, incubation time,

concentration of bio-barcode DNA and incubation time, fluorescence intensity of bio-barcode assay was increased.

3.2.1. Hybridization temperature

One of the important factors that influence detection of *S. aureus* is the probe hybridization temperature with the genomic DNA. In the present study, the appropriate temperature for hybridization was determined. The results showed that the fluorescence intensity increased with enhance in reaction temperature from 20 °C to 65 °C and then decreased quickly (Fig. 4 A). The temperature of the hybridization of probe was slightly less than the melting temperature (Tm). In this study, the appropriate temperature was 54 °C, whereas the melting temperature (Tm) of probe and fluorescence bio-barcode DNA was 55 °C.

3.2.2. PH

In the present study, the pH of the reaction mixture was determined. The results showed that the appropriate pH was 7.5. According to the results, hybridization of probes, barcode DNA with genomic DNA were affected via change of pH. Under weak alkaline condition, DNA hybridization was performed better. Then, pH 7.5 was selected as optimum pH for the experiments (Fig. 4 B).

3.2.3. Probes

In Fig. 4 C, the effect of different concentrations of probes was showed on the sensor response of *S. aureus* (5×10^5 CFU mL^{-1}). The fluorescence intensity was enhanced with increase of different concentrations of probes from 1-30 nM. The optimum fluorescence intensity was showed at 15 nM of probes. When probes concentrations were greater than 15 nM, there were no obvious changes in the fluorescence intensity. Then, the probes concentration (15 nM) was used for all of the experiment steps.

3.2.4. Bio-barcode DNA

The bio-barcode DNA is one of the other recognition elements for the detection of *S. aureus*. Hence, the amount of bio-barcode DNA conspicuously has effects on the sensitivity of diagnosis nanomaterials. Under optimum conditions (3.5×10^5 CFU mL^{-1}), the amount of bio-barcode DNA ranging from 1-20 nM was optimized. The fluorescence intensity was increased, when the amount of the bio-barcode DNA was enhanced from 1-10 nM (Fig. 4 E). The highest fluorescence intensity was obtained, when the amount of the bio-barcode DNA was 7.5 nM (Fig. 4 D). Thus, the amount of bio-barcode DNA, 7.5 nM was adopted in the further experiments.

3.2.5. NPs

Fluorescence intensity was increased with raised of NPs concentration (Fig. 5 E and F). According to the results, the fluorescence intensity was not obvious more than 7.5 mM and 5 mg mL^{-1} of AuNPs and MNPs concentrations, respectively. Therefore, the concentrations of AuNPs and MNPs were fixed at 7.5 mM and 5 mg mL^{-1} respectively, in the following experiments. These concentrations were sufficient for the detection of the target DNA.

3.3. Fluorescence spectra

Under optimum conditions, the limit of detection of nanomaterial's system was determined. In order to achieve this purpose, the *S. aureus* culture was serially diluted. The concentration of *S. aureus* was prepared from 10^9 to 10^1 CFU mL^{-1}. Subsequently, the genomic DNA of *S. aureus* was extracted at different concentrations. In the presence of the nanomaterial's system (bio-barcode DNA-AuNP-probe1 and MNP-probe2), the genomic DNA at different concentrations was added to the reaction mixture. With increase of the concentration of target DNA, the emission rate was significantly raised (Fig. 5 A). The results showed the linear relationship between the concentration of target DNA and fluorescence intensity. Finally, the LODs and the R^2 were calculated for target DNA. Detection of target DNA was done using the fluorescence spectrophotometry at λ ex/λ em = 595/613 nm based on signals of bio-barcode DNA. In the present study, the presence of MNPs and AuNPs in the reaction mixture would lead to interference with the fluorescence signal of dye; hence the nanoparticles were removed from the reaction mixture by a magnetic field and centrifuged before measuring the fluorescence intensity. In order to increase the sensitivity for detection of genomic DNA, the excitation slit width was regulated from 10 to 5 nm.

Fig.4

Good linear relationship between different concentrations of genomic DNA from 10^2 to 10^9 CFU mL^{-1} was observed (Fig. 5 B). The regression equation was $Y = 2.54X + 22.3$, with the correlation coefficient of 0.9855 for target DNA (Fig.5 C). The limit of detection (LOD) was determined based on 3SD/b formula in which the SD is the standard deviation of the blank control sample and the b is the slope of the calibration curve. The limit of detection was found to be 86 CFU $mL^{-1}$for *S. aureus* protein A. The present method was compared with some other methods that were previously indicated in Table 1 (23-27). The results showed that the bio-barcode DNA assay is sensitive, specific, simple, and very inexpensive. Also, the limit of detection this method is better than other methods that the reported to now [20 and 22].

Fig.5

3.4. Specificity

In the present study, six bacteria strains (*P. aeruginosa* ATCC27853, *S. dysentery* PTCC 1188, *S. aureus* ATCC 29213, *K. pneumoniae* ATCC7881, *E. coli* ATCC 25922 and *S. epidermidis* ATCC12228) were used to test the specificity of nanomaterial's system. The result showed that the sample containing target DNA *S. aureus* and target DNA mixed other strains such as *S. aureus*, *E. coli*, *P. aeruginosa*, *S. dysentery*, *K. pneumoniae* and *S. epidermidis* have the highest change in fluorescence intensity ratio than other bacteria strains (Fig. 6A). More importantly, the sample was contained *S. aureus* (target DNA) was mixed with other bacteria. Also, the samples containing target DNA other strains were not increased fluorescence intensity (Fig. 6B, C, D, E and F). Also, Fig. 6A showed a substantial change in the fluorescence intensity. These results indicated that the nanomaterial's system based on bio-barcode DNA assay used in the present study could detect the *S. aureus* target DNA with high specificity.

3.5. Stability of method

In the present study, stability of nanomaterial's system was determined. Briefly, the nanomaterial's system was stored for 15 days, 4, 8, 16, 24 and 32 weeks at 25 °C. The nanomaterial's system was tested against *S. aureus* and fluorescence intensity was measured by the fluorescence spectroscopy. The result showed that the nanomaterial's system could be stable after 24 weeks of storage at 25 °C. The fluorescence intensity of the nanoparticles was slightly weaker at 32 weeks in compare to 24 weeks. The low fluorescence intensity was not important (Fig. 6B). The results showed that the nanomaterial's system remains fixed when stored at 25 °C, for 24 weeks.

Fig.6

3.8. Real samples

In order to determine the accuracy of bio-barcode DNA assay, the recovery of genomic DNA in the clinical samples were isolated and measured according section 2.9. The results were presented in Table 2. The results were confirmed target DNA (protein A) in three groups including: stool, urine and blood culture samples. Also, each sample was replicated three times. The genomic DNA concentrations were showed in Table 2. The recoveries of genomic DNA were obtained for stool, urine and blood culture samples in the range of 98-106.5%, 97.5-103.3% and 96.4-105.3%, respectively. These results indicated that the nanomaterial's system was exacted and repeatable for detection of *S. aureus* protein A in clinical samples.

4. Conclusions

In the present study, a nanomaterial's system was designed was for high sensitive diagnosis of *S. aureus* protein A gene. Firstly, MNPs and AuNPs nanoparticles were synthesized and conjugated with probes DNA

and bio-barcode DNA. Thus, characterization of MNP and AuNPs were confirmed by UV-Vis spectrophotometry, SEM and DLS. UV-Vis spectrophotometry, SEM and DLS were confirmed the amine group, probes and bio-barcode DNA on the surface of nanoparticles. Fluorescence intensity was measured by fluorescence spectrophotometry and the linear relationship there with genomic DNA was confirmed. The LOD nanomaterial's system in both PBS and real samples was 86 CFU mL^{-1} with correlation coefficient of R^2 = 0.9855. The results of this study showed that our method has high potential for the diagnosis of *S. aureus* and pathogenic bacteria in real samples [26-31].

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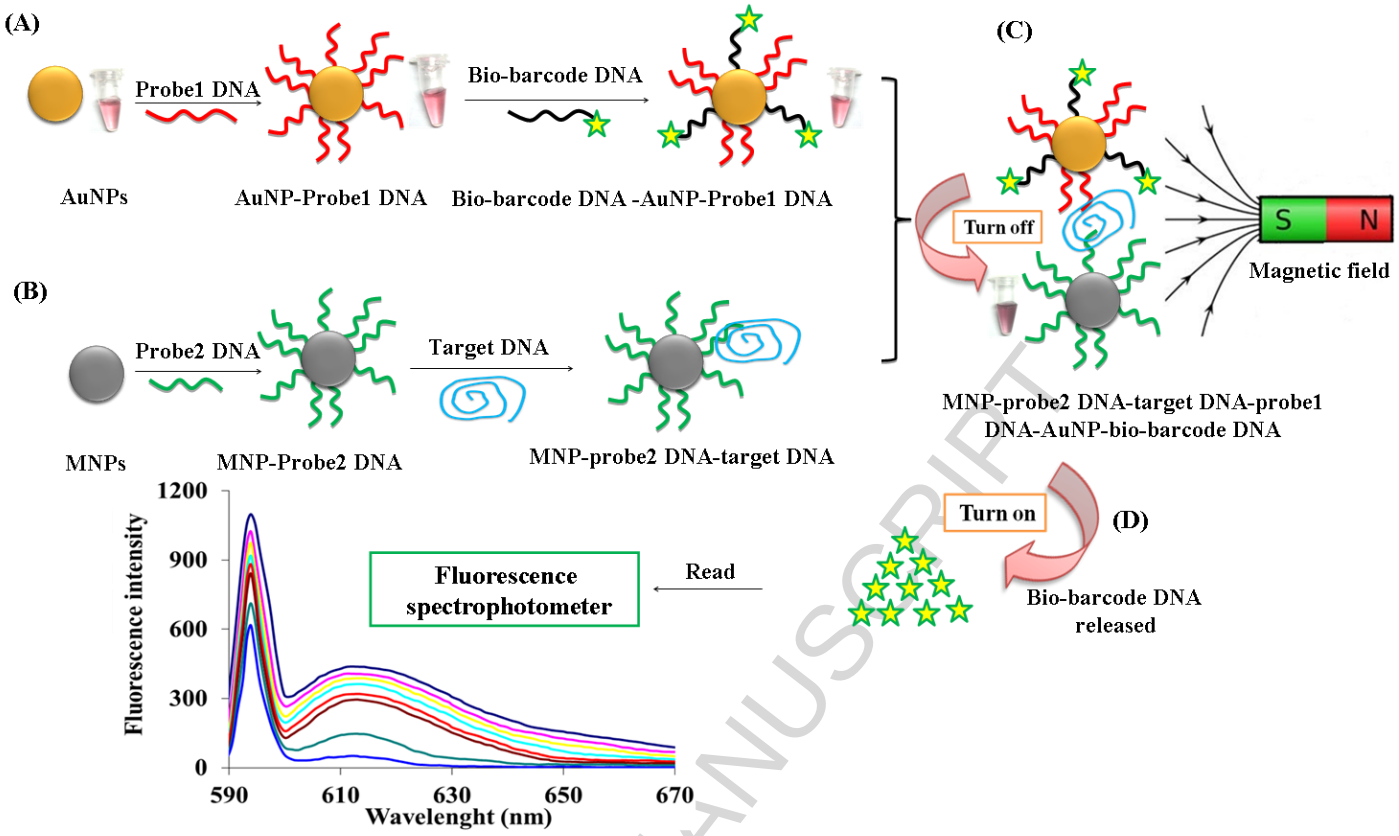

Fig.1. Schematic illustration of nanomaterial's method; (A) functionalization of AuNPs with probe1 and bio-barcode DNA; (B) functionalization of MNPs with probe2 and genomic DNA; (C) MNP-probe2-genomic DNA-probe1-AuNPs-bio-barcode DNA complex; (D) Bio-barcode DNA was released and read with fluorescence spectrophotometry.

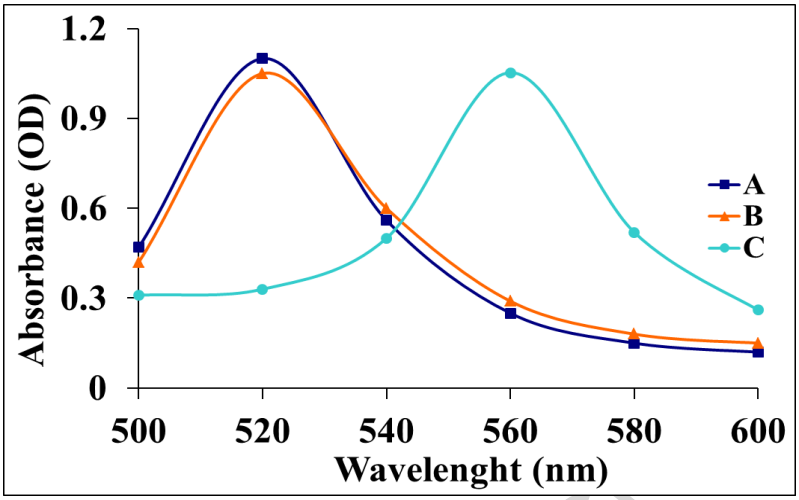

Fig.2. UV-Vis spectrophotometry; (curve A) AuNPs; (curve B) bio-barcode DNA-AuNPs-probe1 and (curve C) bio-barcode DNA-AuNPs-probe-genomic DNA.

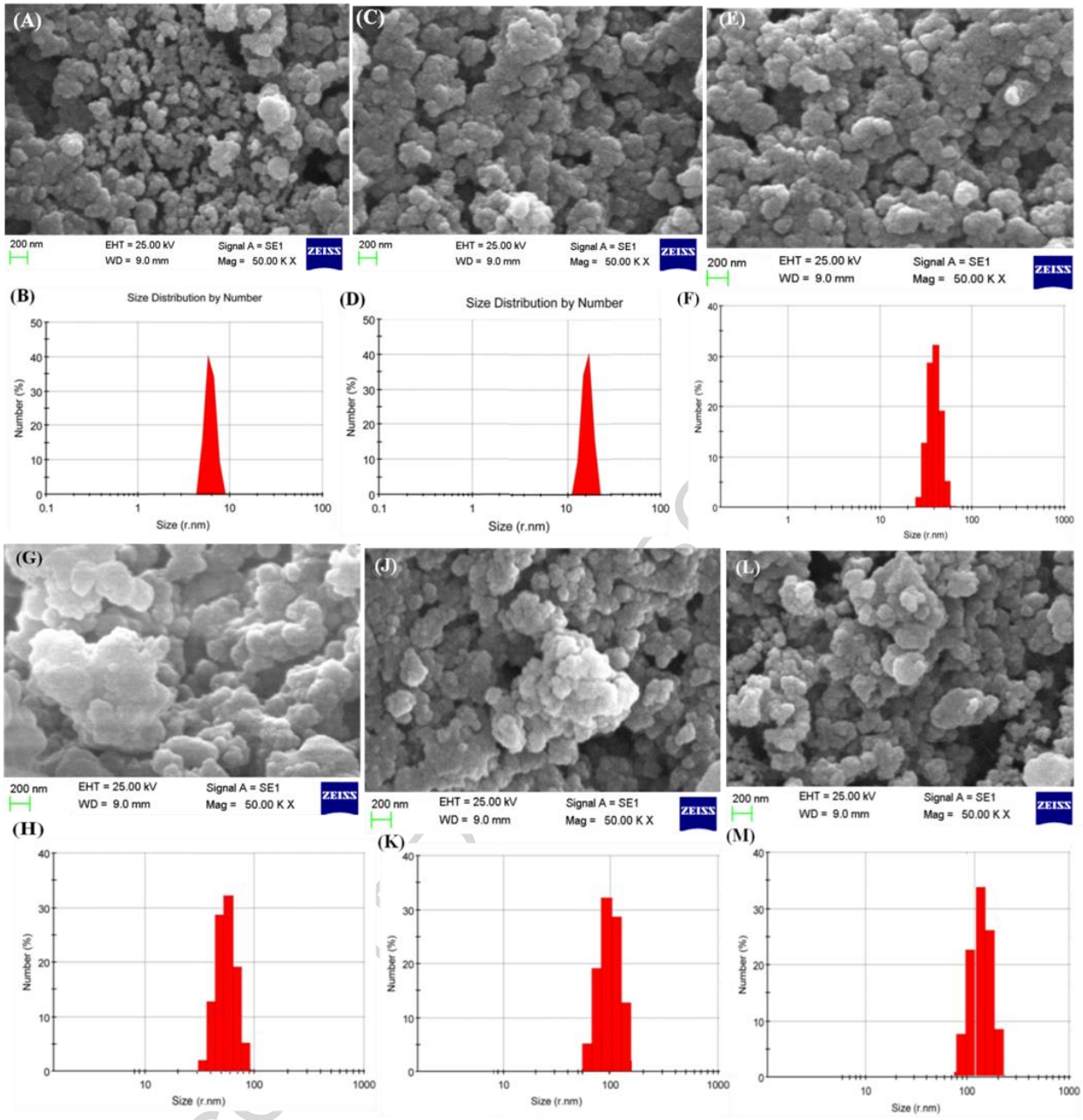

Fig.3. SEM and DLS of NPs: (A and B); AuNPs, (C and D); bio-barcode DNA-AuNPs-probe1, (E and F); pure MNPs, (G and H); SMCC-APTES-TEOS-MNPs (J and K); MNPs-TEOS-APTES-SMCC-probe2-target DNA, (L and M); MNPs-TEOS-APTES-SMCC-probe2-target DNA-probe1-AuNPs-bio-barcode DNA sandwich complex.

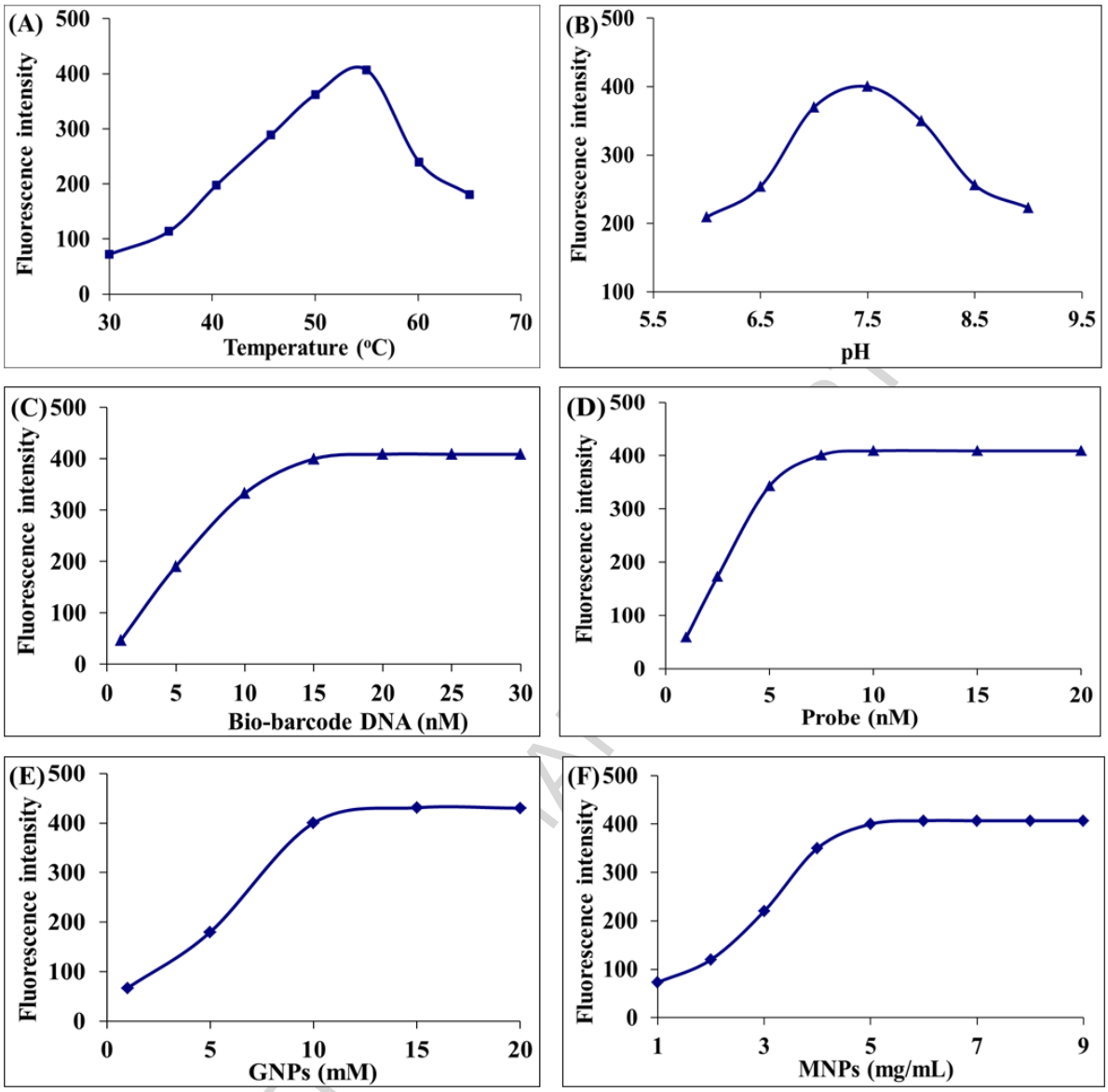

Fig. 4. Optimization of reaction conditions, (A) Effect of temperature on hybridization, (B) Effect of pH on hybridization, (C) Effect of bio-barcode DNA concentration on hybridization, (D) Effect of DNA probes concentration on hybridization (E) GNPs concentration and (F) MNPs concentration for detection of *S. aureus* protein A.

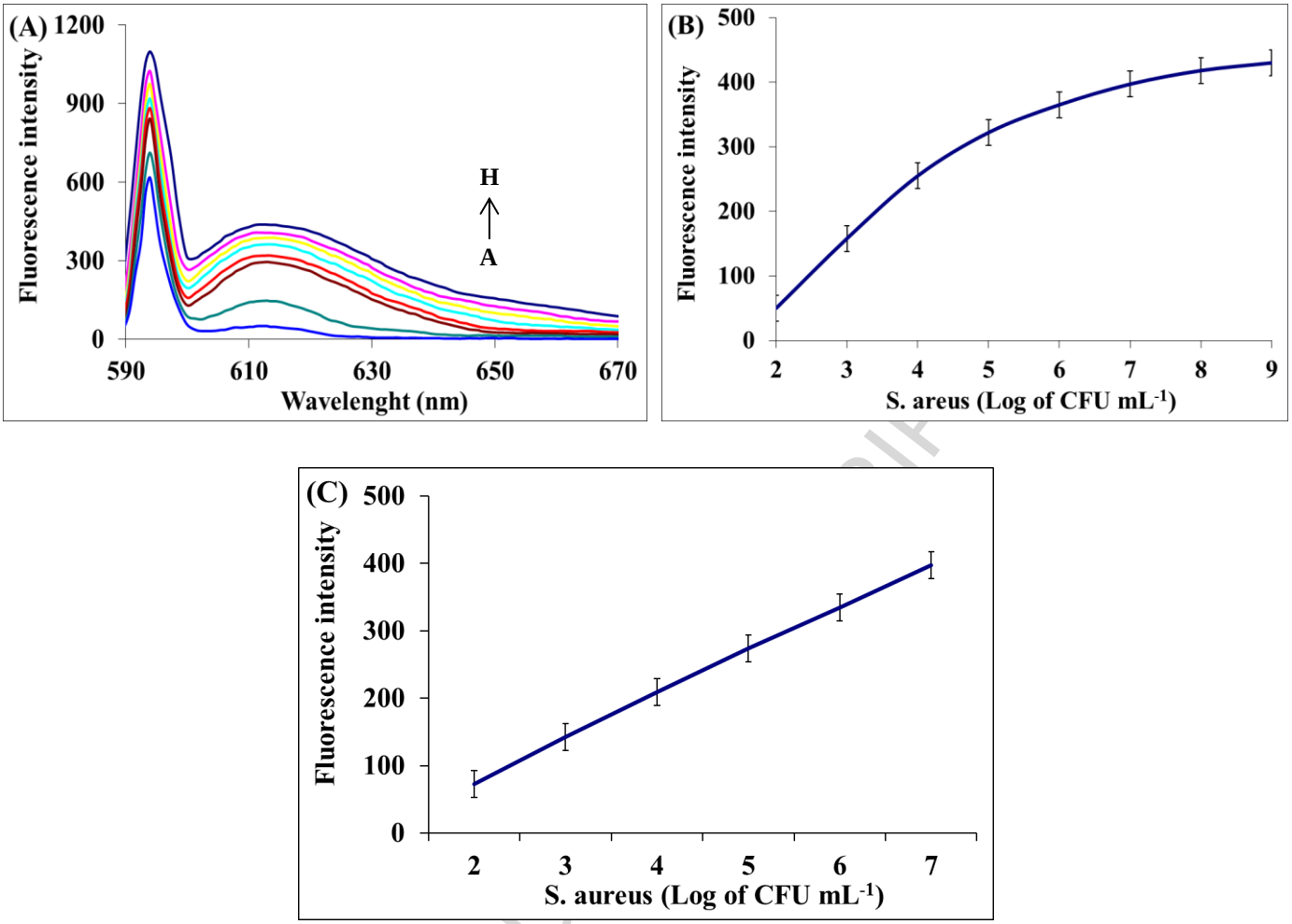

Fig.5. (A) Sensivity of nanomaterial's method; the concentrations of *S. aureus* were range from A to H; 2.8 × 10^2, 2.3 × 10^3, 3.2 × 10^4, 3.5 × 10^5, 3.2 × 10^6, 3.5 × 10^7, 3.4 × 10^8, 3.8 × 10^9 CFU mL^{-1} respectively. (B) The linear relationship between fluorescence intensity and *S. aureus* concentration. C= 2.8 × 10^2, 2.3 × 10^3, 3.2 × 10^4, 3.5 × 10^5, 3.2 × 10^6, 3.5 × 10^7, 3.4 × 10^8 and 3.8 × 10^9 CFU mL^{-1}. (C) The linear relationship between fluorescence intensity and *S. aureus* concentrations ranging from 10^2 - 10^7 CFU mL^{-1}.

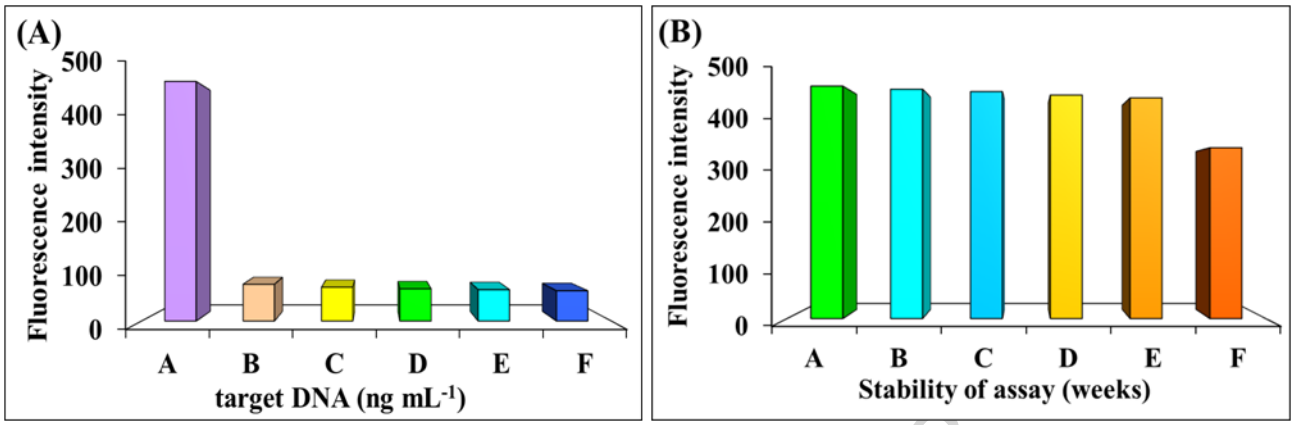

Fig.6. (A) Specificity of nanomaterial's method; (Column, A) the target DNA of *S. aureus* as positive control with target DNA mixed other strains such as *E. coli*, *P. aeruginosa*, *S. dysentery*, *K. pneumoniae* and *S. epidermidis* as negative controls, (Column, B) target DNA of *E. coli*, (Column, C) target DNA of *P. aeruginosa*, (Column, D) target DNA of *S. dysentery*, (Column, E) target DNA of *K. pneumoniae* and (Column, F) target DNA of *S. epidermidis* were all 3.5×10^5 CFU mL^{-1}. (B) Stability of nanomaterial's system for detection of *S. aureus*; (Column, A); 15 days, (Column, B); 4 weeks, (Column, C); 8 weeks, (Column, D); 16 weeks, (Column, E); 24 weeks and (Column, F); 32 weeks.

Table1. Compare of detection limit of *S. aureus* using different methods.

Method	Detection limit	References
Silica nanoparticle-probe assay	682 CFU/ml	[23]
Colorimetric assay	4×10^4 CFU/ml	[24]
Loop-mediated isothermal amplification (LAMP)	6.4×10^3 CFU/ml	[25]
Quantum dots	1×10^2 CFU/ml	[27]
enzyme-linked immunosorbent assay (ELISA)	1×10^4 CFU/ml	[6-7]
PCR assay	1×10^3 CFU/ml	[8]
Bio-barcode DNA assay	86 CFU/ml	Our method

Table2. The recovery and RSD value of detecting target DNA (Protein A).

Analyses	**Added target DN (ng mL^{-1})**	**Found (ng mL^{-1})**	**Recovery (%)**	**RSD (%)**
Urine				
1	10	10.18	101.8	4.03
2	20	19.5	97.5	6.0
3	30	31.0	103.3	6.5
Blood culture				
1	10	9.64	96.4	4.5
2	20	20.7	103.5	6.1
3	30	31.5	105.3	7.2
Stool				
1	10	9.8	98.0	4.0
2	20	19.7	98.5	5.8
3	30	31.96	106.5	8.2