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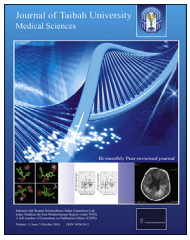

Original Article

# Signal enhancement in ELISA: Biotin-streptavidin technology against gold nanoparticles

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**الملخص**

**أهداف البحث:** تهدف هذه الدراسة لمقارنة البيوتين - ستربيتوفيدين و تقنية جزيئات الذهب في تقنية إليزا لتحسين الحساسية والدقة.

**طرق البحث:** أجرينا طريقتين لتحسين تقنية إليزا البيوتين - ستربيتوفيدين لتعزيز حد كشف بسبب ارتفاع ربط الانجذاب وترافق الجزيئات الحيوية للذهب لتحسين الكشف. تم مقارنة هذه الطريقتين باستخدام المضادات التي تفرز الهدف المستضدي-٦ مبكرا من السل الفطري للتفاعل مع مضاد الهدف المستضدي-٦ الجسم المضاد.

**النتائج:** بعد استعراض نتائج حد كشف الهدف المستضدي-٦ كانت هي نفسها بوجود أو عدم وجود جزيئات الذهب، بسبب تحقيق الإشباع مع ربط البوتين وستربيتوفيدين. ولكن لوحظ وجود امتصاصية عالية في وجود جزيئات الذهب.

**الاستنتاجات:** يمكن استخدام اقتراح تعديل إليزا للفحص الأساسي لأنواع مختلفة من الأمراض. كما أظهرت هذه الدراسة تقنيات عدة يمكن تطبيقها لتحسين الكشف والدقة بواسطة إليزا.

**الكلمات المفتاحية:** إليزا؛ جزيئات الذهب؛ مرض السل؛ بيوتين؛ ستربيتافيدين

**Abstract**

**Objectives:** The aim of this study is to compare the use of biotin-streptavidin and gold nanoparticle (GNP) technologies in enzyme linked immunosorbent assay (ELISA) techniques to improve its sensitivity and accuracy.

**Methods:** We evaluated two ELISA methods to improve the sensitivity and accuracy. Biotin-streptavidin technology was selected to enhance the ELISA limit of detection due to the high binding affinity of biotin-streptavidin. GNP-conjugated biomolecules were selected to improve detection by ELISA. To evaluate these two methods, the early secreted antigenic target-6 (ESAT-6) from *Mycobacterium tuberculosis* and the anti-ESAT-6 antibody were used.

**Results:** The detection limit of ESAT-6 was the same with and without GNP due to the saturation of biotin and streptavidin binding. However, higher absorbance was noticed using GNP only.

**Conclusion:** The proposed modified ELISA can be used to screen different types of common diseases. Additionally, this study showed how several new techniques can improve the detection and accuracy of ELISA.

**Keywords:** Biotin; ELISA; GNP; Streptavidin; Tuberculosis



## Introduction

Enzyme Linked Immunosorbent assay (ELISA) is widely used to detect antigens relating to disease using an

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appropriate antibody. ELISA is easier to perform, inexpensive, involves safe materials and gives accurate results.[1,2] Many disease-causing agents are detected by ELISA, including early secreted antigenic target-6 (ESAT-6),[3] glucose levels,[4] duck tembusu virus,[5] histoplasmosis,[6] and human fetuin A.[7] Because ELISA is widely used to screen for disease, improving ELISA techniques is important to enhance the ability to detect diseases. ELISA detection limits depend on factors such as capture antibody immobilization, pH, temperature, ligand binding affinity and analyte. Importantly, surface functionalization and biomolecular interactions can help to improve the limit of detection. In the surface functionalization process, the capture and detection antibodies are important to consider because less capture antibody leads a reduction in sensitivity. Many researchers have attempted to improve ELISA detection by immobilizing higher amounts of antibodies or antigens on an ELISA plate. Antibodies and antigens can bind ELISA plates through electrostatic, chemical or physical interactions. Vashit et al.[8] improved ELISA detection by immobilizing more antibody on an ELISA plate in just one step.

On the other hand, biomolecular affinity also plays an important role in improving the detection of ELISA. In this case, the maximum binding of an analyte and ligand can be improved, increasing the limit of detection. Biotin-streptavidin technology has been effectively used to improve ELISA detection due to the strong affinity between biotin and streptavidin because it has a dissociation constant ($K_d$) in the femtomolar range. Biotinylated probes are generally used to tag antibodies, deoxyribonucleic acid (DNA) or peptides for subsequent detection using enzyme-conjugated streptavidin. Because biotin and streptavidin interact strongly, more analyte molecules can be captured on an ELISA plate can be captured. On the other hand, nanomaterial application of the biosensor was used to improve detection and stabilize analyte molecules. Among other materials, gold nanoparticle (GNP) is a very attractive material because it easy to use and is stable. It was demonstrated that the use of various sensors, such as surface plasmon resonance (SPR), waveguide mode sensor, SPR-based Biacore and Raman spectroscopy, GNP improved the limit of detection.[9–13] Making use of the good characteristics of GNP, here, we used GNP-conjugated biomolecules to improve ELISA. A GNP conjugated primary antibody and secondary antibody as well as streptavidin-HRP (Streptavidin-Horseradish Peroxidase) were used to improve detection of the analyte. The goal is to use antibody conjugation with GNP to increase antibody capture, leading to improved analyte binding, induced high sensitive detection. GNP conjugated biomolecules are also very stable, even in crude samples, such as serum. In this work, we compared and attempted to improve the detection of biomolecules with ELISA-assisted biotin-streptavidin technology and GNP.

The model protein that was chosen for detection in ELISA is ESAT-6 from *Mycobacterium tuberculosis*. Tuberculosis (TB) is a life-threatening disease that damages lungs in humans.[13,14] TB spreads very fast via air-borne droplets, and the infection is difficult to control. Different methods have been developed to detect TB, such as the tuberculin skin test, microscopic analyses or blood tests.[15–17] When TB enters the bloodstream, it is difficult to detect in its earliest stages. Therefore, it is necessary to develop a novel method to detect TB during the earlier stages in blood-borne infection to avoid spreading contagion and to implement cures quickly. The method used in this study is useful for detecting different types of diseases with the use of an appropriate antibody by ELISA.

## Materials and Methods

### *Reagents and biomolecules*

ELISA plates were purchased from Becton Dickinson (France). ESAT-6 antibody and protein were purchased from Sino Biological, Inc. (China). ELISA 5X coating buffer purchased was from Biolegend. Anti-mouse ESAT-6 antibody was obtained from Santa Cruz Biotechnology (USA), anti-mouse-IgG-HRP (anti-mouse Immunoglobulin G-HRP) was obtained from Thermo-Scientific (USA), bovine serum albumin (BSA) was obtained from Promega (USA), biotinylated anti-mouse-IgG was obtained from Invitrogen (USA), streptavidin-HRP was obtained from Thermo-Scientific (Japan), and GNPs (10 and 80 nm) were obtained from Sigma−Aldrich. The HRP substrate was obtained from Promega (USA), and the ELISA reader was obtained from R & M Chemicals (U.K.).

### *Preparation of GNP-biomolecule complexes*

GNP-conjugated biomolecules were prepared as follows. Precisely 1 mL [one optical density, (1 OD)] of a GNP (sizes 10 or 80 nm) solution was added to 100 µl of mouse ESAT-6 antibody, anti-mouse-IgG-HRP, biotinylated anti-mouse-IgG antibody or streptavidin-HRP at a 100 µg/mL concentration, and then, the mixture was stirred for 30 min at room temperature (RT). Excess antibody was removed by centrifugation at 15,000× *g* for 15 min at 4 °C. Uncovered areas on GNP molecules were blocked with 2% BSA, and excess BSA was removed by centrifugation. Finally, GNP-conjugated antibodies were suspended in 1 mL of phosphate-buffered saline (PBS) and stored at 4 °C for further use.

### *Determination of GNP-conjugate stability by colourimetric assay*

To determine the stability of the GNP conjugates, we used a colourimetric assay. Different concentrations of sodium chloride (NaCl) were used to determine the GNP stability. NaCl concentrations of 30, 60, 120, 250, 400, and 800 mM were mixed with GNPs, and their stability was checked based on well aggregation. Once the NaCl concentration was determined (800 mM), it was added to the 1:500 dilution of ESAT-6 antibody-GNP, anti-mouse-IgG-HRP-GNP, biotinylated anti-mouse-IgG-GNP, streptavidin-HRP-GNP, conjugated GNPs, or the control (i.e., GNPs without ESAT-6 antibody). After 10 min, photographs were taken, and the colourimetric results were obtained using a nanophotometer. A wavelength scan was performed from 400 to 700 nm, and the data were graphed using Excel.

*Detection of ESAT-6 by biotin-streptavidin technology*

To detect ESAT-6 by using biotin-streptavidin technology, ELISA plates were first coated with ESAT-6 (0–500 nM, diluted in coating buffer). Next, the wells were blocked with 3% BSA before addition of the ESAT-6 antibody (1:1000). Biotinylated anti-mouse-IgG (1:1000) was added to bind the ESAT-6 antibody, and streptavidin-HRP (1:10,000) was added and resolved using the HRP-substrate to detect the presence of ESAT-6. Between each step, the wells were washed five times with washing buffer. All ELISAs were carried out at room temperature (RT). The optical density (OD) of the substrate was measured using a plate reader, and photographs were taken (using 3× optical zoom) 15 min after the substrate was added.

*Comparisons of biotin-streptavidin technologies with or without GNP*

For comparison with biotin-streptavidin technologies and to determine the effect of GNP, one of the following three different methods was evaluated. For the first method, ESAT antibody-GNP was used instead of the ESAT-6 antibody. The second method used ESAT-6 antibody-GNP and biotinylated-anti-mouse-IgG-GNP instead of the ESAT-6 antibody and biotinylated anti-mouse-IgG. The third method used biotinylated anti-mouse-IgG, streptavidin-HRP, ESAT-6 antibody-GNP, biotinylated anti-mouse-IgG-GNP, and streptavidin-HRP-GNP instead of the ESAT-6 antibody. These three methods were compared to conventional ELISA detection of ESAT at 250 nM. Ten nanometre (10 nm)-sized GNPs were used for all experiments. The remaining biomolecular concentrations and parameters were the same as those of conventional ELISA.

*Limit of detection for ESAT-6*

To determine the limit of detection of the ESAT-6 protein, we titrated from 8 nM to 250 nM. The detection limit was also compared between biotin-streptavidin technology and GNP technology. The formula used to calculate the limit of detection was: average Optical Density (Blank) − Standard Deviation (Blank).

*GNP Size based improvement in ESAT-6 detection*

In the previous experiments, we used 10 nm GNP-conjugated biomolecules for ESAT-6 detection. To improve ELISA, we tested GNP sizes of 80 nm and 10 nm. Both 10 and 80 nm GNP-conjugated biomolecules were able to detect 250 nm ESAT-6. Therefore, 80 nm GNP-conjugated biomolecules were subsequently used to check the limits of detection of ESAT-6. We next titrated the ESAT-6 concentrations to determine the detection limits under these GNP conditions and compared them to conventional ELISA. The remaining biomolecular concentrations and parameters were the same as those used for conventional ELISA.

## Results

In this study, we conjugated GNPs with biomolecules to improve the detection of ESAT-6 in ELISA and compared these results from those determined using biotin-streptavidin technology. Figure 1a is the schematic representation of a biotin-streptavidin based ELISA. Three different combinations of GNP-conjugated biomolecules were compared under these conditions (Figure 1b–d).

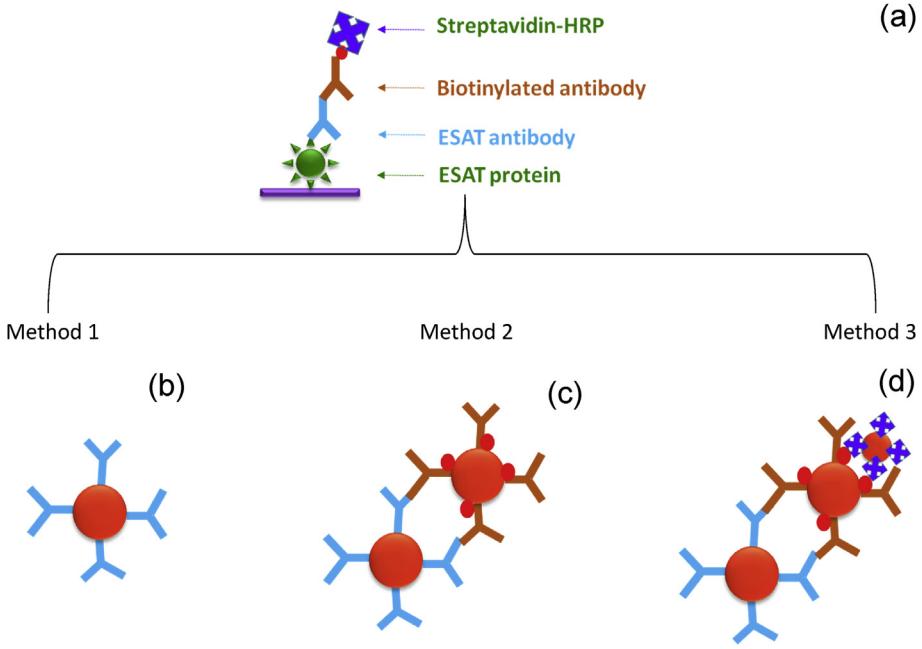


**Figure 1: Schematic representation of ESAT-6 detection by ELISA method assisted by biotin-streptavidin technology and GNP-assisted ELISA**. (a) Biotin-streptavidin based ELISA, (b) ELISA with ESAT-6 antibody-GNP, (c) ELISA with ESAT-6 antibody-GNP and biotinylated anti-mouse-IgG-GNP, (d) ELISA with ESAT-6 antibody-GNP, biotinylated anti-mouse-IgG-GNP, and streptavidin-HRP GNP.

*Detection of ESAT-6 by ELISA: biotin-streptavidin technology against GNP*

ESAT-6 protein was detected by biotin-streptavidin ELISA and compared with GNP-based ELISA. In these ELISA methods the final interactions were monitored by streptavidin-HRP. In this experiment, the effect of the biotin-streptavidin interaction was assessed for ELISA without GNPs and for the assays with 10 nm sized GNPs conjugated to the ESAT-6 antibody, biotinylated anti-mouse-IgG, or streptavidin-IgG-HRP (Figure 1). First, we confirmed the stability of the prepared GNPs (10 and 80 nm). The stability of the prepared ESAT-6 antibody-GNP, biotinylated anti-mouse-IgG-GNP, and streptavidin-IgG-GNP all exhibited excellent colourimetric stability with 800 mM NaCl. GNP-conjugated molecules remained red, whereas GNPs lacking molecules turned from red to purple due to aggregation. GNP-conjugated biomolecules had a maximum absorption of approximately 520 nm with dispersion, whereas when GNP aggregated, the absorbance peak appeared at a wavelength of approximately 630 nm (Figure 2).

Next, we assessed the ability of the three different methods for their ability to detect 250 nM ESAT-6. Compared to the biotin-streptavidin ELISA results, all three methods showed higher absorbance values for the same ESAT-6 concentrations, especially the third method (Figure 3), because more antibodies were able to bind GNP and thus improved ESAT-6 detection; the streptavidin-HRP

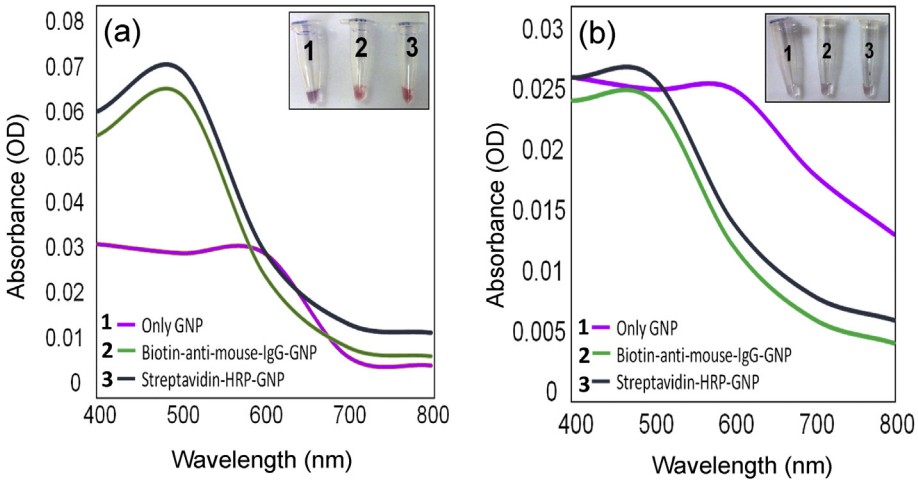


**Figure 2: Stability assay of prepared GNP-conjugated biomolecules in the presence of NaCl**. (a) Eighty nanometre GNPs were mixed with the appropriate protein and 800 mM NaCl. The spectrum was read and photographs were taken 15 min after NaCl was added. GNP-conjugated molecules showed excellent stability with a peak at ~520 nm, and they retained the original red colour. GNPs only showed aggregation at ~630 nm and exhibited aggregation with a purple colour.

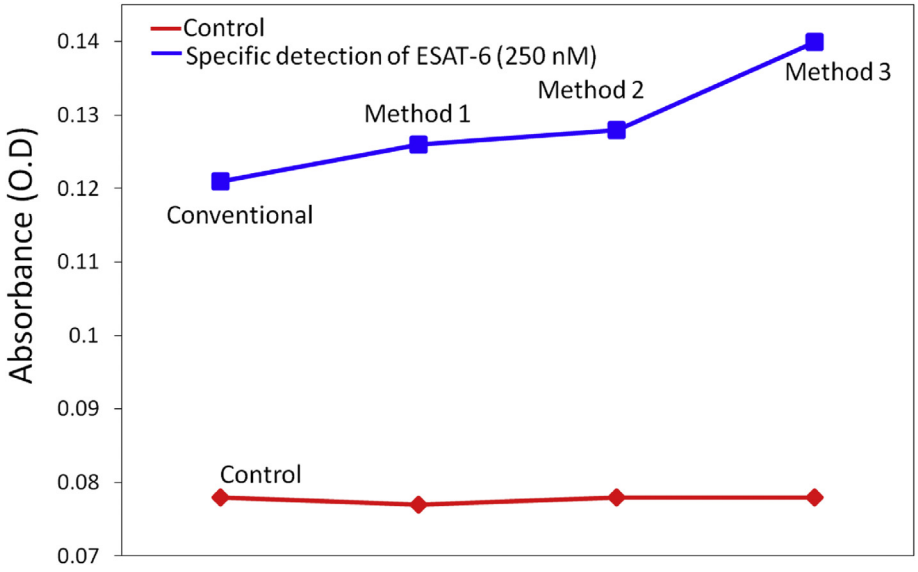


**Figure 3: Comparison between the conventional and GNP mediated ELISA**. Two-hundred-fifty nanomolar ESAT-6 was detected by conventional method and compared with three different methods with different combinations of GNP-conjugated GNPs. Method 3 showed high sensitivity compared with the other methods.

conjugated GNP especially increased the binding of biotin and streptavidin.

*Limit of detection of ESAT-6*

Because GNP-conjugated biomolecules improved the detection of ESAT-6, we next titrated ESAT-6 from 8 to 250 nM to determine the limit of detection of ESAT-6 by all three methods. As shown in Figure 4, all three methods (including the biotin-streptavidin ELISA) were able to detect as little as 8 nM ESAT-6 (Figure 4). However, ELISA without GNP was able to detect significantly lower (1 nM) concentrations of ESAT-6 compared to assays using ESAT-6 antibody-GNP or ESAT-6 antibody-GNP and biotinylated anti-mouse-IgG-GNP. The detection of ESAT-6 at 120 and 250 nM was the same due to saturation by ESAT-6. Interestingly, streptavidin-GNP improved ESAT-6 detection for all concentrations. Saturation of ESAT-6 began at 60 nM because streptavidin-GNP-HRP has a greater chance of binding.

*ESAT-6 detection improved using 80 nm GNP*

For comparison, we used 80 -nm sized GNPs to generate ESAT-6 antibody-GNP, anti-mouse-IgG-GNP, biotin-anti-mouse IgG-GNP and streptavidin-HRP-GNP. First, we checked whether 250 nM ESAT-6 was detected best by 10 or 80 nm sized GNP (Figure 5). Because the third method gave the best results previously, we used this method to compare the GNP sizes. As shown in Figure 5, 80 nm GNP increased the detection limits compared to 10 nm GNP because more antibodies were able to bind the 80 nm GNP. Because 80 nm GNP gave the best results, we determined their detection limits for ESAT-6 compared to conventional ELISA. The limits of detection reached 1 nM in these experiments without GNPs; however, GNPs increased the limits of detection for all of the tested concentrations of ESAT-6 (Figure 6). Compared with 10 nm GNP, 80 nm GNP increased the limit of detection using the same concentration of ESAT-6. Additionally, there was a significant increase in the limit of detection for all of the concentrations of ESAT-6 using 80 nm GNP (Figure 6).

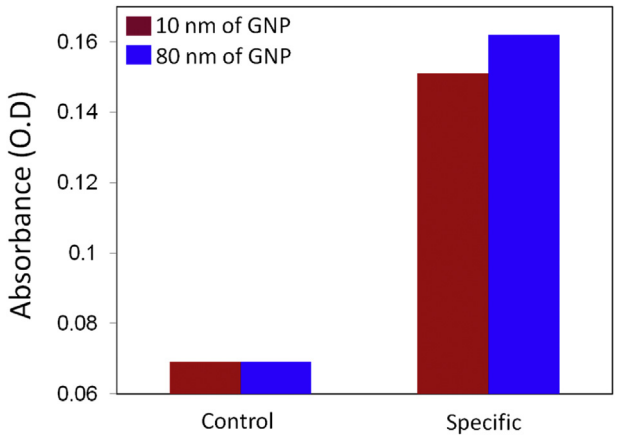


**Figure 5: Comparison of different sized GNPs**. Ten and eight nanometre GNPs were used to evaluate detection of ESAT-6. Two-hundred-fifty nanomolar ESAT-6 was detected by both 10 and 80 nm sized GNP. Eighty nanometre GNP shows the best results.

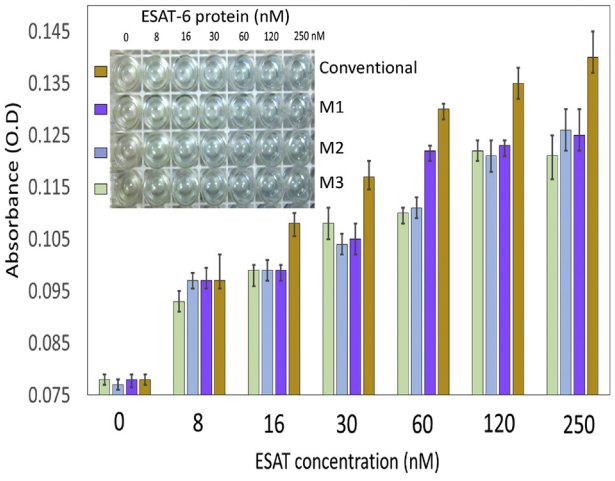


**Figure 4: Limit of detection of ESAT-6**. ESAT-6 was titrated with concentrations ranging from 0 to 500 nM. (a) Conventional ELISA, (b) ELISA with ESAT-6 antibody-GNP, (c) ELISA with ESAT-6 antibody-GNP and biotinylated anti-mouse-IgG-GNP, (d) ELISA with ESAT-6 antibody-GNP, biotinylated anti-mouse-IgG-GNP, and streptavidin-HRP-GNP. Ten nanometre GNPs were used in all experiments. The figure inset shows the colour change of the solution after adding the HRP substrate. As the concentration of ESAT-6 increased, the colour intensity also increased.

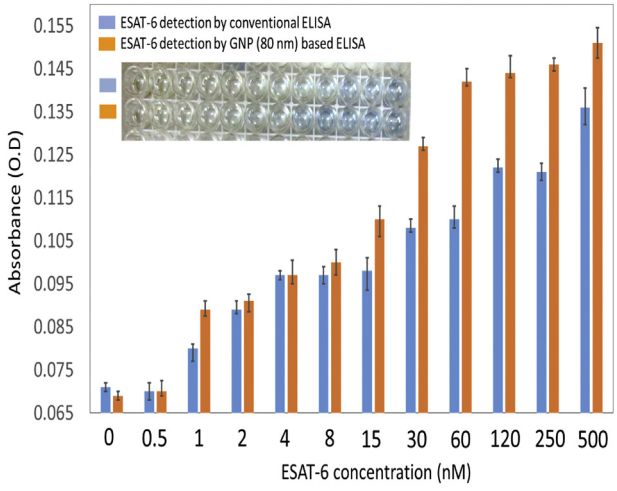


**Figure 6: Limit of detection determined by conventional ELISA assisted by GNP based ELISA**. ESAT-6 was titrated with the concentrations ranging from 0.5 to 500 nM. Graphical representation of OD with the above experiments. Limit of detection for both conventional and GNP-assisted ELISAs were similar, but the use of GNPs increased the OD for all concentrations. Eighty nanometre GNP was used in all experiments. The figure inset shows the colour change of the solution after adding the HRP substrate. As the concentration of ESAT-6 increased, he colour intensity also increased.

## Discussion

ELISA is a precise detection system for a wide range of targets when the correct antibody is used. Because ELISAs can detect biomolecules in crude samples, such as serum, improving binding affinities for different biomolecules to reach maximum sensitivity can be helpful for detecting disease. Biotin-streptavidin is already widely used in many biotechnology fields due to their strong interactions; it is particularly useful in biosensor development to improve the detection of the system. Moreover, nanoparticles, such as GNP, have also been used in a similar way to improve detection. In this study, we used this commonly used technology to improve the limit of detection of ELISA. Moreover, nanoparticles have also been used for a long time as biosensors to improve the limit of detection. Among nanoparticles, GNP is a very popular particle in sensor development. ELISA combined with GNP displays improved biomolecular detection. Han et al.[18] used a GNP-conjugated detection antibody and DNA for the detection of protein; these conjugations were successfully used to simultaneously detect two different biomarkers in human serum.

The biotin-streptavidin interaction was expected to improve the limit of detection of ESAT-6 because biotin and streptavidin have a strong binding affinity with a femtomolar dissociation constant.[19] If only a small amount of biotinylated anti-mouse-IgG binds to the ESAT-6 antibody, streptavidin has a greater chance of binding ESAT-6 because streptavidin has four biotin-binding sites. Thus, ESAT-6 should be detected at even lower concentrations. Among the three methods tested, method three worked best because more antibodies were able to bind GNP and improved ESAT-6 detection, especially streptavidin-HRP conjugated GNP, which increased the binding affinity between biotin and streptavidin. Regarding sensitivity, all three methods were able to detect as little as 8 nM of ESAT-6, including biotin-streptavidin ELISA because the strong biotin-streptavidin interaction improves the sensitivity of the assay. Because 10-nm sized GNP in the above assay improved the optical density of the detection of ESAT-6, the same experiment was carried out with 80 nm GNP because we thought that more antibodies could bind 80 nm GNP. As expected there was an increased ability to detect ESAT-6 with 80 nm GNP.

The experiments described herein showed that there is no change of the limit of detection without GNP due to the saturated binding of biotin and streptavidin. Simultaneously, the visible detection of ELISA was greatly improved when biomolecules were immobilized on the GNP surface. GNPs have often been used in sensor development to enhance the detection of a given compound. For example, a mixture of an antibody with GNP or an aptamer with GNP was found to improve the limit of detection to the lower femtomolar range using a surface Plasmon-based Biacore system.[9,11] Lakshmipriya et al.[11] found that GNP-conjugated anti-mouse-IgG improved the limit of detection of Factor IX protein to the low picomolar range in a SPR-based Biacore system. Gopinath et al.[20] reported that antibody-conjugated GNP improved influenza detection in the waveguide mode sensor. GNPs are also commonly used in the Raman-scattering system to detect biomolecules and in commercial detection kits (e.g., immunochromatography pregnancy test). In the present study, our GNP-assisted ELISA successfully detected ESAT-6 with a high performance typical of that of other demonstrated GNP-based assays. This strategy can be similarly used for other important targets.[21–27]

## Conclusions

ESAT-6 is an early secreted protein by *M. tuberculosis*; here, we detected ESAT-6 by both conventional ELISA and GNP-assisted ELISA with appropriate antibodies. Compared with biotin-streptavidin based ELISA, GNP based ELISA improved the optical density for all concentrations of ESAT-6, but the limit of detection was found to be the same using both methods. Use of 80 nm GNP-conjugated biomolecules improved the detection of ESAT-6 compared with 10 nm. Thus, GNP-based ELISA was able to detect ESAT-6 at lower concentrations (1 nM) and is suitable for diagnosing the early stage of infection. Moreover, this strategy should be applicable to the early detection of other diseases, such as influenza and Human Immunodeficiency Virus.

## Conflict of interest

The authors have no conflict of interest to declare.

## Authors' contributions

TL performed the experiments, analysed the data, wrote the manuscript; SCBG designed the experiments, analysed the data, wrote the manuscript; UH supervised the work; THT supervised the work and provided the materials; and all of the authors read and approved the final version of the manuscript.

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## References

1. Dixit CK, Vashist SK, O'neill FT, O'reilly B, MacCraith BD, O'kennedy R. Development of a high sensitivity rapid sandwich ELISA procedure and its comparison with the conventional approach. **Anal Chem 2010**; 82: 7049–7052. http://dx.doi.org/10.1021/ac101339q.
2. Dixit CK, Vashist SK, MacCraith BD, O'Kennedy R. Multi-substrate-compatible ELISA procedures for rapid and high-sensitivity immunoassays. **Nat Protoc 2011**; 6: 439–445. http://dx.doi.org/10.1038/nprot.2011.304.
3. Shams H, Weis SE, Klucar P, Lalvani A, Moonan PK, Pogoda JM, et al. Enzyme-linked immunospot and tuberculin skin testing to detect latent tuberculosis infection. **Am J Respir Crit Care Med 2005**; 172: 1161–1168. http://dx.doi.org/10.1164/rccm.200505-748OC.
4. Liu QL, Yan XH, Yin Xm, Situ B, Zhou HK, Lin L, et al. Electrochemical enzyme-linked immunosorbent assay (ELISA) for α-fetoprotein based on glucose detection with multienzyme-nanoparticle amplification. **Molecules 2013**; 18: 12675–12686. http://dx.doi.org/10.3390/molecules181012675.

5. Li X, Li G, Teng Q, Yu L, Wu X, Li Z. Development of a blocking ELISA for detection of serum neutralizing antibodies against newly emerged duck Tembusu virus. **PLoS One 2012**; 7: e53026. http://dx.doi.org/10.1371/journal.pone.0053026.
6. Guimaraes AJ, Pizzini CV, De Matos Guedes HL, Albuquerque PC, Peralta JM, Hamilton AJ, et al. ELISA for early diagnosis of histoplasmosis. **J Med Microbiol 2004**; 53: 509–514. http://dx.doi.org/10.1099/jmm.0.05469-0.
7. Vashist SK. A sub-picogram sensitive rapid chemiluminescent immunoassay for the detection of human fetuin A. **Biosens Bioelectron 2013**; 40: 297–302. http://dx.doi.org/10.1016/j.bios.2012.07.067.
8. Perumal V, Hashim U, Gopinath SCB, Haarindraprasad R, Foo KL, Balakrishnan SR, et al. "Spotted Nanoflowers": gold-seeded zinc oxide nanohybrid for selective bio-capture. **Sci Rep 2015**; 5: 12231. http://dx.doi.org/10.1038/srep12231.
9. Kim S, Lee J, Lee SJ, Lee HJ. Ultra-sensitive detection of IgE using biofunctionalized nanoparticle-enhanced SPR. **Talanta 2010**; 81: 1755–1759. http://dx.doi.org/10.1016/j.talanta.2010.03.036.
10. Gopinath SCB, Awazu K, Fujimaki M, Shimizu K, Shima T. Observations of immuno-gold conjugates on influenza viruses using waveguide-mode sensors. **PLoS One 2013**; 8: 1–10. http://dx.doi.org/10.1371/journal.pone.0069121.
11. Lakshmipriya T, Horiguchi Y, Nagasaki Y. Co-immobilized poly(ethylene glycol)-block-polyamines promote sensitivity and restrict biofouling on gold sensor surface for detecting factor IX in human plasma. **Analyst 2014**; 139: 3977–3985. http://dx.doi.org/10.1039/c4an00168k.
12. Lakshmipriya T, Fujimaki M, Gopinath SCB, Awazu K, Horiguchi Y, Nagasaki Y. A high-performance waveguide-mode biosensor for detection of factor IX using PEG-based blocking agents to suppress non-specific binding and improve sensitivity. **Analyst 2013**; 138: 2863–2870. http://dx.doi.org/10.1039/c3an00298e.
13. Brites D, Gagneux S. Co-evolution of *Mycobacterium tuberculosis* and *Homo sapiens*. **Immunol Rev 2015**; 264: 6–24. http://dx.doi.org/10.1111/imr.12264.
14. Geluk A, van Meijgaarden KE, Franken KLMC, Subronto YW, Wieles B, Arend SM, et al. Identification and characterization of the ESAT-6 homologue of *Mycobacterium leprae* and T-cell cross-reactivity with *Mycobacterium tuberculosis*. **Infect Immun 2002**; 70: 2544–2548. http://dx.doi.org/10.1128/IAI.70.5.2544.
15. Dorman SE. New diagnostic tests for tuberculosis: bench, bedside, and beyond. **Clin Infect Dis 2010**; 50(Suppl 3): S173–S177. http://dx.doi.org/10.1086/651488.
16. Bothamley GH. Serological diagnosis of tuberculosis. **Eur Respir J** 1995;(Suppl 20): 676s–688s.
17. Brock I, Munk ME, Kok-Jensen A, Andersen P. Performance of whole blood IFN-gamma test for tuberculosis diagnosis based on PPD or the specific antigens ESAT-6 and CFP-10. **Int J Tuberc Lung Dis 2001**; 5: 462–467. Available: http://www.ncbi.nlm.nih.gov/pubmed/11336278.
18. Han KC, Yang EG, Ahn DR. A highly sensitive, multiplex immunoassay using gold nanoparticle-enhanced signal amplification. **Chem Commun 2012**; 48: 5895. http://dx.doi.org/10.1039/c2cc31659e.
19. Green NM. Advances in protein chemistry. **Adv Protein Chem 1975**; 29. http://dx.doi.org/10.1016/S0065-3233(08)60411–8.
20. Gopinath SCB, Awazu K, Fujimaki M, Shimizu K. Evaluation of anti-A/Udorn/307/1972 antibody specificity to influenza A/H3N2 viruses using an evanescent-field coupled waveguide-mode sensor. **PLoS One 2013**; 8: e81396. http://dx.doi.org/10.1371/journal.pone.0081396.
21. Balakrishnan SR, Hashim U, Gopinath SCB, Poopalan P, Ramayya HR, Veeradasan P, et al. Polysilicon nanogap lab-on-chip facilitates multiplex analyses with single analyte. **Biosens Bioelectron 2015**. http://dx.doi.org/10.1016/j.bios.2015.10.075. Elsevier.
22. Gopinath SCB, Hayashi K, Kumar PKR. Aptamer that binds to the gD Protein of Herpes Simplex Virus 1 and efficiently inhibits viral entry. **J Virol 2012**; 86: 6732–6744. http://dx.doi.org/10.1128/JVI.00377-12.
23. Gopinath SCB, Shikamoto Y, Mizuno H, Kumar PKR. Snake-venom-derived factor IX-binding protein specifically blocks the gamma-carboxyglutamic acid-rich-domain-mediated membrane binding of human factors IX and X. **Biochem J 2007**; 405: 351–357. http://dx.doi.org/10.1042/BJ20061737.
24. Anbu P, Gopinath SCB, Hilda A, Mathivanan N, Annadurai G. Secretion of keratinolytic enzymes and keratinolysis by *Scopulariopsis brevicaulis* and *Trichophyton mentagrophytes*: regression analysis. **Can J Microbiol 2006**; 52: 1060–1069. http://dx.doi.org/10.1139/w06-067.
25. Gopinath SCB, Balasundaresan D, Akitomi J, Mizuno H. An RNA aptamer that discriminates bovine factor IX from human factor IX. **J Biochem 2006**; 140: 667–676. http://dx.doi.org/10.1093/jb/mvj203.
26. Kumarevel T, Gopinath SCB, Nishikawa S, Mizuno H, Kumar PKR. Identification of important chemical groups of the hut mRNA for HutP interactions that regulates the hut operon in *Bacillus subtilis*. **Nucleic Acids Res 2004**; 32: 3904–3912. http://dx.doi.org/10.1093/nar/gkh725.
27. Kumarevel T, Tanaka T, Nishio M, Gopinath SCB, Takio K, Shinkai A, et al. Crystal structure of the MarR family regulatory protein, ST1710, from Sulfolobus tokodaii strain 7. **J Struct Biol 2008**; 161: 9–17. http://dx.doi.org/10.1016/j.jsb.2007.08.017.


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