Supporting Information for:

SERS-based Rapid Assay for Sensitive Detection of Group A *Streptococcus* by Evaluation of the Swab Sampling Technique

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Procedure for synthesis of magnetic gold nanoparticles

These nanoparticles had a spherical core-shell structure which occurred with hydroxylamine reduction of $HAuCl_4$ on the surface of EDTA immobilized iron (magnetite Fe_3O_4) nanoparticles in the presence of CTAB solution. As it was previously described in the literature (Tamer et. al., 2010), magnetite nanoparticles were synthesized at first with a slight change. For this purpose, 0.64 M $FeSO_4.7H_2O$ and 1.28 M $FeCl_3$ were dissolved in deionized water for the precipitation of Fe (III) and Fe (II). After, 1M NaOH was added dropwise for 40 min and black precipitation was collected with a magnet and washed with deionized water. For obtaining oxidized Fe_3O_4 nanoparticles, the resulting solution was washed with 2M $HClO_4$ and left in the same solution for 3 hours. Then, the Fe_3O_4 were centrifuged at 10,000 rpm for 20 min and washed with deionized water for three times. For the deposition of the gold layer, 10 mg of pre-prepared Fe_3O_4 nanoparticles were dispersed in EDTA solution and after a 10 min of centrifugation at 8000 rpm, 7 mL of 0.1 M CTAB, 3 mL of 0.1 M $HauCl_4$ and 150 mg of hydroxylamine added in turn by mixing in a sonicator. In the end, reduction of Au(III) into Au(0) enabled the formation of the gold layer onto the Fe_3O_4 surface. The final solution was left in the shaker for 24 hours and when the color was dark red, they were ready to use in assays.

Procedure for synthesis of spherical gold nanoparticles

First of all, 50 mL of distilled water was boiled in an oil bath and 20 μL of 30% $HAuCl_4$ was added under vigorous stirring. Then, 5 mL of 40 mM sodium citrate solution was added and the solution kept boiling for 20 minutes. In the end, the color of the solution changed to wine red as the confirmation of the spherical AuNPs. The solution was cooled at room temperature and kept in the fridge for further modification.

Procedure for synthesis of rod shaped gold nanoparticles

For this purpose, a two-step protocol, seed-mediated growth technique was followed to synthesize rod-shaped gold nanoparticles. First, 4.75 mL of 0.1 M CTAB solution and 500 μL of 0.01 M $HauCl_4$ solution were mixed to form the seed solution. The synthesis of AuNPs started with reducing Au(III) to Au(0) by adding 600 μL of 0.01 M ice-cold $NaBH_4$ rapidly and this solution was allowed to stand for 30 min to form the seed. Second, to prepare gold nanorods, growth solution was prepared by mixing 500 μL of 0.01 M $HauCl_4$, 4.75 Ml of 0.1 M CTAB and 60 μL of 0.01 M $AgNO_3$. The resulting color was deep yellow and after one minute, 100 μL of ascorbic acid was added and the solution turned colorless. To obtain the final nanorod solution, 10 μL of seed solution was added and after one hour, the color was turned to dark blue. Both magnetic and rod-shaped AuNPs were characterized with UV-visible spectroscopy and transmission electron microscope (TEM).

Procedure for the modification of AuNPs

For this purpose, magnetic AuNPs were mixed with 20 mM of 11-MUA for 24 hours. Then, the nanoparticles were washed by centrifugation with MES buffer pH 6.5 and then freshly prepared 0.05 M of NHS and 0.1 M EDC were added and waited for 45 min to activate the carboxyl groups. After the activation, magnetic AuNPs were ready to interact with 0.1 mg/mL of streptavidin for an hour. Then, the magnetic AuNPs were washed again with MES buffer pH 6.5, and 0.1 mg/mL of polyclonal anti-Group A *Streptococcus* antibody added and waited for an hour for the modification. In the end, to block the unreacted activated esters, the magnetic AuNPs interacted with 10% of ethanolamine in PBS buffer for 30 minutes. The washing procedure was performed twice prior to a final resuspension of the AuNPs with PBS buffer and they were stored at 4^{o}C before use. SERS tagged rod shaped Au-NPs were also modified with the same procedure. However, they were modified with 20 mM of DTNB prepared in ethanol, instead of 11-MUA for 24 hours. The rest of the antibody conjugation was followed as described above.

Modification of gold nanoparticles for SERS-based LFIA test.

Spherical AuNPs modified with SERS-tag and anti-GAS antibody for using at conjugation pad. These NPs were modified with DTNB and antibody by the same method explained above. The concentration of antibody was 0.5 mg/mL in this step and % 5 BSA was preferred for blocking the inactivated groups. In the end, AuNPs were washed three times and the pellet was suspended in PBS pH 7.4 containing 0.1 M trehalose and 0.05 % Tween 20 to enable easy dissolve of modified AuNPs by the lateral flow.

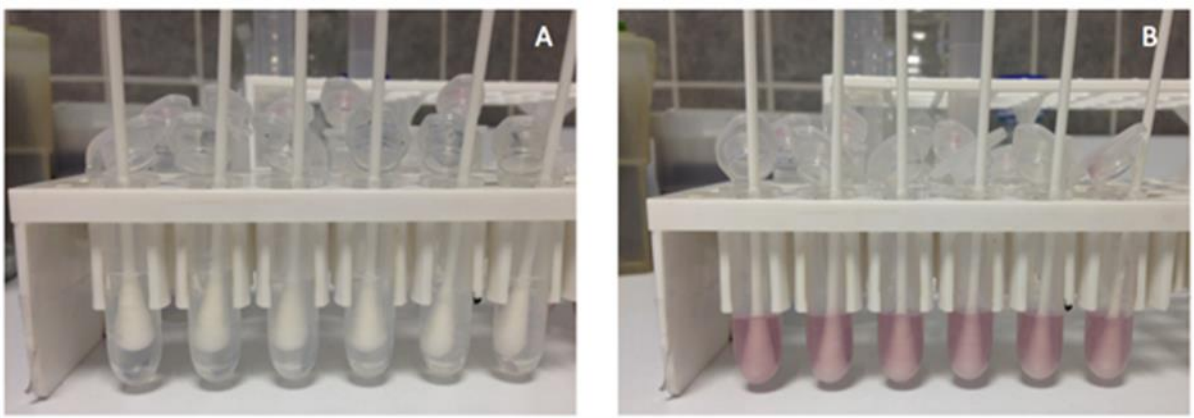

Figure S1. A) Cotton swabs in dilutions of different Group A *Streptococcus pyogenes* (GAS) B) Cotton swabs with GAS in the modified magnetic gold nanoparticles

Figure S2. Image of a plate for counting colonies of Group A *Streptococcus pyogenes* on the Colombia agar.

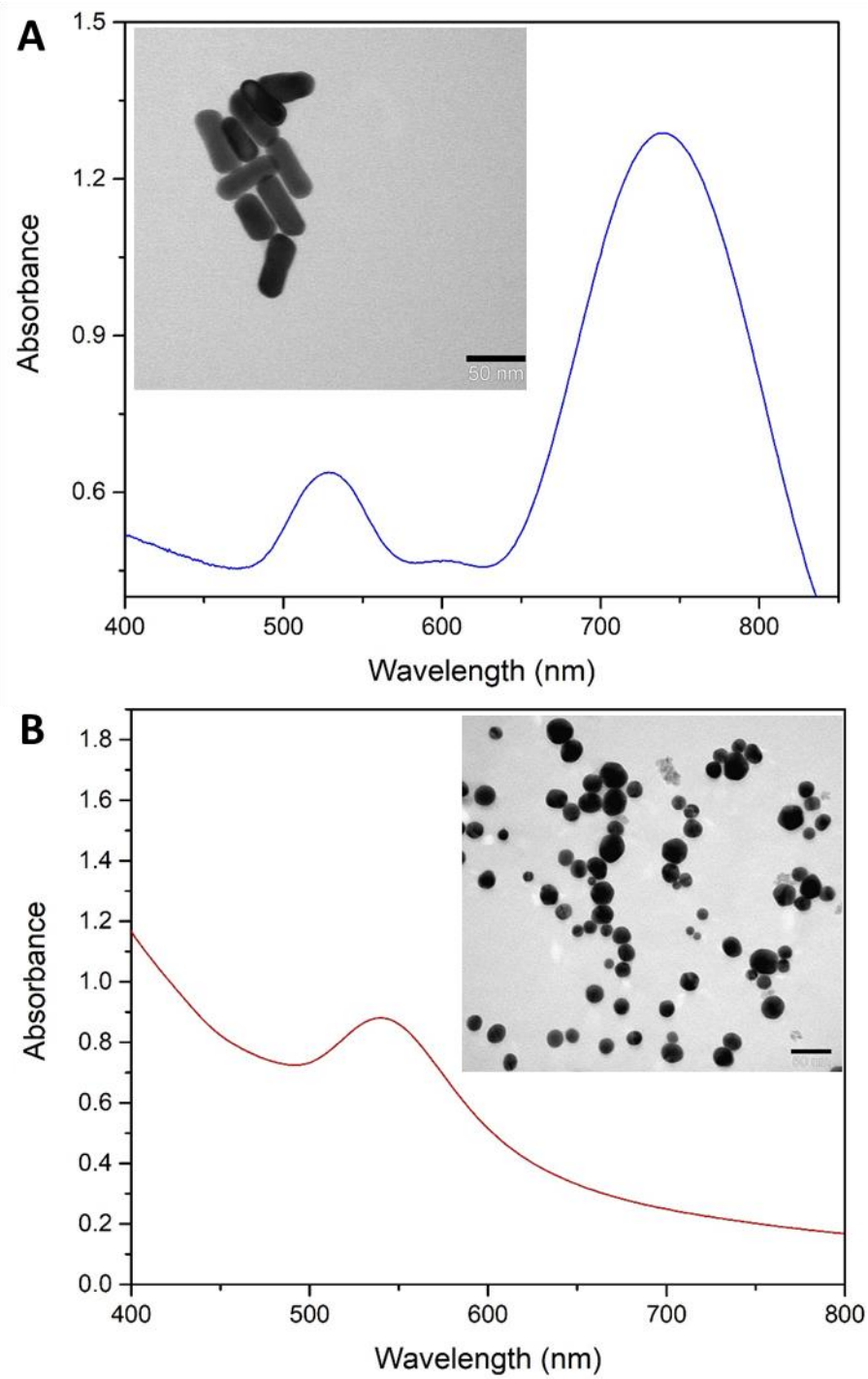

Figure S3. A) UV-vis spectra of rod-shaped gold nanoparticles and TEM image of the rod-shaped gold nanoparticles in a 50 nm scale (inset). B) UV-vis spectra of magnetic gold nanoparticles and TEM image of them in a 50 nm scale (inset).

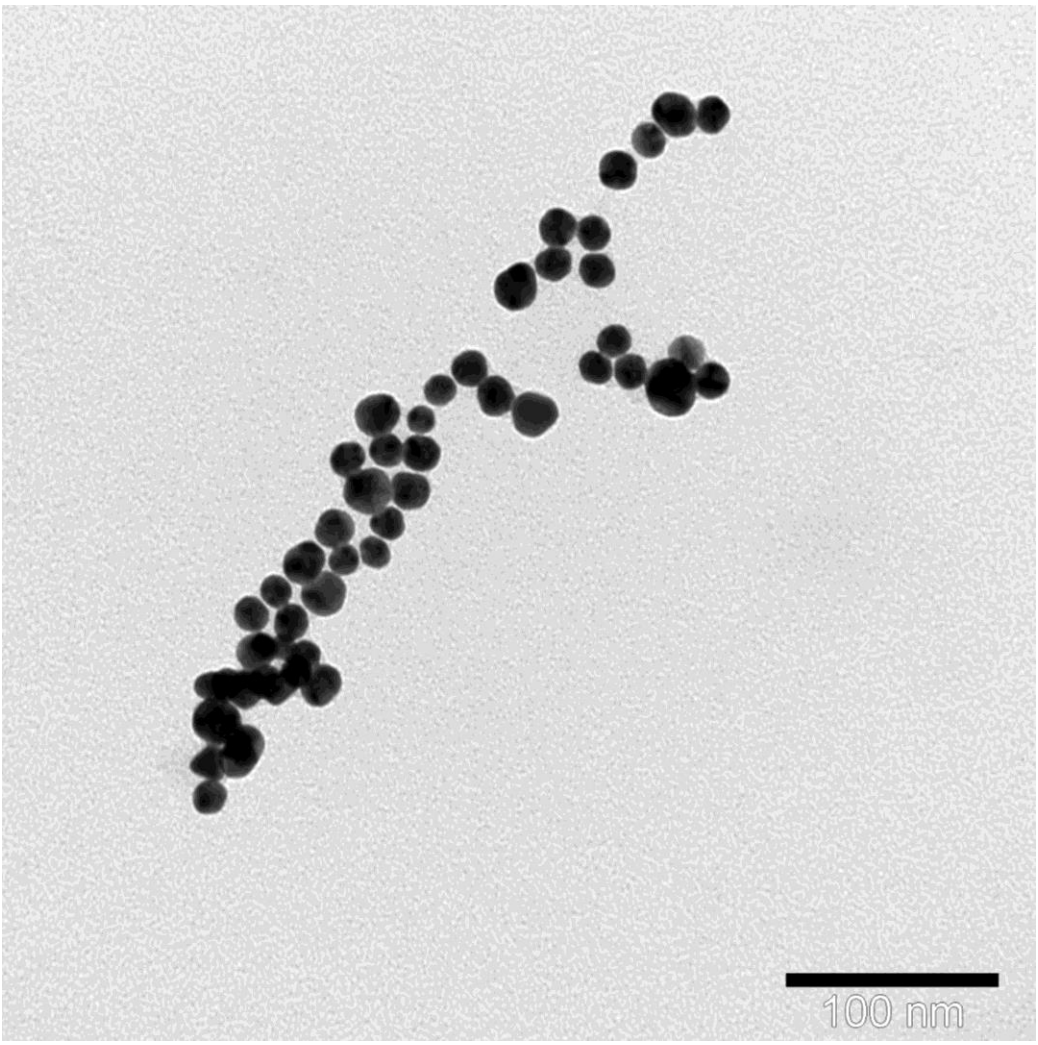

Figure S4. TEM image of the spherical gold nanoparticles in a 100 nm scale.

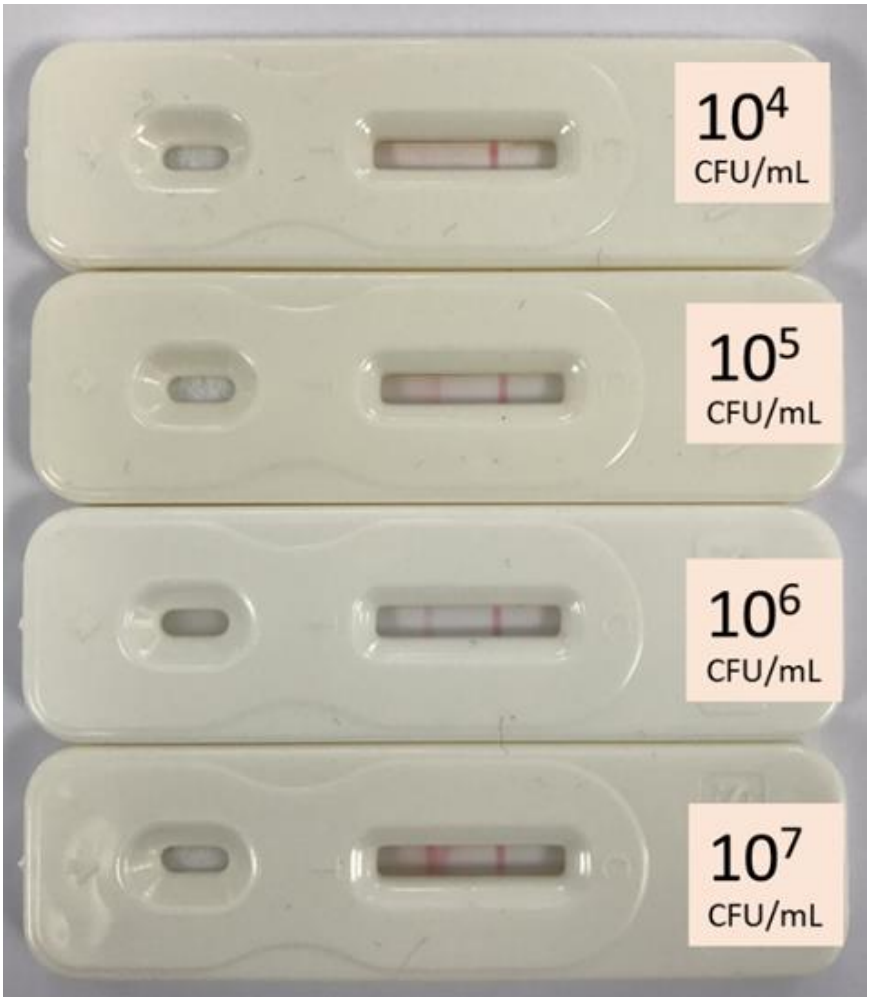

Figure S5. Detection of whole-cell *S.pyogenes* in the range of 10^4 – 10^7 CFU/mL with commercial test strips. Left lines for the test lines and the right lines for the control lines of LFIA.