Supporting Information

Detection of total count of *Staphylococcus aureus* using anti-toxin antibody labelled gold magnetite nanocomposites: a novel tool for detection, identification and bacterial separation

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Synthesis of Au seed stabilized Fe$_3$O$_4$ nanoparticles.

A 10 ml solution of EDBE-Fe$_3$O$_4$ (20 mg) suspension was first sonicated for 1 h using an ultrasonic probe with a pH 4 and then mixed with 20 ml of gold seed solution and stirred for 2 h. After 2 h of stirring, Au NPs could be electrostatically attracted onto the surface of EDBE-Fe$_3$O$_4$ NPs, leading to formation of the Au seed coated Fe$_3$O$_4$ particles as reported by Rose et al$^1$. The gold seed was synthesized by mixing of a 20 mL aqueous solution containing $2.5 \times 10^{-4}$ M HAuCl$_4$ and $2.5 \times 10^{-4}$ M trisodium citrate. To this solution was added 0.6 mL of ice cold 0.1 M NaBH$_4$ with stirring$^2$. The Au seed stabilized EDBE-Fe$_3$O$_4$ nanoparticles were magnetically separated from excess Au colloid solution and rinsed 5 times with Milli-Q water. The particle surfaces were then functionalized again with EDBE by dispersing 100 µl of EDBE solution into the gold seed stabilized EDBE-Fe$_3$O$_4$ for 4 h stirring and magnetically separated by rinsing 5 times and dispersing in 20 mL of Milli-Q water with an ultrasonic probe.

Characterizations of the nanocomposites by DLS and Zeta potential Analysis.

The Dynamic light scattering (DLS) and Zeta potential analysis of these Fe$_3$O$_4$-NH$_2$ and Au-Fe$_3$O$_4$ nanoparticles were further performed to monitor the formation of gold immobilized magnetite nanoparticles. After functionalization with EDBE, the particle size and zeta potential of Fe$_3$O$_4$-NH$_2$ was observed as 150-170 nm and +32.2 mV (table S1) at pH5
indicating that after EDBE modification, magnetite nanoparticle achieved amenable NH₂
groups for further conjugation. After the formation of Au-Fe₃O₄ nanocomposites, the size
was found to be increased than the previously obtained size of Fe₃O₄-NH₂ (200-220 nm) as
well as the corresponding zeta of this gold-magnetite was decreased from highly positive
value to lower negative one. This indicates that the presence of gold layer on the Au-Fe₃O₄
nanocomposites somewhat lowers the stability of these functionalized nanoparticles by
increasing the agglomeration tendency between these particles.

**Table S1.** Hydrodynamic (HD) size, PDI and zeta potential of the EDBE-Fe₃O₄, Au-EDBE-
Fe₃O₄, Antibody-Au-Fe₃O₄

<table>
<thead>
<tr>
<th>Nanoparticles</th>
<th>Size (nm)</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDBE-Fe₃O₄</td>
<td>160</td>
<td>+27.4</td>
</tr>
<tr>
<td>Au-EDBE-Fe₃O₄</td>
<td>220</td>
<td>-21.5</td>
</tr>
<tr>
<td>Antibody-Au-Fe₃O₄</td>
<td>315</td>
<td>-7.7</td>
</tr>
</tbody>
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After antibody modification of MPA-Au-Fe₃O₄ nanocomposites, the particle size and
surface charge of these nanoparticles was checked by DLS and Zeta potential analysis. This
results indicates that the increase in size and decrease in ζ was due to the successful
modification of the nanocomposites by anti-toxin antibody which significantly exhibit
particle agglomeration reflecting these values.
Fig. S1 EDX pattern of the Au-Fe$_3$O$_4$.

References
