Supplementary Material (ESI) for The Analyst

Electronic Supporting Information

for

Gold(III) enhanced chemiluminescence immunoassay for detection of antibody against ApxIV of *Actinobacillus pleuropneumoniae*

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The Choice of Dissolve Acid

The CLIA is based on the enhancement of $AuCl_4^-$ for the luminol-NaOH system. Therefore, it is the primary step to dissolve the gold nanoparticles from the gold nanoparticle-Rabbit anti-Pig IgG conjugate to $AuCl_4^-$. This was achieved with the use of HCl-NaCl-Br_2 solution, which was proved to be more efficient than other solution, such as HNO_3-HCl solution, NaCl-Br_2 solution and HCl-NaCl solution, and so on.

The conditions of gold dissolution with the use of HCl-HNO_3 have been studied, which is shown in Fig.S1 and Fig.S2. As the concentration of HCl increased, the signal/noise ratio increased between 0.25 M and 3 M, and then decreased in the range of 3 to 6 M HCl (Fig.S1). Hence, subsequent work employed 3 M HCl. Second, the signal/noise ratio increased when the concentration of HNO_3 was increased from 0.33 to 1 M, and then decreased quickly (Fig.S5). Thus, 1 M HNO_3 was selected for the following experiments.

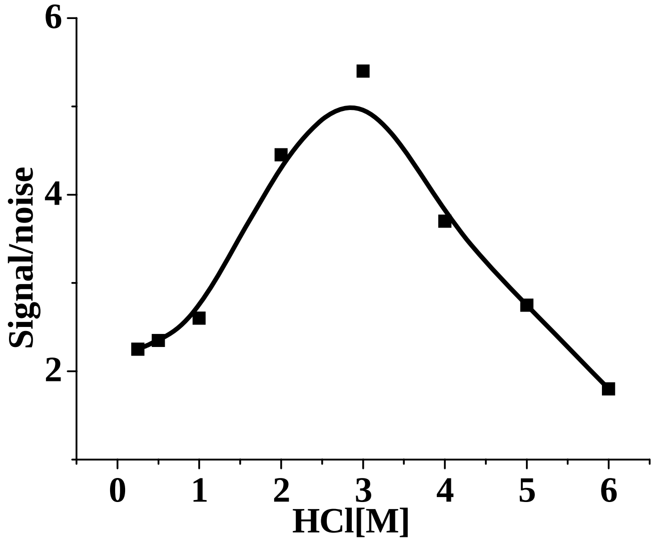

Fig.S1. Signal/noise ratio vs the concentration of HCl. Experimental conditions: 50 μL of 2.32 × 10^{-9} M gold nanoparticles (15 nm) was dissolved in 50 μL of HCl-HNO_3 solution (final concentration, different concentrations of HCl-1.0 M HNO_3), and then 90 μL of the resultant solution was injected into glass tubes containing 1.0 × 10^{-6} M luminal solution (dissolved in 0.1 M NaOH) for CL measurement.

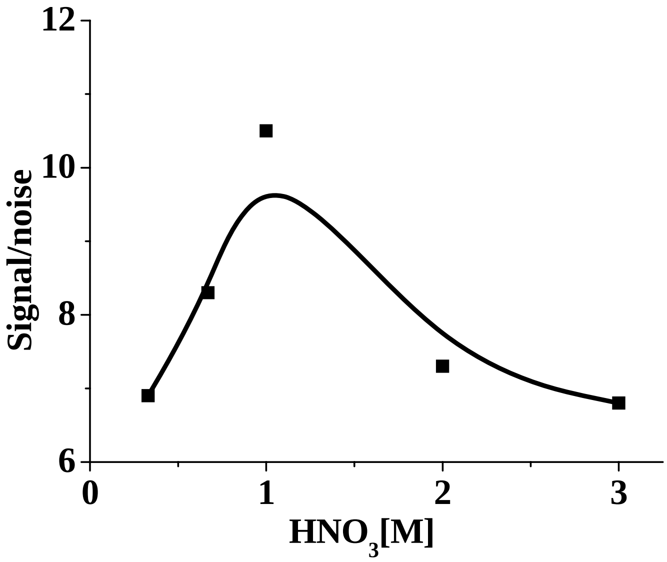

Fig.S2. Signal/noise ratio vs the concentration of HNO_3. Experimental conditions: 50 μL of 2.32 × 10^{-9} M gold nanoparticles (15 nm) was dissolved in 50 μL of HCl-HNO_3 solution (final concentration, different concentrations of HNO_3-3.0 M HCl), and then 90 μL of the resultant solution was injected into glass tubes containing 1.0 × 10^{-6} M luminal solution (dissolved in 0.1 M NaOH) for CL measurement.

The conditions of gold dissolution with the use of HCl-NaCl-Br_2 have been studied. First, as the concentration of HCl increased, the signal/noise ratio increased between 1.5×10^{-3} M and 1.2×10^{-2} M, and then maintained almost the same in the range of 1.2×10^{-2} to 1.0×10^{-1} M HCl (Fig.S3). Hence, subsequent work employed 5.0×10^{-2} M HCl. Second, the signal/noise ratio increased when the concentration of NaCl was increased from 3.7×10^{-3} to 1.5×10^{-2} M, and then decreased quickly (Fig.S4). Thus, 1.5×10^{-2} M NaCl was selected for the following experiments. Third, the signal/noise ratio increased when the concentration of Br_2 was increased and reached its maximum at 2.5×10^{-4} M. In the other hand, the signal/noise ratio decreased with increasing the concentration of Br_2 when it was higher than 2.5×10^{-4} M (Fig.S5). Thus, 2.5×10^{-4} M Br_2 was chosen for the following experiments.

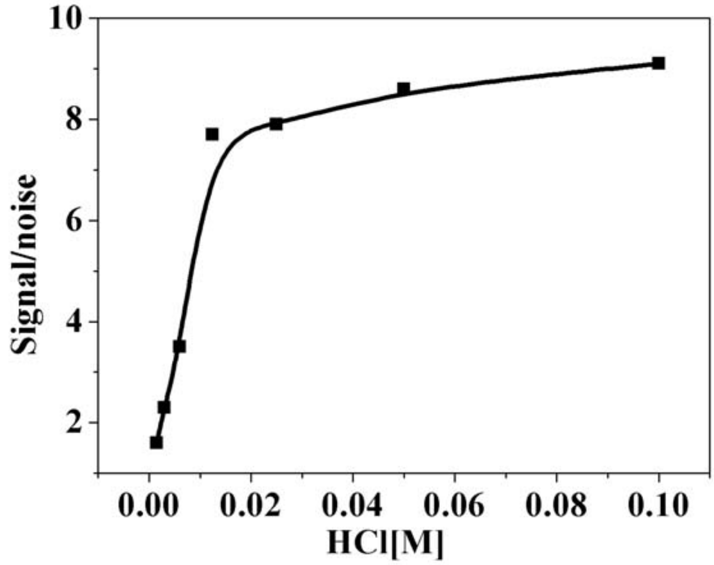

Fig.S3. Signal/noise ratio vs the concentration of HCl. Experimental conditions: 50 μL of 2.32×10^{-9} M gold nanoparticles (15 nm) was dissolved in 50 μL of HCl-NaCl-Br_2 solution (final concentration, different concentrations of HCl-0.5 M NaCl-1.0×10^{-4} M Br_2), and then 90 μL of the resultant solution was injected into glass tubes containing 1.0×10^{-6} M luminal solution (dissolved in 0.3 M NaOH) for CL measurement.

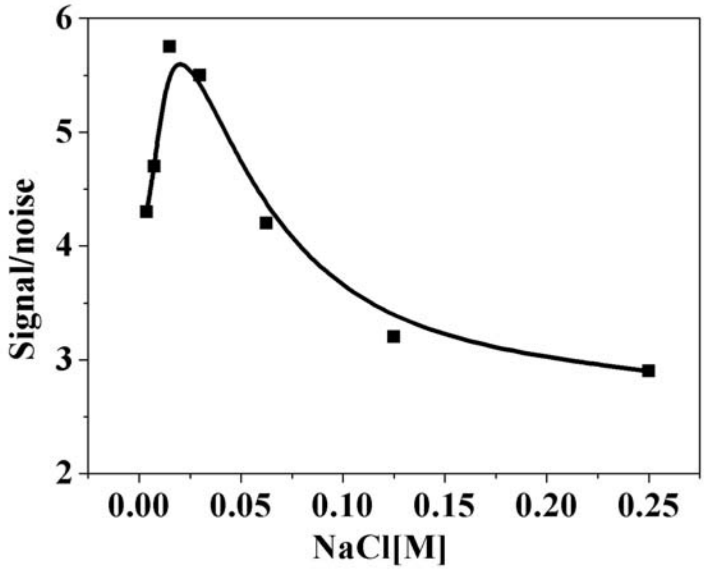

Fig.S4. Signal/noise ratio vs the concentration of NaCl. Experimental conditions: 50 μL of 2.32 × 10^{-9} M gold nanoparticles (15 nm) was dissolved in 50 μL of HCl-NaCl-Br_2 solution (final concentration, 5.0 × 10^{-2} M HCl-different concentrations of NaCl-1.0 × 10^{-4} M Br_2), and then 90 μL of the resultant solution injected into glass tubes containing 1.0 × 10^{-6} M luminal solution (dissolved in 0.3 M NaOH) for CL measurement.

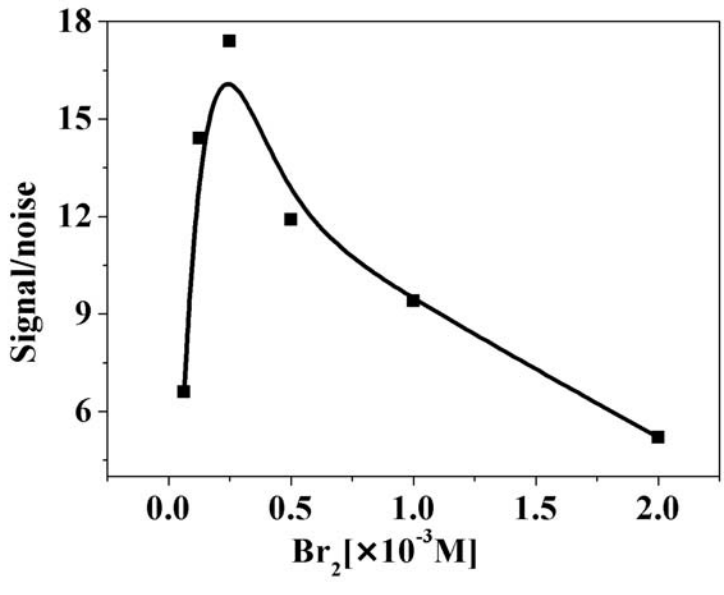

Fig.S5. Signal/noise ratio vs the concentration of Br_2. Experimental conditions: 50 μL of 2.32 ×

10^{-9} M gold nanoparticles (15 nm) was dissolved in 50 μL of HCl-NaCl-Br_2 solution (final concentration, 5 × 10^{-2} M HCl-1.5 × 10^{-2} M NaCl -different concentrations of Br_2), and then 90 μL of the resultant solution was injected into glass tubes containing 1.0 × 10^{-6} M luminal solution (dissolved in 0.3 M NaOH) for CL measurement.

The conditions of gold dissolution with the use of other acid have been studied, which is shown in Fig.S6. The HCl-NaCl-Br_2 solution is proved to be more efficient than other solution, so we choose HCl-NaCl-Br_2 solution to dissolve gold nanoparticles.

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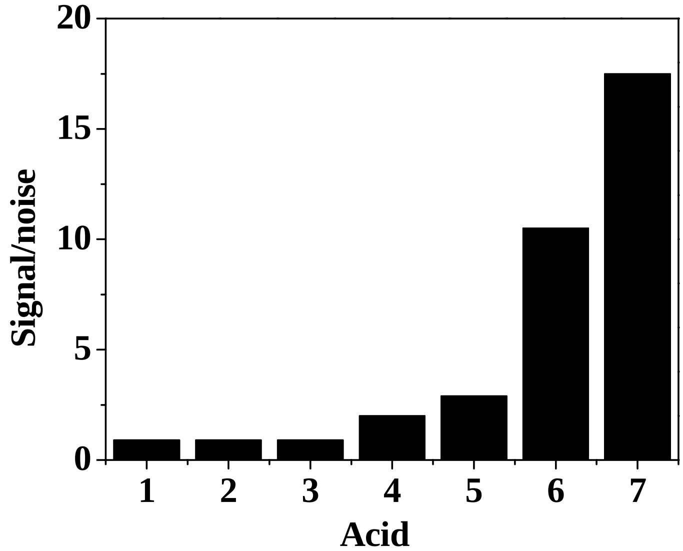

Fig.S6. Signal/noise ratio vs the kinds of acid. Experimental conditions: (1) 1M H_2SO_4; (2) 1M HNO_3; (3) 2.5 × 10^{-4} Br_2-5.0 × 10^{-2} M HBr; (4) 2.5 × 10^{-4} M Br_2-5.0 × 10^{-2} M HCl; (5) 3M HCl; (6) 1M HNO_3-3M HCl and (7) 5.0 × 10^{-2} M HCl-1.5 × 10^{-2} M NaCl-2.5 × 10^{-4} M Br_2

CL reaction conditions

CLIA for detection of antibody against ApxIV is based on the catalytic effect of $AuCl_4^-$ to alkaline luminol solution (initiating the CLIA emission) reaction. Therefore, the effect of the concentration of NaOH on the CL intensity was investigated over the range of 8.0 × 10^{-3} to 2 M. It was found that CL intensity reached a maximum value when NaOH concentration was 1.0 × 10^{-1}

M (Fig.S7). Thus, 1.0×10^{-1} M NaOH was selected for the following experiments. The effect of luminol concentration on the CL intensity was studied. The results showed that the CL signal/noise ratio increased between 5.0×10^{-9} M and 5.0×10^{-7} M with the increasing of luminol concentration, and then maintained almost the same in the range of 5.0×10^{-7} to 2.0×10^{-6} M luminol (Fig.S8). Hence, 1.0×10^{-6} M luminol was selected for subsequent work.

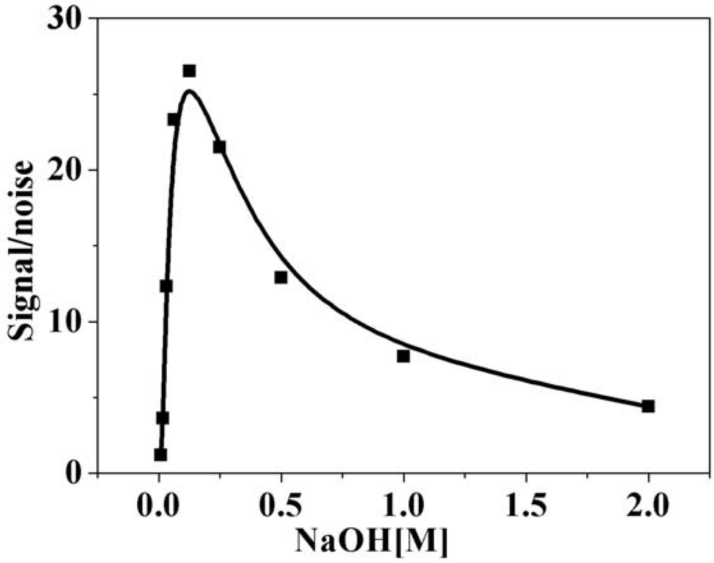

Fig.S7. Signal/noise ratio vs the concentration of NaOH. Experimental conditions: 50 μL of 2.32×10^{-9} M gold nanoparticles (15 nm) was dissolved in 50 μL of HCl-NaCl-Br_2 solution (final concentration, 5.0×10^{-2} M HCl-1.5×10^{-2} M NaCl-2.5×10^{-4} M Br_2), and then 90 μL of the resultant solution was injected into glass tubes containing 1.0×10^{-6} M luminal solution (dissolved in different concentration of NaOH) for CL measurement.

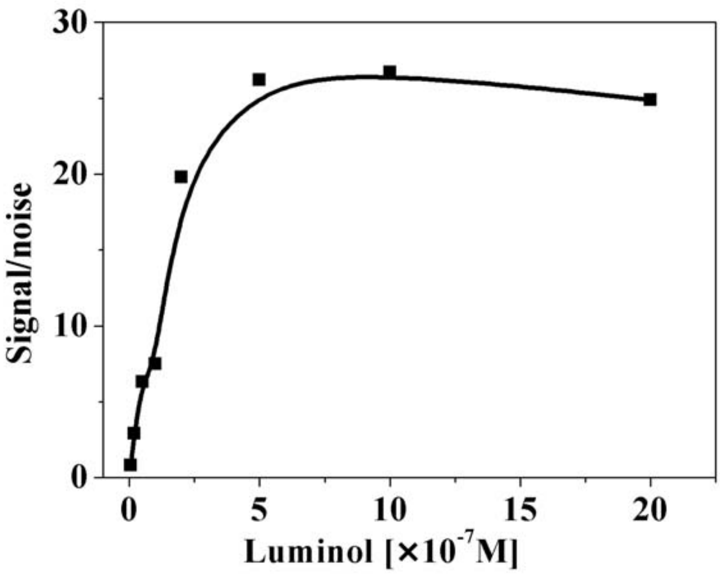

Fig.S8. Signal/noise ratio vs the concentration of luminol. Experimental conditions: 50 μL of 2.32 × 10^{-9} M gold nanoparticles (15 nm) was dissolved in 50 μL of HCl-NaCl-Br_2 solution (final concentration, 5.0 × 10^{-2} M HCl-1.5 × 10^{-2} M NaCl-2.5 × 10^{-4} M Br_2), and then 90 μL of the resultant solution was injected into glass tubes containing different concentrations of luminol (dissolved in 0.1 M NaOH) for CL measurement.