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-HNO₃ solution (final concentration, different concentrations of HCl-1.0 M HNO₃), 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 HNO₃. Experimental conditions: 50 μL of 2.32 × 10⁻⁹ M gold nanoparticles (15 nm) was dissolved in 50 μL of HCl-HNO₃ solution (final concentration, different concentrations of HNO₃-3.0 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-Br2 have been studied. First, as the concentration of HCl increased, the signal/noise ratio increased between $1.5 \times 10^{-3}$ M and $1.2 \times 10^{-2}$ M, and then maintained almost the same in the range of $1.2 \times 10^{-2}$ to $1.0 \times 10^{-1}$ M HCl (Fig.S3). Hence, subsequent work employed $5.0 \times 10^{-2}$ M HCl. Second, the signal/noise ratio increased when the concentration of NaCl was increased from $3.7 \times 10^{-3}$ to $1.5 \times 10^{-2}$ M, and then decreased quickly (Fig.S4). Thus, $1.5 \times 10^{-2}$ M NaCl was selected for the following experiments. Third, the signal/noise ratio increased when the concentration of Br2 was increased and reached its maximum at $2.5 \times 10^{-4}$ M. In the other hand, the signal/noise ratio decreased with increasing the concentration of Br2 when it was higher than $2.5 \times 10^{-4}$ M (Fig.S5). Thus, $2.5 \times 10^{-4}$ M Br2 was chosen for the following experiments.

Fig.S3. Signal/noise ratio vs the concentration of HCl. Experimental conditions: 50 μL of $2.32 \times 10^9$ M gold nanoparticles (15 nm) was dissolved in 50 μL of HCl-NaCl-Br2 solution (final concentration, different concentrations of HCl-0.5 M NaCl-$1.0 \times 10^{-4}$ M Br2), and then 90 μL of the resultant solution was injected into glass tubes containing $1.0 \times 10^{-6}$ 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-Br₂ solution (final concentration, 5.0 × 10⁻² M HCl-different concentrations of NaCl-1.0 × 10⁻⁴ M Br₂), 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 Br₂. Experimental conditions: 50 μL of 2.32 ×
10^9 M gold nanoparticles (15 nm) was dissolved in 50 μL of HCl-NaCl-Br2 solution (final concentration, 5 × 10^{-2} M HCl-1.5 × 10^{-2} M NaCl -different concentrations of Br2), 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-Br2 solution is proved to be more efficient than other solution, so we choose HCl-NaCl-Br2 solution to dissolve gold nanoparticles.

Fig.S6. Signal/noise ratio vs the kinds of acid. Experimental conditions: (1) 1M H2SO4; (2) 1M HNO3; (3) 2.5 × 10^{-4} Br2-5.0 × 10^{-2} M HBr; (4) 2.5 × 10^{-4} M Br2-5.0 × 10^{-2} M HCl; (5) 3M HCl; (6) 1M HNO3-3M HCl and (7) 5.0 × 10^{-2} M HCl-1.5 × 10^{-2} M NaCl-2.5 × 10^{-4} M Br2

CL reaction conditions

CLIA for detection of antibody against ApxIV is based on the catalytic effect of AuCl4^- 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}
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-Br₂ solution (final concentration, 5.0 × 10⁻² M HCl-1.5 × 10⁻² M NaCl-2.5 × 10⁻⁴ M Br₂), 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 \times 10^{-2} \text{ M HCl} - 1.5 \times 10^{-2} \text{ M NaCl} - 2.5 \times 10^{-4} \text{ 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.