Supporting information for

Bioluminescence assay for rapid detection of live *Staphylococcus aureus* based on the enrichment of egg yolk antibody modified magnetic metal organic framework immunobeads

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Materials and apparatus

All reagents are analytically pure. Iron (II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$, 99%), Iron (III) chloride tetrahydrate ($FeCl_3 \cdot 6H_2O$, 99%), Zirconium tetrachloride ($ZrCl_4$, ≥99.9%), 3-aminopropyltriethoxysilane (APTES), ammonium hydroxide solution (28 wt%), 2-aminoterephthalic acid (99.5%), 4-dimethylaminopyridine (DMAP), glutaraldehyde (50 wt%), Dimethylbenzene (≥ 99.9%), methanol (MeOH), ethanol (EtOH), N, N-dimethyl formamide (DMF), Mercaptoacetic acid (MAA, 98%) were obtained from Aladdin Industrial Corporation, Shanghai, China. Phosphate buffer solution (PBS), carbonate buffer solution (CBS, 1 $mol \cdot mL^{-1}$, pH 9.6), 0.1%sterile peptone broth, Tween 20, and bovine albumin (BSA, MW: 200,000) were purchased from Sigma-Aldrich (Shanghai) Trading Co., Ltd (Shanghai, China). The yolk antibody IgY (titer 1:10, 240 kDa) was obtained by our group by extracting antibodies from eggs with immunized SPF laying hens (SPF: animals without specific pathogens and reared in single cages).

The ATP bioluminescence sensor was purchased from Meicheng Biotechnology Co., Ltd (Ningbo, China). Fourier transform infrared (FT-IR) spectra were recorded on a Thermo Nicolet 6700 FT-IR spectrometer (Madison, USA). UV-Vis spectral analysis was conducted using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). The powder X-ray diffraction (XRD) pattern was analyzed on Bruker AXS D8 ADVANCE XRD (Karlsruhe, Germany). The transmission image was recorded on the JEM 2100F transmission electron microscope (TEM, JEOL, Tokyo, Japan). Surface morphology was analyzed by a scanning electron microscope (SEM, Hitachi SU-70, and Tokyo, Japan).

Preparation of egg yolk antibody IgY

The yolk antibody IgY was extracted by immunization from egg yolk [1]. SPF laying hens were first immunized with inactivated Staphylococcus aureus. Two months later, eggs from immunized hens were collected. The yolk was separated from the egg white and poured into a measuring cylinder. Dilute with 9 times the volume of acidified sterile deionized water (pH 5.0). Mix uniformly and leave at 4 °C for 12 h. Then, the solution was centrifuged at 15,000 rpm for 15 min to extract the supernatant. Afterwards, sodium sulfate was added to the aqueous supernatant solution to make a final concentration of 18 %. The solution was left at room temperature (25 °C) for 1 h and then centrifuged at 10,000 rpm for 15 min to obtain the precipitate. Finally, the precipitate was dissolved in 1 ml of phosphate-buffered saline (PBS). After dialysis and filtration, the antibody solution was obtained after 12 hours. The yolk antibody is then diluted according to the experimental requirements.

Bacterial culture and plate counting

Standard bacterial strains of *E. coli* (CMCC 44484), *S.A* (ATCC 43300), and *S.T* (ATCC 14028) were purchased from BIOBW Biotechnology Co., Ltd, (Beijing, China). *E. coli* and *S.A* were cultured according to the previously reported method (Zhang et al. 2021; Zhang et al. 2020). In summary, the strains were taken from -80°C storage, seeded in LB broth, and cultured overnight under constant shaking at 37 °C in a Stab S2 incubator (RADOBIO Scientific CO., Ltd., Shanghai, China) for ~12 h. Active strains were then selected and further transferred to new culture media. After a second incubation for ~10 h to achieve mid-exponential phase, the cell numbers were determined using the colony counting method. We prepared 10-fold diluted culture by mixing the 50 μL aliquots with 450 μL fresh LB broth. Five consecutive 10-fold dilutions were applied to the most recent diluted cultures. For each of these 10 to 10^6-fold diluted cultures, three 100 μL aliquots were placed on three LB agar plates (9.0 cm diameter). The plates were sealed with parafilm and incubated in the incubator at 37 °C. Then, the number of colonies on the overnight plates with 30-300 colonies was counted. The densities ($CFU \cdot mL^{-1}$) of the un-diluted cultures were calculated from the average colony counts and the magnitude of culture dilution. The cultures were then immediately diluted to achieve the desired cell concentrations for further use, or otherwise centrifuged to harvest the cells. All broth powders and agar plates were purchased from Hope Bio-Technology Co., Ltd., (Qingdao, China), and prepared with ultrapure water. The culture and plate counting of *P. rettgeri*, which was isolated from activated sludge by our research group (Shi et al. 2021), were the same as for *E. coli* and *S.A*. The *L. bulgaricus* strain was cultured and enriched with MRS broth (Apostolidis et al. 2007) according to the manufacturer's instructions. Other preparation methods for use were similar to the above statements.

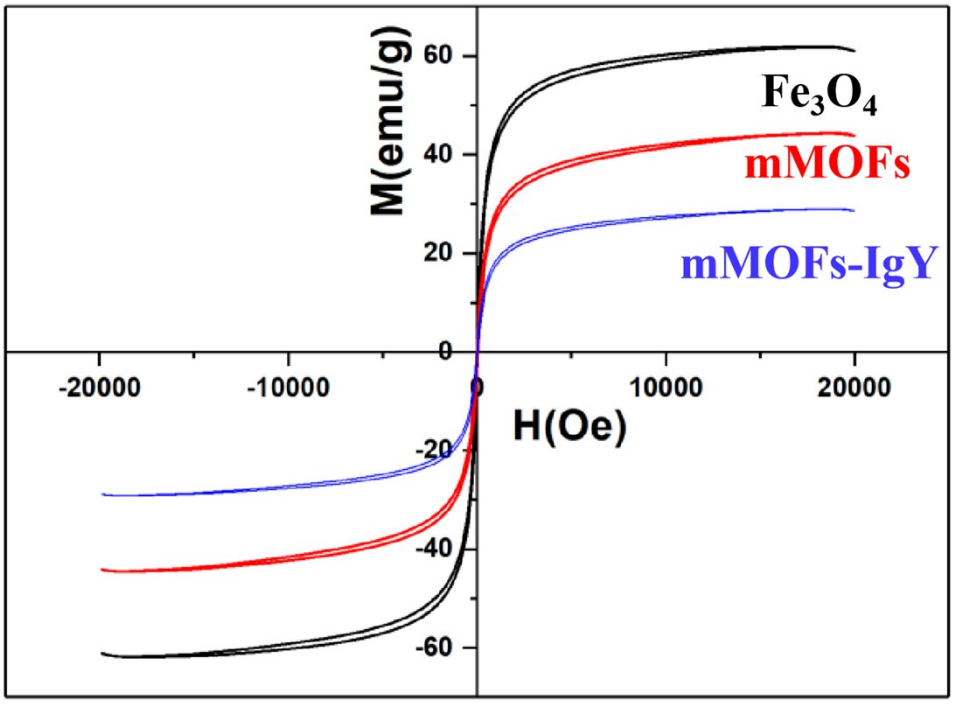

Fig.S1 The hysteresis curve of different magnetic material.

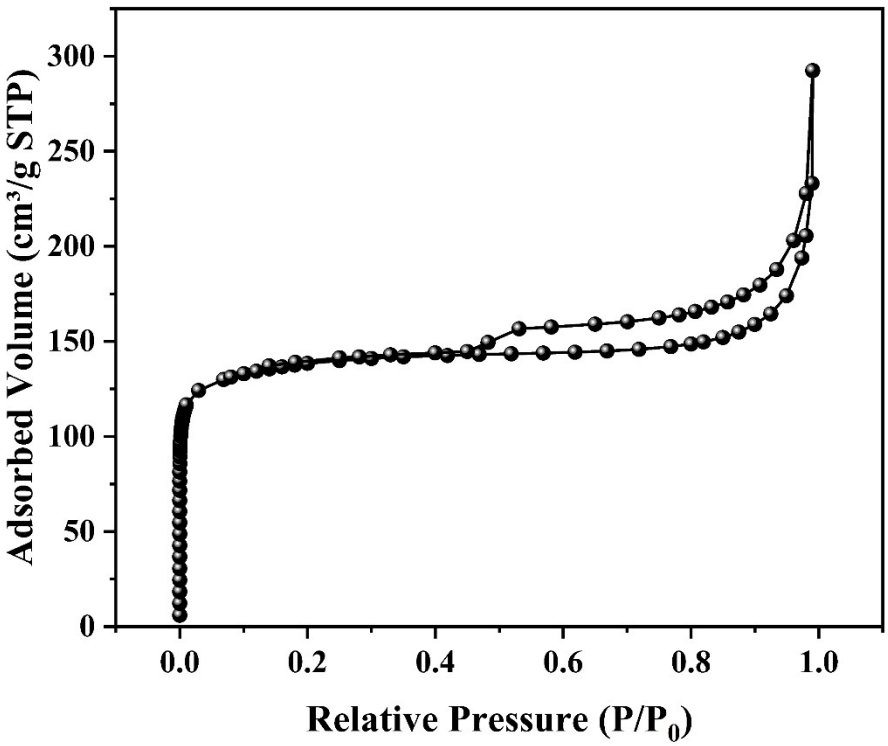

Fig.S2 porosity distribution of mMOF.

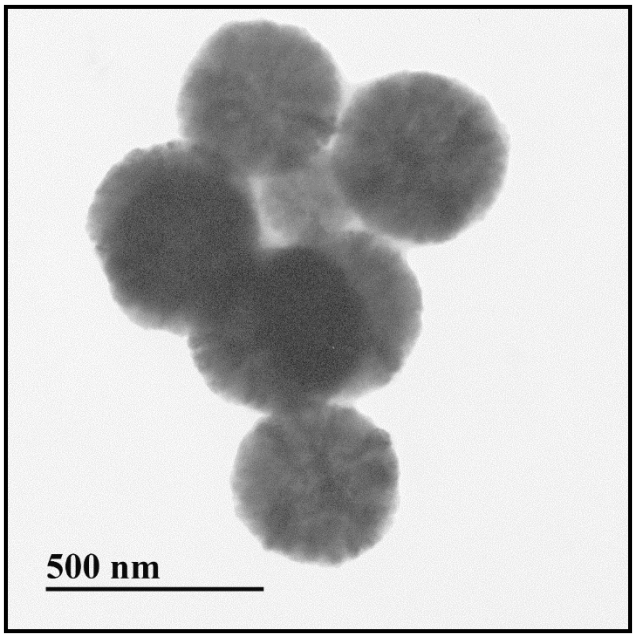

Fig.S3 TEM images of Fe_3O_4.

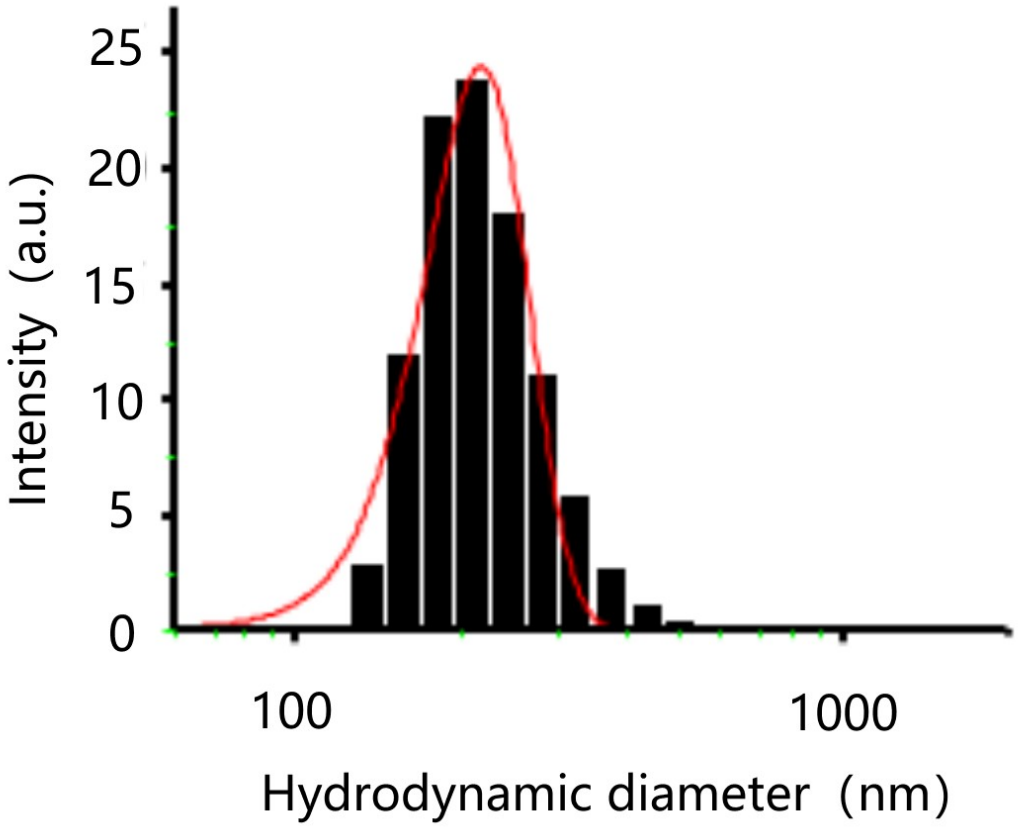

Fig.S4 The diameter distribution of mMOF-IgY.

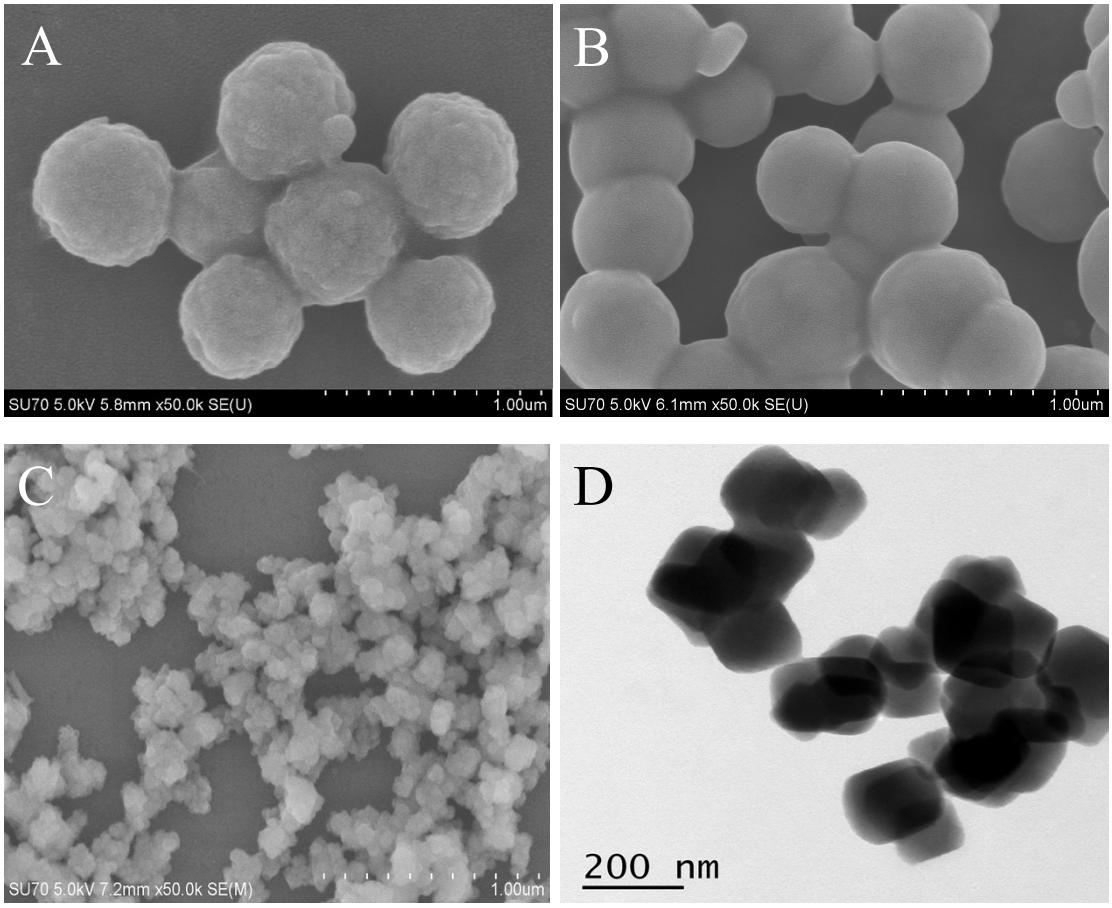

Fig. S5 SEM of (A) $mSiO_2$ and (B) $mSiO_2$-IgY; (C)SEM and (D) TEM of UiO-66-NH_2.

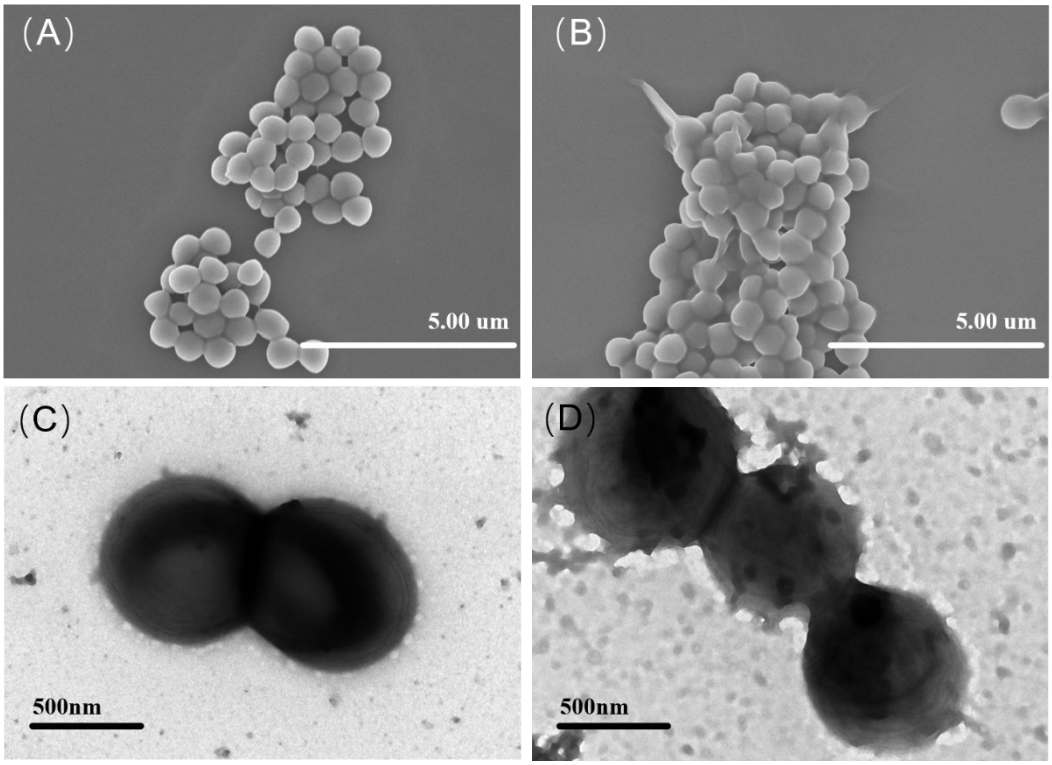

Fig.S6 (A) SEM and (C) TEM of *S.A*; (B) SEM and (D) TEM of *S.A* was incubated with IgY after1 h.

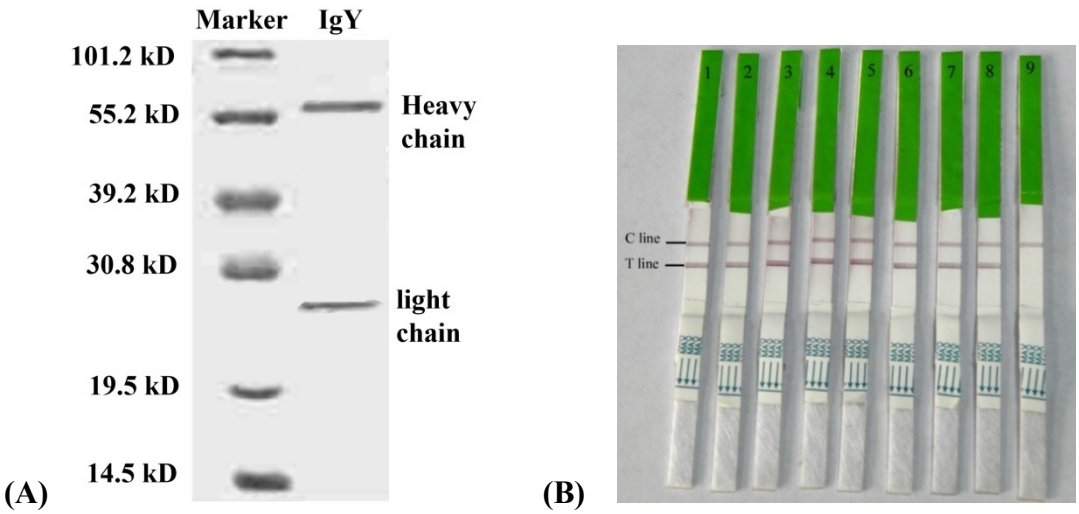

Fig.S7 (A) The electrophoresis gel of IgY (Marker: standard protein with different molecular weight, IgY: Two bands appeared from top to bottom, which was originated from heavy chain of IgY, and light chain of IgY in the presence of tris (2-carboxyethyl) phosphine (TCEP). The heavy chain was 56 kD that was ascribed to the Fc fragment and the light chain was 28.4 kD that was assigned to the Fab fragment.); (B) Specificity test of competitive immunochromatographic strip of *S.A* at different concentration (number 1-8 represents concentrations 10^{1}-10^{8} CFU·mL^{-1}, number 9 represents concentration 0 CFU·mL^{-1} as control);

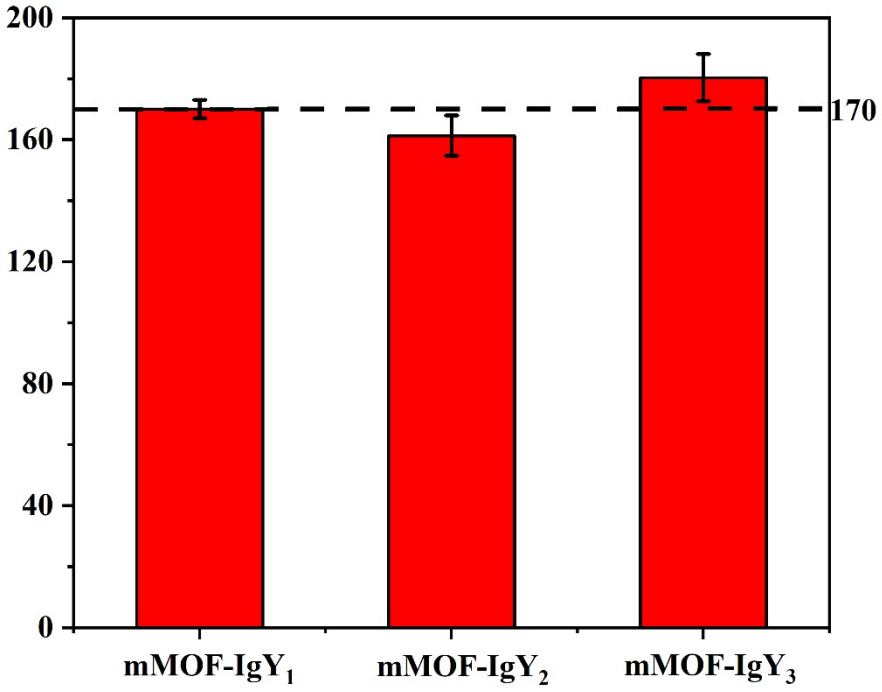

Fig.S8 The bioluminence light signal of by mMOF labeled with three batches of antibodies for capturing 10^3 CFU·mL^{-1} *S.A*.

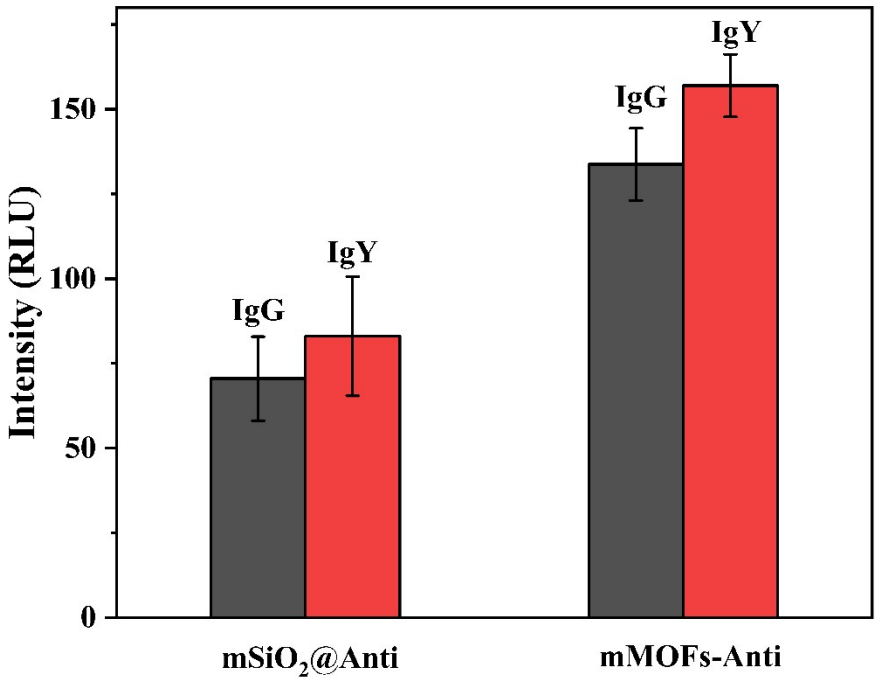

Fig. S9 Capture of *S. A* by IMB conjugated with different antibodies (IgG and IgY).

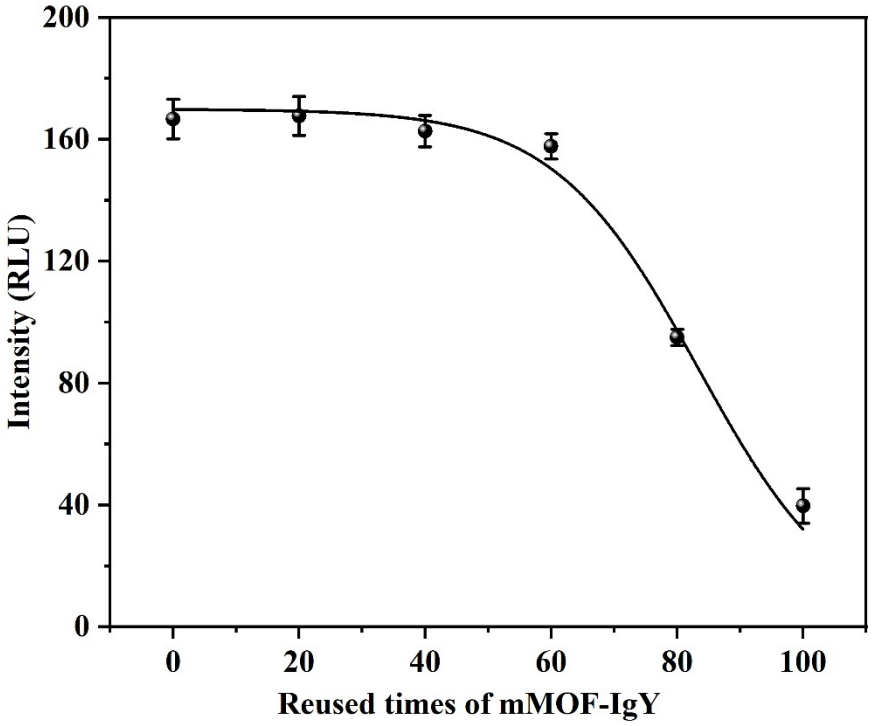

Fig. S10 The reusability of mMOF-IgY for detection of 10^3 CFU ·mL^{-1} *S.A*.

Fig. S11 Magnetic separation process (A) before and (B) after (C) Photographs of the standard on-site detection procedures.

References

[1] W. Gan, Z. Xu, Y. Li, W. Bi, L. Chu, Q. Qi, Y. Yang, P. Zhang, N. Gan, S. Dai, T. Xu, Rapid and sensitive detection of Staphylococcus aureus by using a long-period fiber grating immunosensor coated with egg yolk antibody. Biosens. Bioelectron. 199 (2022) 113860.