Supplementary Material

Integrating 3D-printed read-out platform with a quantum dot-based immunoassay for the Avian Influenza A (H7N9) virus

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1. Material and methods

1.1 Materials

Avian influenza virus H7N9 antigen were purchased from Harbin Weike Biotechnology Development Co., Ltd. IsoQuick™ Kits for Mouse Monoclonal Isotyping was purchased from Sigma-Aldrich (Wuhan, China). Myeloma cells (SP2/0) were purchased from Shanghai Cell Biology (Shanghai, China).75% alcohol and PEG1500 were purchased from Beijing Dingguo changsheng Biotechnology Co., Ltd. (Beijing, China). chloroauric acid ($HAuCl_4 .3H_2O$), bovine serum albumin (BSA), and trisodium citrate dihydrate were obtained from Sigma-Aldrich (Guangzhou, China).

1.2 Animal Immunity

A bottle of H7 antibody was dissolved in 500 μL saline and the isopyknic freund's complete adjuvant. The Balb/c mice (8 weeks old) were hypodermic injected respectively with 200 μL H7 antigen every two weeks. After 3 times injection, the antigen without adjuvant were injected into enterocoelia.

1.3 Cells fusion

The SP2/0 cells were chosen to prepare the cells suspension. Four days after booster immunization, the eyes of mice were excised to get the blood as positive serum. Mice were killed by cervical dislocation, placed in 75% alcohol for 10 min, and fixed on the clean bench with the abdomen facing up. And then, the spleen cells were taken out and ground to collect the spleen cells suspension. SP2/0 cells were mixed with the spleen cells at ration of 1:5 and fused in 1 mL PEG1500 at 37℃. The reaction ended by 30 mL incomplete medium. After the reaction, the mixed cells, were suspended in HAT medium and spread out on the feeder layer, cultivated in incubator with 60% CO_2 at 37℃. Until the medium became yellow or the cloned cells were distributed on the well with the area of over 1/10, 100 μL cell solution were used for antibody detection.

1.4 Hybridoma selection

The H7 antigen were used as the detection antigen, and the positive clones were detected by indirect ELISA and HI test. A positive hybridoma cells, named 6B3, was obtained from 3 subclones.

1.5 Ascites preparation and Titer detection

The Balb/c mice (10 weeks old) were injected respectively with 0.5 mL aseptic liquid paraffin into celiac. After a week, the hybridoma cells 6B3 in the logarithmic growth phase diluted with PBS were injected into celiac respectively with 5×10^5 cells. When the abdomen of the mouse was significantly upheaval, ascites were collected and centrifuged at 2500 g for 10 min, and then stored at −70° C. The titer of mouse ascites antibody was monitored by indirect ELISA (iELISA) and Hemagglutination inhibition (HI) assays. Subsequently, the subclasses of the antibody were identified by the method described in the manual for monoclonal antibody subclass kits. The results showed that the monoclonal antibody 6B3 is IgG1.

Cell Strain	HI titers	Antibody titer
6B3	2^{19}	1：509600

1.6 H7 monoclonal antibody purification

The monoclonal antibody ascites was purified by Protein G affinity chromatography

(AC). Before purification the volume of the ascites was 8mL. After purifying, 150 mg of monoclonal antibody was obtained. It means that each milliliter ascites has 18 mg monoclonal antibody, indicating that the selected hybridoma cells 6B3 has a strong ability of secreting monoclonal antibodies.

1.7 Preparation of mAb-AuNPs

Typically, 50 mL ultrapure water was heated to boil with magnetic stirring and 0.5 mL of 1 wt % $HAuCl_4$ solution was added. Subsequently, 1.0 mL of 1 wt % sodium citrate were introduced. The solution was heated for 10 min with vigorous stirring, the solution was cooled at 25°C. 1ml of AuNPs was adjusted to 8.5 with 0.25 M K_2CO_3, and then 8 μL of 1 mg. mL^{-1} AIV H7N9-mAb were added. The mixture incubated in a rotary mixer for 30 min. 100 μL of 100 mg. mL^{-1} BSA were used to block the surface of AuNPs. After reacted for 30 min, the excess reagents were removed via centrifugation at 11000 rpm/15 min. The precipitate was resuspended in 200 μL of 15 mM PBS buffer (pH 7.4), and stored at 4 °C for future use.

1.8 Fabrication of Colloidal gold immunochromatography strip for AIV H7N9 virus detection.

The Colloidal gold ICSs composed of sample pad, conjugation pad, NC membrane, absorption pad, and a plastic backing. The conjugation pad was prepared by dispensing a desired volume of mAb-AuNPs onto the pad using the dispense system, and dried at 37 ℃. 0.5 mg. mL^{-1} of AIV-mAb and mg. mL^{-1} of goat anti-mouse mAb was dispensed onto NC membrane as the test line (T-line) and control line (C-line) using the dispense system, and dried at 37 ℃. All of the parts were assembled on the plastic backing, and each part has an overlap of 2 mm. Finally, the Colloidal gold ICSs were cut to widths of 3.8 mm by a programmable HGS201 strip cutter and placed in a plastic case for later use. The gray values of the T-line signals obtained by Image J.

1.9 The performance of Colloidal gold immunochromatography strip for AIV H7N9 virus detection.

100 μM containing a series concentration of AIV H7N9 virus was added to the sample pad. The dried mAb-AuNPs was suspends in water and migrated toward the absorption pad by capillary action. After 15 min, the image of Colloidal gold ICSs were

recorded using a mobile phone CCD camera.

Results

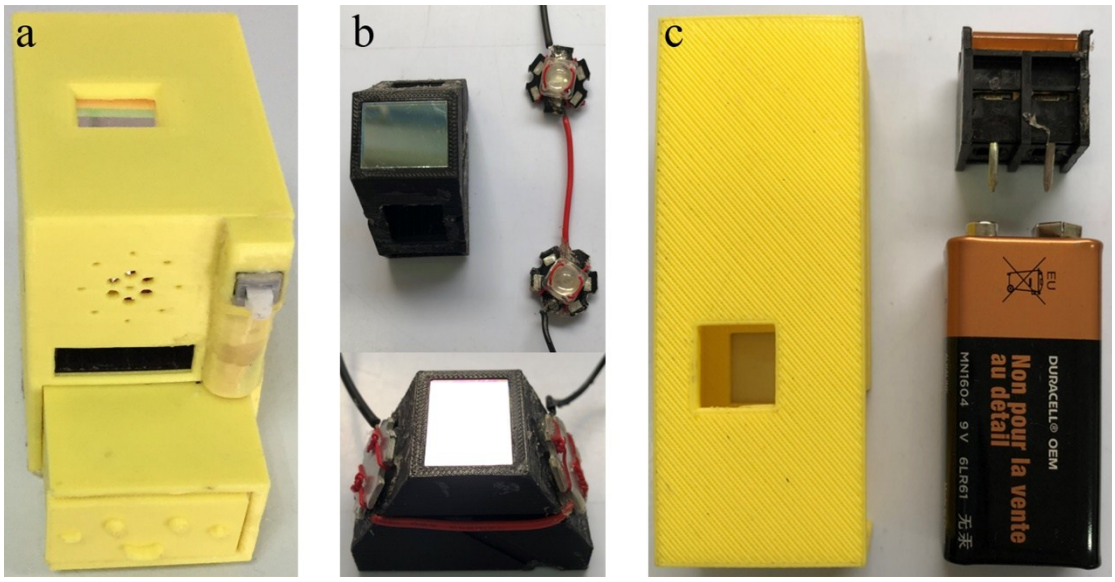

Figure S1. Schematic illustrations demonstrating (a) Scheme of 3D-printed read-out platform; (b) The composed of core component, included two LED lights and an optical filter; (c)The battery case consisted of lithium ion and a switch.

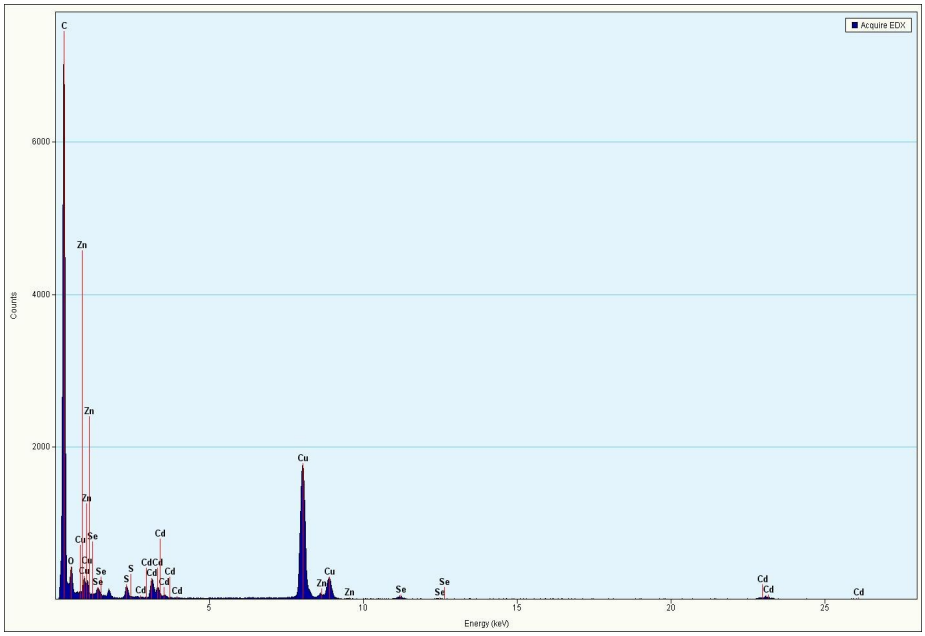

Figure S2. Composition of QDNBs by EDS analysis.

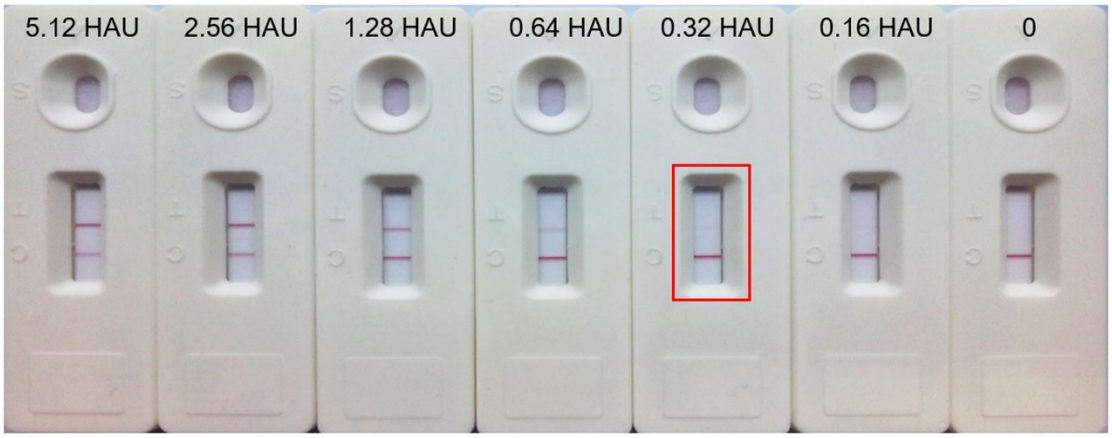

Figure S3. Images of Colloidal gold immunochromatography strip for different concentrations of AIV H7N9 virus detection.

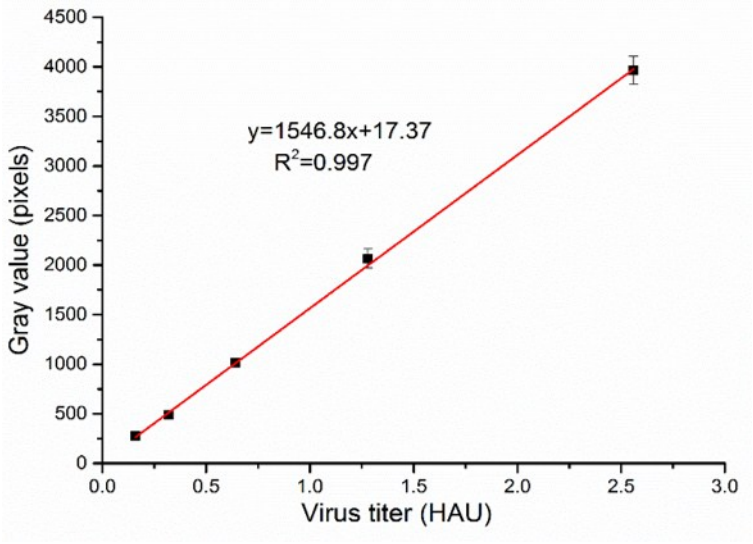

Figure S4. Calibration curve of Colloidal gold immunochromatography strip for AIV H7N9 virus detection. Each value presents the mean from 3 independent experiments (n=3).

Table S1. Intra-Assay and Inter-Assay Precision.

	Spiked concentrations (HAU)	Measured Value (Mean, HAU)	SD	CV (%)
Intra-Assay Precision	0.2	0.192	0.0145	7.25
Inter-Assay Precision	0.2	0.187	0.017	8.5
Intra-Assay Precision	0.1	0.113	0.0063	6.3
Inter-Assay Precision	0.1	0.108	0.0096	9.6

Intra-Assay Precision	0.05	0.056	0.00435	8.7
Inter-Assay Precision	0.05	0.059	0.0064	12.8