



Rapid identification of A29L antibodies based on mRNA immunization and high-throughput single B cell sequencing to detect Monkeypox virus

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ABSTRACT

With the large number of atypical cases in the mpox outbreak, which was classified as a global health emergency by the World Health Organization (WHO) on 23 July 2022, rapid diagnosis of mpox and diseases with similar symptoms to mpox such as chickenpox and respiratory infectious diseases in the early stages of viral infection is key to controlling the spread of the outbreak. In this study, antibodies against the monkeypox virus A29L protein were efficiently and rapidly identified by combining rapid mRNA immunization with high-throughput sequencing of individual B cells. We obtained eight antibodies with a high affinity for A29L validated by ELISA, which were used as the basis for developing an ultrasensitive fluorescent immunochromatographic assay based on multilayer quantum dot nanobeads (SiTQD-ICA). The SiTQD-ICA biosensor utilizing M53 and M78 antibodies showed high sensitivity and stability of detection: A29L was detected within 20 min, with a minimum detection limit of 5 pg/mL. A specificity test showed that the method was non-cross-reactive with chickenpox or common respiratory pathogens and can be used for early and rapid diagnosis of monkeypox virus infection by antigen detection. This antibody identification method can also be used for rapid acquisition of monoclonal antibodies in early outbreaks of other infectious diseases for various studies.

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KEYWORDS Mpox detection; mRNA immunization; high-throughput single B cell sequencing; antibodies; immunochromatographic assay

Introduction

Mpox is a zoonotic infection caused by the monkeypox virus (MPXV). The first detection of the infection in humans was in 1970 [1], and it was classified as a global health emergency by the WHO on 23 July 2022 [2]. As of 19 December 2023, a total of 92,783 cases of monkeypox virus infections have been confirmed worldwide since 1 January 2022, with 171 deaths [3]. Monkeypox virus infection may have an incubation period of 5–21 days and present with non-specific symptoms such as fever, flu-like symptoms, and lymph node symptoms early in the course of the disease, and a skin rash on the face and body within 1–5 days of fever. It is easily misdiagnosed as other diseases, such as chickenpox and COVID-19, which makes the risk of hidden transmission of MPXV high. The main routes of human-to-human transmission are respiratory droplet transmission [4], body fluids, and direct contact with skin lesions and crusts [4–6]. Identification of monkeypox virus infection by a number of means is important for treating

and preventing its mass transmission [7–11]. The common method for monkeypox virus diagnosis is nucleic acid detection from swab specimens taken from patient rashes, lesions, and scabs [12]. Although the accuracy of monkeypox virus nucleic acid testing is high, a professionally equipped laboratory and technicians are needed, which is not available in some MPXV-infected areas [13]. Furthermore, as the early symptoms of some patients may only be fever, weakness, or headache [14, 15], swab samples of rashes, lesions, and crusts cannot always be obtained, and therefore, establishment of a highly sensitive pharyngeal swab antigen detection method is needed for the rapid diagnosis of mpox before the rash appears [16]. Immunochromatography assay (ICA) has the advantages of rapidity, accessibility, and easy operation for rapid diagnosis of viral infections [17]. Since traditional ICA (AuNP) based colorimetric analysis has the disadvantages of limited sensitivity and quantitative ability [18, 19]. In recent years, a luminescent material named quantum dots (QDs) has been introduced into

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the ICA system to replace colorimetric labels by its advantages of high stability, narrow fluorescence emission spectra, and strong luminescence intensity, and it can provide strong and quantifiable fluorescence signals for ICA [20]. The fluorescent ICA established by adsorbing three layers of carboxylated quantum dots on the surface of monodispersed SiO₂ prepared by silica QD nanocomposites (SiTQD) as a pre-signal probe may improve the sensitivity of ICA, which is a method that may be used for highly sensitive and rapid detection of viral samples [19].

High sensitivity and specificity of antibodies are required for in vitro antigen diagnosis [21]. Traditional monoclonal antibody identification protocols usually involve protein immunization to stimulate the production of specific B cells in mice, which in turn fuse with myeloma cells to produce hybridoma clones. However, there are some limitations of this method, such as the protein purification process required to immunize mice is time-consuming; purifying some proteins can be difficult; and the natural conformation of the protein may be altered by adjuvants [22–24]. Non-specific fusion during the fusion of B cells with myeloma is present and may result in the loss of B cells that produce high-affinity antibodies [25]. With the maturation of mRNA technology, complex protein purification processes can be avoided by mRNA in vivo expression of antigenic proteins, which has been shown

to induce a positive humoral immune response [26–28]. Therefore, production of monoclonal antibodies by immunizing mice with mRNA could avoid some of the shortcomings of recombinant proteins.

In this study, we innovatively combined rapid mRNA immunization with high-throughput sequencing of single B cells, thus establishing a rapid method for the identification of monkeypox virus A29L (a surface protein of monkeypox virus) monoclonal antibodies. Eight strains of antibodies were obtained that bind specifically to A29L. We also developed an ultra-sensitive fluorescent immunochromatographic method based on multilayer quantum dot nanobeads as a monkeypox virus A29L pharyngeal swab diagnostic reagent (Figure 1).

Results

1. Preparation and characterization of the A29L mRNA-LNP

In this study, A29L mRNA [29] containing a 5' cap-1, 5'- and 3'-untranslated regions, and a 120 nt poly-A tail at the 3' (Figure 2A) was used. The mRNA was authenticated by an Agilent 2100 Bioanalyzer system (Supplementary Figure 1) and was shown to be correctly translated in cells by western blot analysis (Figure

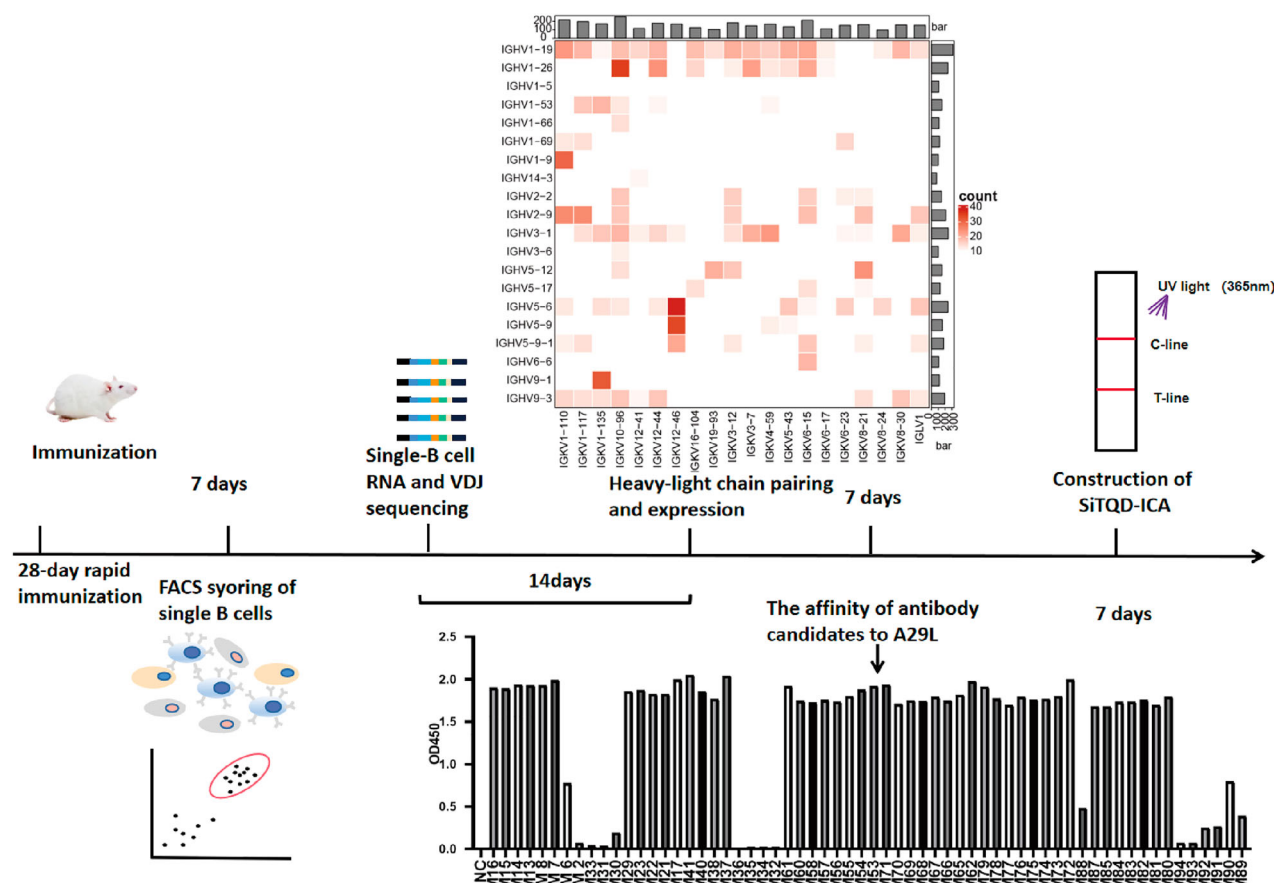


Figure 1. Schematic overview of the rapid screening for monkeypox virus A29L antibodies and antigen diagnosis.

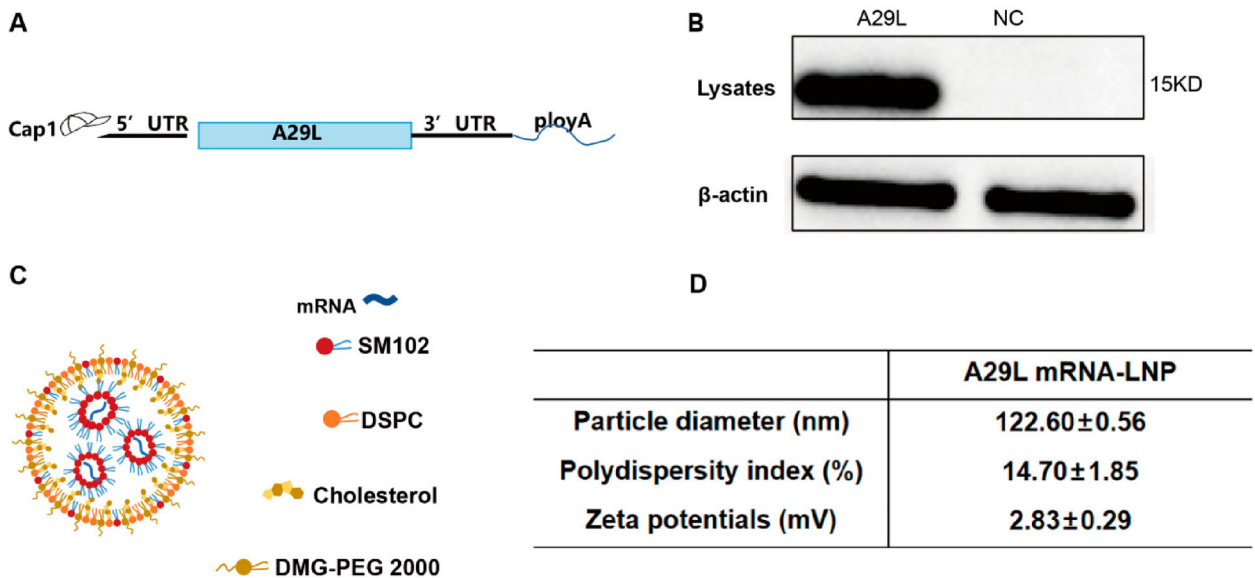


Figure 2. Design and characterization of mRNA for immunization. (A) Schematic of A29L mRNA, containing a 5' cap-1, the 5'- and 3'-untranslated regions (UTRs), an open reading frame (ORF), and a 120 nt poly-A tail at the 3' end. (B) The MPXV antigen A29L was expressed by mRNA in HEK293 T cells. Cells were transfected with A29L mRNA (1 μ g/mL) for 24 h using a Trans IT[®] mRNA Transfection kit. (C) Schematic representation of the LNP-encapsulated A29L mRNA. (D) The physicochemical parameters of A29L-mRNA-LNP.

2B). A29L mRNA-LNP were formulated as lipid nanoparticles by mixing A29L mRNA with four types of lipids for successful expression of A29L protein in vivo [30] (Figure 2C). The A29L mRNA-LNP showed an average particle size of 122.6 ± 0.56 nm and a potential of 2.83 ± 0.29 mV (Figure 2D).

2. Immunization and isolation of single B cells

To induce an immune response in mice, BALB/c mice were immunized every 2 weeks by intramuscular injection with 10 μ g A29L mRNA-LNP. Serum antibody titers were measured on day 10 after the second immunization. The OD value using ELISA was still >1 after a 10,000 dilution of the serum. The results showed that a strong humoral immune response was induced in BALB/c mice after the second immunization (Supplementary Figure 2), satisfying the conditions for single cell sorting.

Mice spleens were isolated on the third day after the third immunization, and single B cells with a BCR specifically recognizing A29L protein were isolated by fluorescence-activated cell sorting. A total of approximately 400,000 enriched single B cell IgG clonotypes from three BALB/c mice were sorted, and 40,000 of these cells were used for subsequent VDJ library and 5' gene expression library construction and sequencing.

3. Cell type identification and clonal expansion analysis of B cells

To assess the signatures of B-cell receptor diversity in B lymphocytes, single B cells isolated from spleens

with BCRs specifically recognizing the A29L protein were used for single cell RNA (scRNA) sequencing and single-cell BCR sequencing. As a result, a total of 11,605 B cells were obtained from the scRNA data after strict filtering, and 1027 variable regions of heavy chains and 328 variable regions of light chains were obtained from 811 clones. Then, we integrated the scRNA-seq and BCR information using the R package scRepertoire, and only the cells with productive paired heavy chains and light chains were retained. Finally, 9123 B lymphocytes were included for further analysis. We performed t-SNE clustering analyses on the obtained B cells and annotated each cluster according to the differential expression of canonical markers, yielding six cell types: follicular B cells, transitional B cells, GC-like B cells, marginal zone B cells, plasma cells, and B1/atMBC (Figure 3A, B). We then explored the clonal expansion pattern of each B cell subset. A cell was considered to be clonally expanded if its clonotype contained at least two cells. As shown in the t-SNE plot, clonally expanded B cells mainly belonged to PC and B1/atMBC (Figure 3C, D). Then, statistical processing of all clonotypes was performed. To further analyze the V gene (encoding variable regions of antibody) usage, we also determined an entire HV and KV gene pairing landscape for the top 20 germlines of HV (V region of the variable domain of IG heavy chains) genes and KV (V region of the variable domain of IG kappa light chains) genes from 2935 pairs in total (Figure 3E). Impressively, several germline combinations were used at a high frequency, such as IGHV5-6: IGKV12-46 and IGHV5-9: IGKV12-46. This suggested that these sequences were probably associated with the mpox

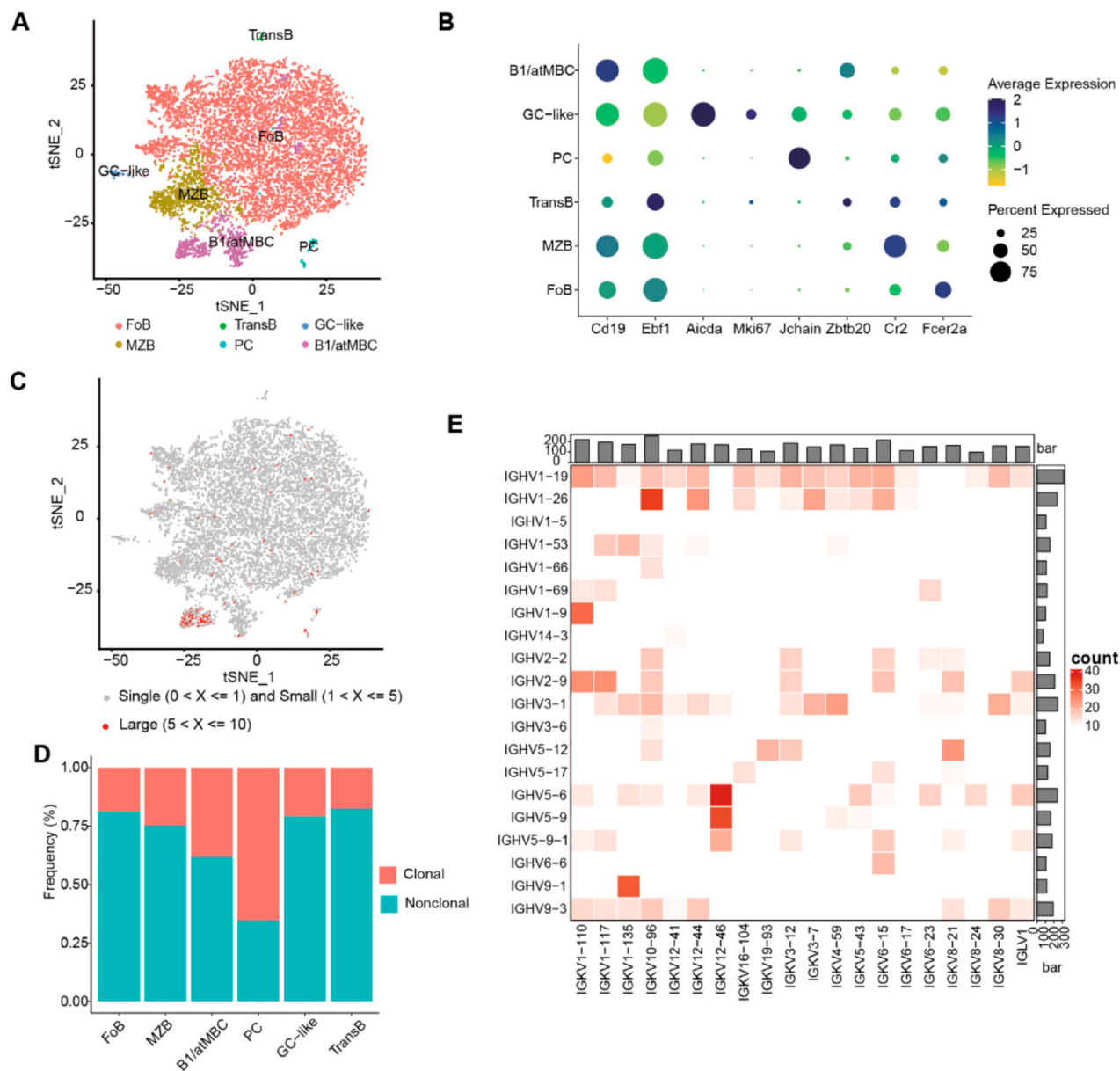


Figure 3. Features of the B cells. (A) The t-SNE projection shows the distribution of six types of B cells. Each dot represents a single cell, coloured according to cell clusters. (B) Dot plots of canonical cell markers across B cell clusters. (C) The t-SNE plot shows the distribution of clonally expanded cells, which are coloured as red. Only the cells with both heavy and light chains are plotted. (D) The proportion of clonally expanded B cells in each B cell cluster. (E) Heatmap showing the usage count of paired V gene combinations in the B cell repertoire.

infection and may have a high affinity for the monkey-pox virus. Thus, we selected a total of 62 BCR sequences from the high-frequency clonotypes for production for subsequent antibody screening.

4. Screening for highly sensitive antibodies

We obtained 62 antibodies at a sufficient concentration by B-cell subtype and VDJ library analysis, and reactivity of the antibodies against A29L was analyzed by ELISA. The results suggested that most of the antibodies showed positive responsiveness to A29L protein (Figure 4A). We selected eight antibodies that were highly positive and measured their affinity

for 29L protein at different concentrations (Figure 4B). All eight antibodies with low EC_{50} values were selected for a subsequent antibody pairing evaluation (Supplementary Figure 3).

To obtain the most sensitive antibody combination, the SiTQD-ICA for monkeypox virus detection was constructed and the performance of different antibody pairs was screened. The SiTQD-ICA included a sample pad, an absorbent pad, a conjugate pad containing immuno-SiTQD, a T-line for captured antibody, and a C-line encapsulated with goat anti-mouse IgG. When a test sample is added dropwise to the sample pad, the solution moves toward the absorbent pad by capillary action, rehydrating the immuno-SiTQD on the conjugate

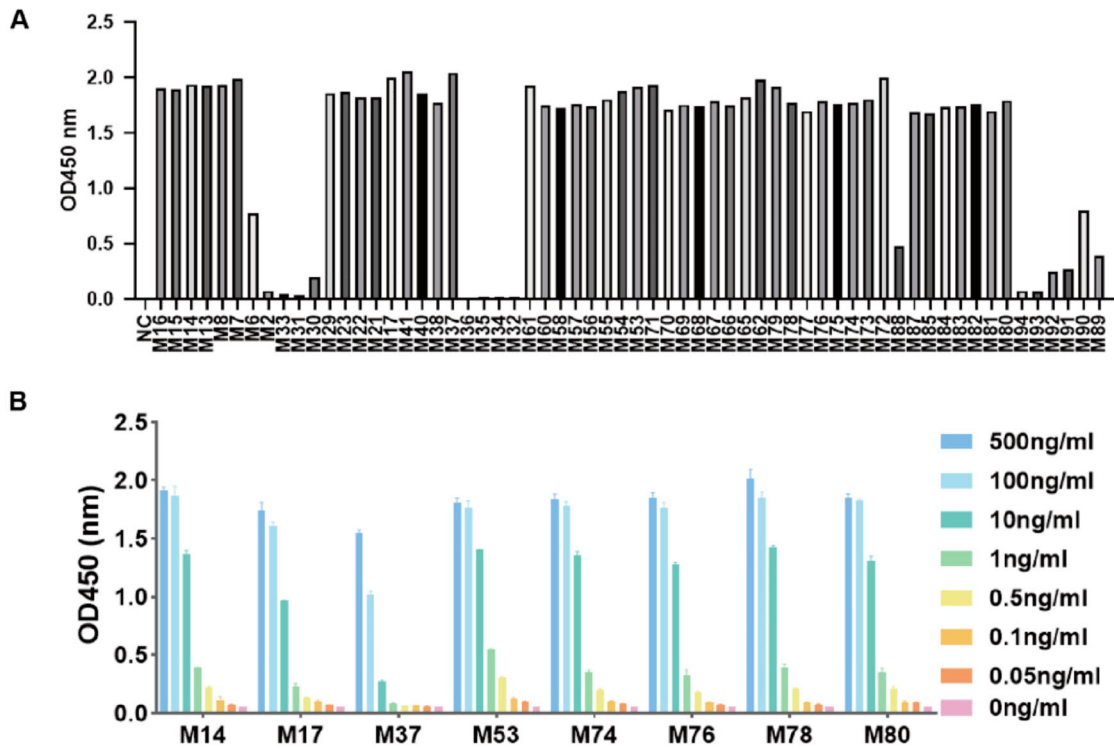


Figure 4. Screening antibodies against A29L. (A) The positive activity of 62 antibodies was preliminarily evaluated by ELISA in which plates were coated with recombinant A29L protein (100 ng/mL^{-1}). (B) Affinity of 12 antibodies with the highest OD values was preliminarily evaluated by ELISA in which plates were coated with recombinant A29L protein (100 ng/mL^{-1}).

pad and binding it to the monkey pox antigen protein. Upon reaching the T-line, the formed SiTQD-antigen immune complex is captured by the T-line and produces a detectable fluorescent signal (Figure 5A, B). Theoretically, the higher the concentration of the target virus, the higher the fluorescence intensity of the corresponding T-line. The excess immuno-SiTQD labels reach the C-line and are captured by goat anti-mouse IgG, thus establishing the validity of the assay.

Briefly, eight antibodies were immobilized on the SiTQD surface by carbodiimide chemistry as immuno-SiTQD labels, while eight antibodies were sprayed on the NC membrane as capture antibodies, constituting a total of 64 antibody pairings. The fluorescence intensity of the candidate antibody pairing for the detection of 1 ng/mL A29L protein is shown in Figure 5C. M53 and M78 were selected for subsequent experiments based on fluorescence intensity. In addition, the performance of M78 and M53 were also verified by WB (Supplementary Figure 4). Results showed that the A29L protein of monkeypox virus could be specifically recognized by mAbs M53 and M78.

5. Optimization of SiTQD-ICA performance

In this study, a SiTQD-ICA using M53 and M78 for monkeypox virus detection was constructed. Several key conditions related to biosensor performance

were optimized. The appropriate running buffer was optimized for its effectiveness at reducing non-specific adsorption of immuno-SiTQD labels²². The signal-to-noise ratio (SNR) was maximized with running buffer containing 1% Tween 20 and 0.5% bovine serum albumin (BSA) in PBS buffer (10 mM , $\text{pH } 7.4$; Supplementary Figure 5). We also optimized the amount of the SiTQD labels. The accompanying figure shows that the highest SNR was achieved with $2 \mu\text{L}$ SiTQD labels under the optimal running buffer conditions (Supplementary Figure 6). To further optimize the sensitivity of SiTQD-ICA, the concentration of the captured antibody loaded on the detection line was optimized, and the accompanying result (Figure 6A) showed that the fluorescence intensity increased with the increase of the T-line antibody concentration. The highest SNR was achieved at a T-line antibody concentration of 1.5 mg/mL (Figure 6B). In addition, the effect of reaction time on the assay was verified, and the results showed that the optimal reaction time was 20 min (Supplementary Figure 7).

6. Sensitivity of the SiTQD-ICA strips

Under optimal conditions, the detection performance of SiTQD-ICA was evaluated by testing a series of specimens containing different concentrations of monkeypox virus A29L protein. As shown in the results (Figure 6C), the fluorescence intensity of the

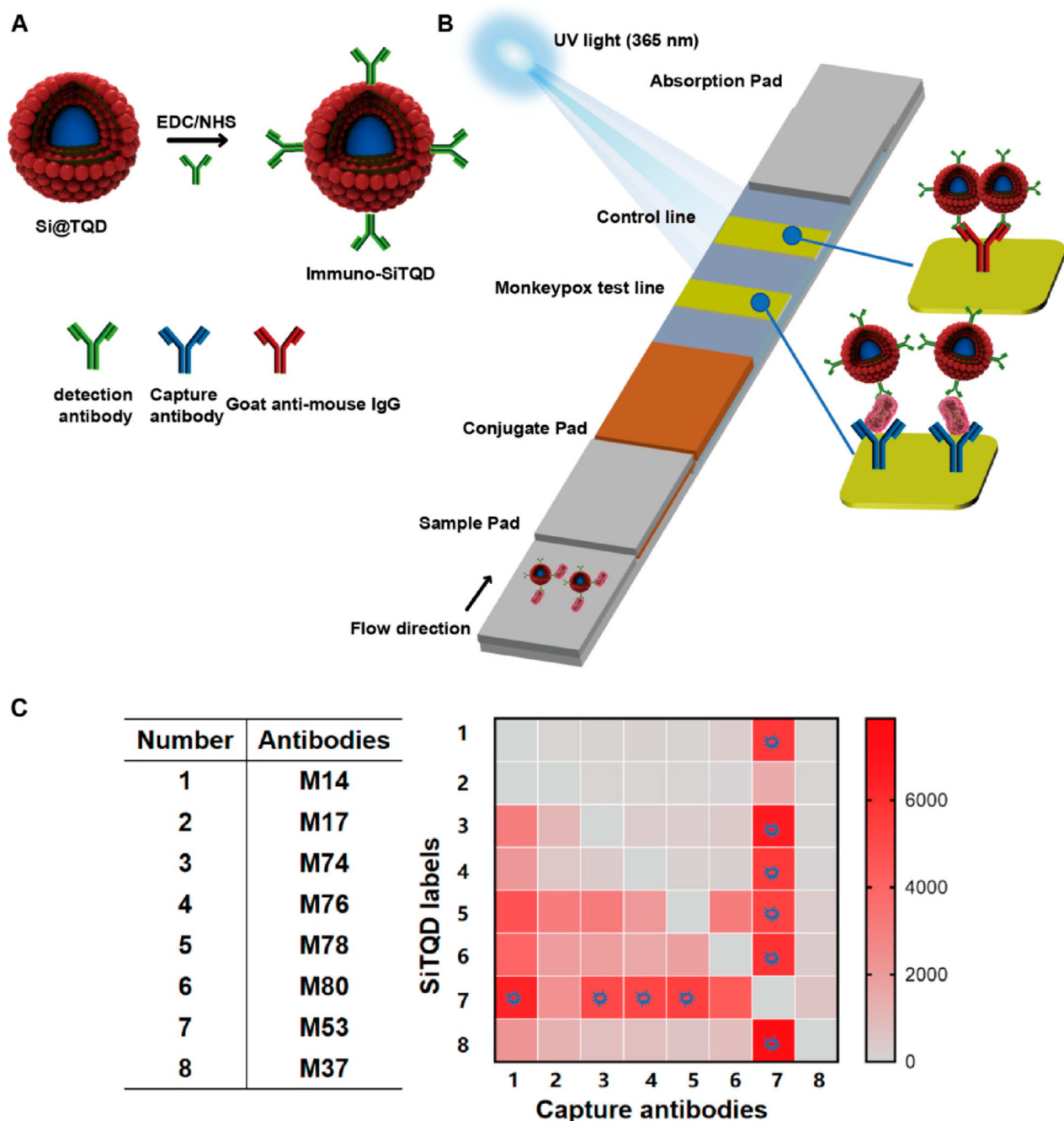


Figure 5. Schema of monkeypox virus detection by SiTQD-ICA. (A) Preparation of immuno-SiTQD probes. (B) Principle of the SiTQD-ICA strip for the detection of monkeypox virus. (C) The fluorescence screening of matching mAbs against A29L.

T-line increased with an increasing antigen concentration in the range of 0.001–10 ng/mL. The minimum antigen concentration at a positive T-line that could be observed by the naked eye was 5 pg/mL.

We achieved quantitative detection of monkeypox virus A29L protein based on the calibration curves for mpox A29L, which were plotted according to fluorescent ICA values (Figure 6D). The results showed that 5 pg/mL was the limit of detection (LOD) of the ICA for monkeypox virus A29L protein according to the IUPAC protocol [31] ($\text{LOD} = y_{\text{blank}} + 3 \times \text{SD}_{\text{blank}}$, where y_{blank} and SD_{blank} are the mean fluorescence intensity and standard deviation of the blank group, respectively). Notably, the fluorescence intensity

saturated at 10 ng/mL of A29L protein concentration, but this did not affect the qualitative detection.

We also used inactivated monkeypox virus added to throat swab samples from healthy volunteers to simulate mpox clinical samples. The results showed that our constructed SiTQD-ICA could detect monkeypox virus and 1×10^4 pfu/mL was the limit of detection (LOD) of the ICA for inactivated monkeypox virus (Figure 6E).

7. Specificity of the SiTQD-ICA strips

The specificity of SiTQD-ICA was analyzed for chickenpox virus and other common respiratory

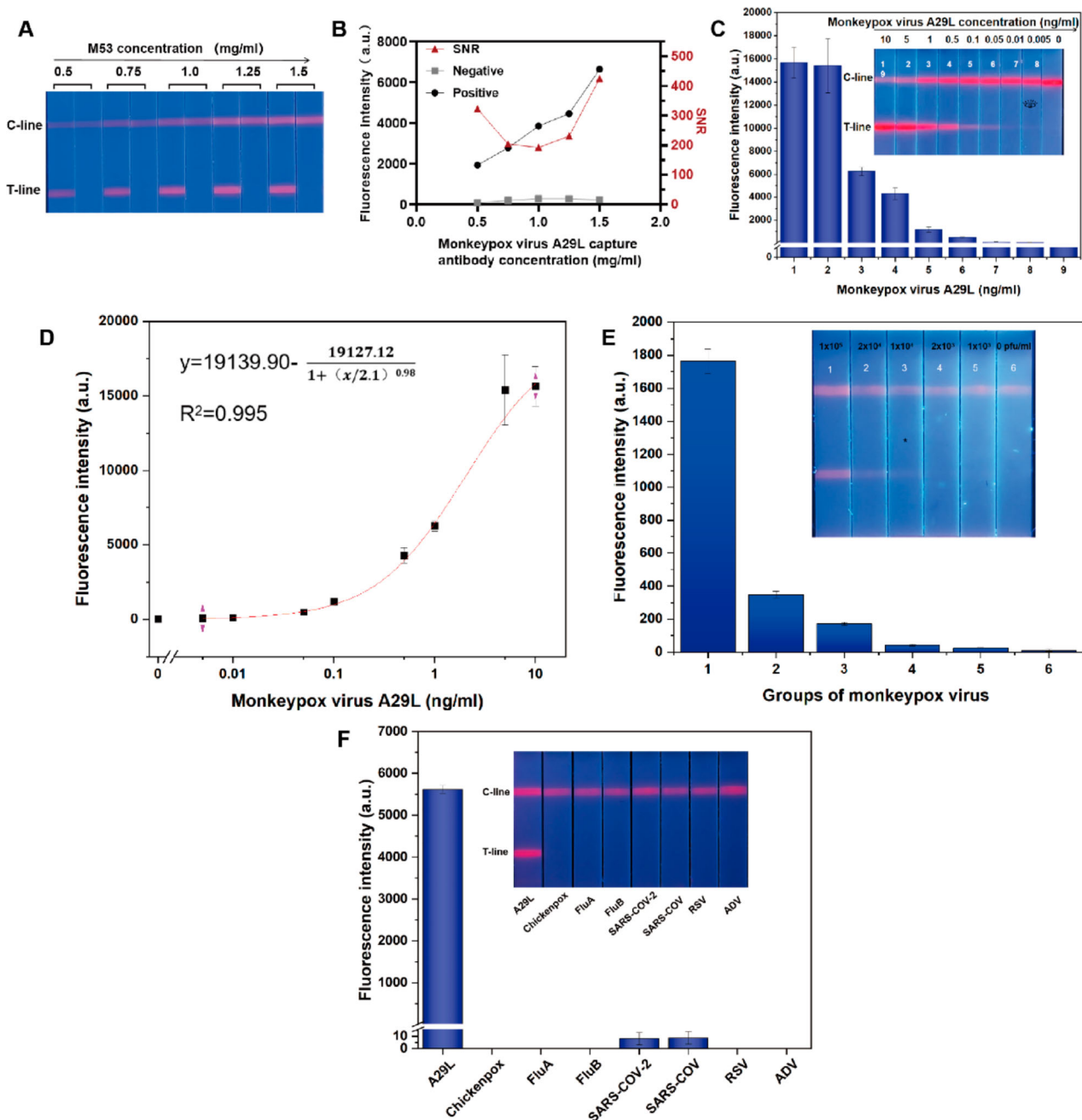


Figure 6. Detection performance of SiTQD-ICA strips. (A) Fluorescence image of different concentrations of capture antibody. (B) Fluorescence intensity and SNR of different concentrations of capture antibody. (C) Test line fluorescence of the SiTQD-ICA strip for monkeypox virus A29L antigen detection. (D) Calibration curves for the SiTQD-ICA strip for monkeypox virus A29L antigen detection. (E) Test line fluorescence of the SiTQD-ICA strip for monkeypox virus detection. (F) Specificity of SiTQD-ICA. All fluorescence intensity were obtained from triplicate wells and are expressed as the mean. Error bars = standard deviation ($n = 3$).

viruses that cause similar symptoms as early symptoms of mpox, including chickenpox virus, influenza A virus, influenza B virus, SARS-COV-2, SARS-COV, respiratory syncytial virus (RSV) and adenovirus (ADV). The results showed that all T-lines were negative, indicating that SiTQD-ICA could distinguish monkeypox virus from other common respiratory viruses or viruses that cause similar symptoms as mpox (Figure 6G). In addition, the reactivity of SiTQD-ICA with vaccinia virus was also analyzed and showed that SIQD-ICA did not bind to inactivated vaccinia virus (Supplementary Figure 8).

Discussion

In this study, M53 and M78 antibodies against the A29L protein were identified by using mRNA immunization and high-throughput single B cell sequencing. Then an SiTQD-ICA for monkeypox virus A29L pharyngeal swab diagnostic method was developed by using M53 as the capture antibody and M78 as the antibody label. The capture antibody concentration and response time were also optimized. The results showed that the antibodies were highly sensitive and specific in this application, with a minimum detection limit of 5 pg/

mL, and distinguished monkeypox virus from common respiratory viruses and chickenpox. This antigen detection method may be used for the initial diagnosis of suspected cases of mpox.

The rapid identification of high-performing monoclonal antibodies is critical for the preparation of antigen detection method in the event of an infectious disease outbreak. To shorten the immunization cycle, we designed and immunized mice every 14 days using mRNA of the monkeypox virus A29L protein instead of the recombinant protein, which avoided a complicated protein expression and purification process and induced a stronger immune response [32]. During the preparation of the fluorescence immunochromatography method, the sensitivity of the varying combinations of capture antibodies and antibody labels was different, and therefore, a large number of candidate antibodies were critical. To improve the efficiency of generating specific monoclonal antibodies, we used high-throughput single-cell sequencing of B cells and sequenced the scRNA as well as scVDJ of a large number of the B cells obtained. We performed lineage analysis to obtain a large number of candidate antibody sequences, which cannot be easily done with hybridoma and single-cell RT-PCR-based technique [33].

In conclusion, we successfully and rapidly identified antibodies specific to monkeypox virus A29L protein by combining mRNA immunization with high-throughput single-cell BCR sequencing to develop a highly sensitive pharyngeal swab antigen detection method. This antibody screening method can also be used for rapid acquisition of monoclonal antibodies in early outbreaks of other infectious diseases for various studies. In addition, the antibodies we identified can be used not only for diagnosis but also for subsequent therapeutic studies.

Materials and methods

1. Ethics statement

All animal studies were reviewed and approved by the Animal Experiment Committee of the Laboratory Animal Center, Academy of Military Medical Sciences (AMMS), China (Assurance Number: IACUC-DWZX-2022-576). All animal studies were conducted strictly in accordance with the guidelines set by the Chinese Regulations of Laboratory Animals and Laboratory Animal Requirements of Environment and Housing Facilities.

2. Cell and cell cultures

HEK293 T cells were cultured in Dulbecco's modified Eagle medium (DMEM; Thermo Fisher) supplemented with 10% fetal bovine serum (FBS; Thermo

Fisher) and penicillin (100 U/mL)-streptomycin (100 mg/mL) (Thermo Fisher). HEK293 T cell cultures were maintained in a 5% CO₂ atmosphere at 37°C.

3. Preparation and characterization of A29L mRNA

A29L mRNA was transcribed by linearizing the A29L DNA as a template, using the T7-FlashScribe™ Transcription kit (Cellscript). The mRNA was capped using a ScriptCap™ Cap 1 Capping System kit with a ScriptCap™ capping enzyme and 2'-O-methyltransferase (Cellscript) according to the manufacturer's instructions. The in vitro transcribed mRNA products were purified by ammonium acetate, redissolved in RNase-free water, and stored at -80°C. Quality control and concentration measurements were performed using an Agilent 2100 Bioanalyzer and an RNA Nano 6000 assay kit (Agilent) according to the manufacturer's instructions. IVT-A29L mRNA (1 µg) was transfected into HEK293 T cells using a Trans IT® mRNA transfection kit (Mirus, MIR 2250) according to the manufacturer's instructions. Lysate was collected, and western blotting was performed.

4. Preparation and characterization of A29L mRNA-LNP

A29L mRNA-LNP formulations were prepared using NanoAssemblr Ignite's (Precision Nanosystems) NxGen microfluidics technology. Briefly, mRNA was solubilized in 20 mM citrate buffer (pH 4.0) and then was mixed with lipids comprising ionized lipids, 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and DMG-PEG2000 dissolved in ethanol (with a molar ratio of 50:10:38.5:1.5). The mixture was then diluted through a 100k MWCO PES membrane (Sartorius Stedim Biotech) against a 10-fold volume of DPBS (pH 7.4) and then diluted to an appropriate concentration and stored at 4°C. The particle size and zeta potential were measured by Litesizer 500 (Anton Paar), according to the manufacturer's instructions.

5. Immunization of mice

In this study, BALB/c mice (6–8 weeks old, weighing 18–22 g, female, *n* = 3) were immunized by intramuscular injection of 20 µg of A29L-mRNA-LNP on day 0 and day 14. Serum antibody titers were measured 10 days after the second immunization. The mice were immunized again 3 days before the spleens were removed.

6. A29L-specific B cell sorting

The spleens of three booster-immunized mice were removed, and single cell suspensions were obtained by

grinding the tissue through a 300-mesh filter. B cells were enriched using an EasySep™ pan-B cell magnetic enrichment kit (STEMCELL). B cells were stained with a panel containing 5 µg of A29L-biotin for 30 min on ice in 1× PBS supplemented with 0.2% BSA, subsequently washed with 1× PBS with 0.2% BSA, and stained with SA-APC (Abcam) for 15 min on ice in 1× PBS supplemented with 0.2% BSA. They were then washed with 1× PBS with 0.2% BSA and stained with IgG-FITC for 15 min on ice in 1× PBS supplemented with 0.2% BSA. They were subsequently washed and resuspended in 400 µL of PBS, and 5 µL of 7AA was added prior to flow sorting. Cells (APC+/FITC+/7AAD−) were then collected in a PBS solution containing 50% FBS and used for downstream 10X Genomics analysis.

7. VDJ library construction

A B cell VDJ library and 5' gene expression library were constructed using a 10× Chromium system (10× Genomics), a Chromium Next GEM Single Cell 5' reagent kit v2 (dual index), and a Chromium Single Cell VDJ Amplification kit (mouse). All steps were according to the manufacturer's instructions. Sequencing was performed by Annoroad.

8. Single-cell RNA/BCR sequencing

Full-length BCR V(D)J segments were enriched from amplified cDNA from 5' libraries using a Chromium Single-Cell V(D)J Enrichment kit according to the official protocol. BCR sequences were assembled by the Cell Ranger VDJ pipeline (version 6.1.2)

9. Single-cell RNA analysis

The Cell Ranger VDJ pipeline (version 6.1.2) provided by 10× Genomics was used for sample demultiplexing, barcode processing, and single-cell 5' gene counting. The scRNA-seq data were aligned to the Ensembl genome GRCm38 reference genome. The unique molecular identifier (UMI) count matrix was integrated and processed using the R package Seurat (4.3.0) [34]. We filtered the cells according to the following criteria: (1) number of UMIs >800; (2) number of genes >500; (3) cells with UMI/gene numbers outside the limit of a mean value +2-fold standard deviation, assuming a Gaussian distribution of each cell's UMI/gene number; and (4) percentage of mitochondrial-expressed genes <10%. To visualize the data, we reduced the dimensionality of all cells and projected the cells into 2D space using t-distributed stochastic neighbour embedding (t-SNE): (1) the LogNormalize method was used to calculate the expression level of genes. (2) Principal component analysis was performed and the top 10 principal

components were used to calculate the first two components of t-SNE. (3) Weighted shared nearest neighbour graph-based clustering was used to find clusters. Each cluster was annotated by their marker genes, which were identified with default parameters via the FindAllMarkers function in Seurat. The clusters showing high expression of markers of two or more cell types were treated as doublets and removed. Finally, six subtypes of B cells were identified for further analysis.

10. Single-cell BCR analysis

Single-cell BCR sequencing data were analyzed with scRepertoire (version 1.6.0) following the official tutorial [35]. For each cell, BCR heavy and light chains were combined based on the cell barcodes. Only cells with both heavy chain and light chains (kappa or lambda) were retained for further analysis. If more than one heavy or light chain was detected in one cell, the chain with the highest number of unique molecular identifiers was retained [36]. A cell was considered to be clonally expanded if its clonotype contained at least two cells. Clones were categorized by the number of cells expressing individual clonotype sequences (single, 0–1 cells; small, 2–5 cells; and large, 6–10 cells).

11. Antibody–antigen affinity assay

The affinity of candidate antibodies for A29L protein was determined by ELISA. Briefly, A29L protein (Sinobiological) at a concentration of 100 ng/mL was placed in 96-well plates overnight at 4°C. Then, 62 antibodies were diluted to 1 µg mL^{−1} as primary antibodies and incubated at 37°C for 2 h. Then, goat anti-mouse IgG-HRP (Beyotime Biotechnology) as the secondary antibody was added and incubated at 37°C for 2 h. Selected antibodies were re-assayed by ELISA. Antibodies with greater sensitivities were selected for fluorescent immunochromatographic assays.

12. Preparation of immuno-SiTQD labels

The quantum dot (QD) nanobead was provided by our laboratory [19]. The A29L antigen-detecting antibody was conjugated with silica-QD nanocomposite with triple QD shell (SiTQD) through carbodiimide chemistry. Briefly, 1 mL of SiTQD NPs were separated from ethanol and then activated for 15 min by sonication in 500 µL 0.1 M MES buffer (containing 1 mM EDC and 2 mM sulfo-NHS). After activation, SiTQD NPs were centrifuged (4,500 rpm, 6 min) to remove the supernatant and then redissolved in 0.2 mL of PBS (0.1 mM, pH 7.4). Then, 15 µg of candidate antibodies was reacted with SiTQD NPs for 2 h.

The SiTQD NP surface was blocked by adding 10% BSA for a 1-h incubation. Finally, the immune SiTQD markers were collected by centrifugation (4200 rpm, 6 min), washed once with PBS, and then dispersed in 1 mL of PBS buffer containing 0.5% sucrose (w/v) and 0.5% BSA (w/v), and stored at 4°C.

13. Preparation of the ICA strip

The ICA strip contains an absorbent pad, a conjugate pad containing candidate immuno-SiTQD labels, and an NC membrane containing a T-line (test line) and a C-line (control line). The conjugate pad was moistened with PBS containing 0.5% Tween 20 and dried at 37°C for 12 h. Immuno-SiTQD labels were uniformly sprayed onto the conjugate pads. A29L capture antibody (1.0 mg/mL) was sprayed on the NC membrane as the T-line, and goat anti-mouse IgG (1.0 mg/mL) was sprayed on the NC membrane as the C-line, at a constant dispensing rate of 0.1 µL/mm by the XYZ spraying platform (Biodot, USA). The ICA strip with the C- and T-lines was dried at 37°C for 3 h, cut into 3-mm strips, and stored in a desiccator.

14. Analytical performance analysis of SiTQD-ICA

To determine the sensitivity of the constructed test strips, A29L recombinant protein was used for analysis. The strips were first tested with a concentration of 1 ng/mL, and the more fluorescent antibody was selected for sensitivity analysis. Then, different concentrations (1000, 500, 100, 50, 10, 5, and 1 pg/mL) of A29L protein were used for sensitivity analysis. The fluorescence intensity was recorded, and the intensity of the non-visible fluorescence to the naked eye was the detection limit.

To determine further the performance of the constructed test strips, inactivated monkeypox virus was used for analysis. The fluorescence intensity was recorded, and the intensity of the non-visible fluorescence to the naked eye was the detection limit.

The specificity of SiTQD-ICA was assessed against other viruses recommended for differentiation by WHO and common respiratory viruses, including chickenpox virus, influenza A virus, influenza B virus, SARS-COV-2, SARS-COV, respiratory syncytial virus, and adenovirus.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Author contributions

S.Q.W., J.Y., S.M.W and J.N.F. conceived and designed the project. H.S.S., Y.Q.M., X.S.Y., L.G., Q.Y.L. per-formed experiments. H.S.S., Q.Y.L., J.R.L., Y.Q.M., X.S.Y., J.W., Z.Z., J.Q.S., J.L., Y.M.C., C.X.Y., J.R.M., J.N.F., analysed data. J.Y. and H.S.S. wrote and finalized the manuscript. All authors read and approved the manuscript.

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