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# Characterization and double antibody sandwich ELISA development of monoclonal antibodies against Schmallenberg virus nucleocapsid protein

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**Research Article**

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# Abstract

Schmallenberg virus (SBV), an emerging Orthobunyavirus transmitted by culicoides, causes congenital malformations, abortions, stillbirths and severe reproductive failures in cattle, sheep and goats, thereby posing substantial risks to the ruminant breeding industry and the safety of cross-border livestock trade. To establish a reliable antigen detection method and supply technical support for quarantine and epidemiological surveillance, we generated two specific monoclonal antibodies ( 4D6 and 5D3) targeting the SBV nucleocapsid (N) protein in the present work. Using the pair of mAbs, we further developed a rapid antigen-capture double antibody sandwich ELISA (DAS-ELISA) for the on-site diagnosis of SBV infection. Sensitivity evaluation revealed that the detection limit was 3.9 ng/mL. No cross-reactions were detected against other prevalent ruminant arboviruses, confirming the excellent specificity of the newly establisehd assay. In summary, this DAS-ELISA enables rapid, precise and high-throughput antigen screening of field-derived SBV specimens, serving as a dependable technical tool for routine SBV surveillance and import/export livestock quarantine.


# Key Points

• We successfully prepared mAbs against SBV N protein.

• We defined a linear epitope of SBV N protein by using mAbs 4D6 and 5D3.

• We preliminarily established a double antibody sandwich ELISA based on mAbs 4D6 and 5D3.

# Introduction

Schmallenberg virus (SBV), a typical arthropod-borne Orthobunyavirus, was first identified in clinically affected dairy cattle in Germany in 2011, and rapidly disseminated throughout European countries via hematophagous *Culicoides* biting midges (Hoffmann et al. 2012). Pregnant ruminants are the primary susceptible hosts (Collins et al. 2015). Infection of SBV occurring during critical gestational stages often causes severe fetal outcomes including congenital malformations, hydranencephaly, arthrogryposis, abortion and neonatal mortality, imposing substantial economic losses on the global livestock industry (Elliott 2014; Wernike et al. 2015).

SBV is classified into the *Simbu* serogroup of the genus *Orthobunyavirus*, family *Peribunyaviridae*, order *Bunyavirales*. Similar to other orthobunyaviruses, SBV possesses three segmented negative-sense single-stranded RNA genome, defined as large (L), medium (M) and small (S) segments, respectively (Afonso et al. 2014). The L segment encodes RNA-dependent RNA polymerase (RdRp) responsible for viral replication and transcription; the M segment encodes envelope glycoproteins Gn/Gc and non-structural protein NSm; the S segment encodes nucleocapsid (N) protein and NSs protein via overlapping open reading frames (Elliott 2014). The N protein is the most abundant, highly conserved and immunodominant structural protein in Simbu serogroup viruses, which encapsidates viral genomic RNA to form ribonucleoprotein complex and regulates viral replication cycle (Ariza et al. 2013).

As a high-priority exotic animal pathogen threatening China, SBV poses a high invasion risk owing to the widespread distribution of vector *Culicoides*, frequent cross-border livestock trade, and extensive circulation of animal-derived products (Wang et al. 2025; Zhai et al. 2018). Timely laboratory detection and regular epidemiological surveillance are core strategies to block SBV entry and subsequent transmission. Compared with variable Gn/Gc glycoproteins, N protein exhibits low mutation frequency among SBV epidemic strains, which is regarded as the optimal target for serological diagnosis and antigen detection (Wernike et al. 2021; Zhang et al. 2015). Currently, routine diagnostic assays for SBV antibody detection include virus neutralization test (VNT), indirect enzyme-linked immunosorbent assay (ELISA) and IFA (Jones S et al. 2019). Though VNT serves as the gold standard for serodiagnosis, its implementation demands high-containment biosafety facilities and viable infectious SBV stocks. Indirect ELISA and IFA commonly employ full-length recombinant N protein as coating antigen, which leads to severe cross-reactivity against other orthobunyaviruses within the Simbu serogroup (Endalew et al. 2019).

Monoclonal antibodies have outstanding specificity, batch stability and precise epitope recognition capability, which are critical for developing antigen-captured diagnostic methods (Näslund et al. 2014). Previous studies confirmed that several anti-SBV-N mAbs had cross-reactivity with AKAV and other Simbu serogroup viruses, severely limiting differential diagnosis application (Zhang et al. 2013). In the present work, we generated two SBV-specific mAbs with no cross-reactivity to other Simbu serogroup viruses, and developed a specific DAS-ELISA exclusively for SBV antigen identification. This work provides technical support for the SBV diagnosis and delivers technical reserves fo differential identification of Simbu serogroup arboviruses as well as national prevention and control of exotic animal disease.This study expands the library of SBV-specific diagnostic antibodies and provides technical support for the differential identification of Simbu serogroup arboviruses, as well as for the national prevention and control of exotic animal diseases.

# Materials and Methods

## Cells and viruses

Baby hamster kidney (BHK-21) cells and SP2/0 myeloma cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, USA) supplemented with 10% and 20% heat-inactivated fetal bovine serum (FBS, Invitrogen, USA), respectively. All cells were incubated in a humidified incubator containing 5% $CO_2$ at 37°C. AKAV TJ2016 strain, BVDV, and BTV were preserved in the lab of Chinese Academy of Quality and Inspection & Testing (CAQIT, Chen et al. 2021).

## Preparation of recombinant SBV N protein

The prokaryotic recombinant plasmid pET28a-SBV-N was constructed via gene synthesis based on the SBV N gene reference sequence deposited in GenBank under accession number JX853181.1. The complete coding sequence of the N gene was 702 bp. The verified recombinant plasmid was

subsequently transformed into Escherichia coli BL21 (DE3) competent cells. Then, the bacteria were cultured and induced at 16℃ or 37℃ with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) for 6 h, and harvested by centrifugation at 6000×g for 10 min at 4℃. Cell pellets were resuspended in lysis buffer and fully disrupted by sonication on ice. The lysate was separated into supernatant (soluble fraction) and precipitate (insoluble inclusion body) by centrifugation at 12,000×g for 15 min at 4℃. Both fractions were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting to evaluate the expression level and solubility of the target protein.

The supernatant containing soluble His-tagged SBV N protein was loaded onto Ni-NTA affinity chromatography resin (TransGen Biotech, China) following the manufacturer's instructions for purification. Eluted protein fractions were pooled and dialyzed overnight against phosphate-buffered saline (PBS) at 4℃ to remove imidazole. The protein concentration was quantified using the BCA protein assay kit (Thermo Fisher Scientific, USA). Purified protein was aliquoted and stored at −20℃ for subsequent immunization, antibody screening and antigen detection assays.

## Western blotting assay

Proteins were separated on a 12% polyacrylamide gel by SDS-PAGE, and then transferred onto polyvinylidene difluoride (PVDF) membranes (Sigma-Aldrich, USA). The membranes were subsequently blocked with 5% skim milk prepared in PBS for 2 h at room temperature (RT, 25 ℃). After washing with PBST, the membranes were incubated with the primary antibody for 1 h at RT. Subsequently, the membranes were washed three times with PBST and incubatd with horseradish peroxidase (HRP)-labeled goat anti-mouse IgG secondary antibody (Solarbio, China) for 1 h at RT. Following another three rounds of PBST washing, After washing with PBST for three times, protein bands were visualized using a chemiluminescence detection kit (Millipore, USA) and the Invitrogen EVOS FL cell fluorescence imaging system (Thermo Fisher Scientific, USA).

## Preparation of monoclonal antibody against SBV N protein

Purified recombinant SBV N protein was used as an immunogen to prepare mouse-derived mAb following a standard immunization procedure (Chen et al. 2023). Briefly, five 6- to 8-week-old female BALB/c mice were immunized intraperitoneally with a mixture of 100 μg recombinant SBV N protein and the equal volume of QuickAntibody-Mouse5W adjuvant (Biodragon, China) according to the instrument. Fourteen days later, the second immunization was performed in the same manner. On day 35 post-initial imunization, mouse sera were collected, and antibody titers were determined by indirect ELISA. Subsequently, the mouse with the highest antibody titer received a final intraperitoneal booster injection without adjuvant. Three days after the final booster immunization, mouse splenocytes were fused with SP2/0 myeloma cells. Hybridoma cells were then screened by HAT selective medium, and positive hybridoma clones were identified via indirect ELISA. Stable positive hybridoma cell lines were obtained by limiting dilution subcloning and preserved in liquid nitrogen for long-term storage.

## Indirect ELISA

Antibody titers of immunized animal sera and hybridoma supernatants were detected by indirect ELISA. Briefly, 96-well plates were coated with 100 μL per well of purified SBV N protein (2 μg/mL) diluted in 0.05 mol/L carbonate buffer (pH 9.6) and incubated overnight at 4℃. The plates were blocked with 3% skim milk-PBS solution overnight at 4℃. After washing with PBS, serially diluted serum samples or hybridoma supernatants were added to the plates and incubated for 1 h at 37℃. Following antother PBS washing step, HRP-conjugated goat anti-mouse IgG (1:5000) was added as the secondary antibody, and the plates were incubated for 1 h at 37℃. Subsequently, TMB chromogenic substrate was added for color development, and the enzymatic reaction was terminated by 2 mol/L $H_2SO_4$. The optical density (OD) value at 450 nm was measured using a Multiskan spectrum microplate reader (Thermo Fisher Scientific, USA).

## Characterization of anti-SBV-N mAbs

Western blotting and IFA assays were performed to verify the specificity and binding activity of screened mAbs. For Western blotting assay, recombinant SBV N and AKAV N proteins (Chen et al. 2023) were separated via SDS-PAGE, and then transferred to PVDF membranes. The mAbs 4D6 and 5D3 were used as primary antibodies and HRP-labeled goat anti-mouse IgG served as secondary antibody. For IFA assay, BHK-21 cells stably exprssing SBV-N protein (previously constructed in our laboratory, Zhang et al. 2015) were fixed with 4% paraformaldehyde. After incubation with the screened mAbs and FITC-labeled goat anti-mouse IgG, cellular fluorescence signals were observed using an EVOS FL fluorescence imaging system (Thermo Fisher Scientific, USA). The subtype of the obtained mAbs were identified using a mouse monoclonal antibody isotyping kit (Biodragon, China).

## Epitope identification of mAbs 4D6 and 5D3

To investigate the binding epitopes of the obtained mAbs 4D6 and 5D3 on SBV N protein, a series of overlapping truncated N fragments were amplified using the primer pairs listed in Table 1 and expressed as glutathione-*S*-transferase (GST)-tagged fusion proteins in *E. coli* (Figure 3). Firstly, three overlapping fragments covering the full-length N protein (amino acids (aa) 1-84, 79-162, 151-233) were amplified and heterologously expressed in *E. coli* BL21 cells. Western blotting was performed to characterize the binding reactivity of mAbs 4D6 and 5D3 with each truncated peptide. Subsequently, the positively reactive fragment was further subdivided into three truncated segments based on the primers in Table 2. Indirect ELISA was performed to evaluate the binding profiles of the two mAbs against each truncated segment.

## Establishment and optimization of DAS-ELISA

To establish the DAS-ELISA mehod, mAb 4D6 was used as the capture antibody, and HRP-conjugated mAb 5D3 was used as the detection antibody. A checkerboard titration assay was performed to optimize the working concentration of the capture antibody and the dilution ratio of the detection antibody. Subsequently, gradient-diluted purified SBV N protein was applied to establish the standard curve and determine the detection limit of the optimized DAS-ELISA.

## Cut-off value determination

A total of 36 SBV-negative healthy bovine serum samples were collected and tested using the optimized DAS-ELISA. The cut-off value was calculated as the mean OD value plus three standard deviations ($\overline{x}$ +3SD). Samples with OD values higher than the $\overline{x}$+3SD were defined as SBV antigen-positive, while those with OD values lower than this threshold were defined as negative.

## Specificity evaluation of DAS-ELISA

For the specificity evaluation of the established DAS-ELISA, several common ruminant arboviruses, including AKAV, BVDV, and BTV, as well as SBV N protein, were detected in parallel. The corresponding OD values were recorded to assess the cross-reactivity and diagnostic specificity of the assay.

## Results

## Prokaryotic expression and purification of recombinant SBV N protein

According to the gene sequence analysis, the full-length SBV N gene (702 bp) was successfully cloned into the pET28a expression vector and designated pET-28a-SBV-N. Subsequently, the recombinant plasmid pET-28a-SBV-N was transformed into competent *E. coli* BL21 (DE3) cells for recombinant protein expression. Following IPTG induction at 16℃ or 37℃, bacterial pellets were collected and lysed by ultrasonication. The bacterial lysate was separated into soluble supernatant and insoluble sediment fractions via high-speed centrifugation. SDS-PAGE was performed to analyze the protein expression profiles of the two fractions. Electrophoresis results demonstrated that the His-tagged recombinant SBV N protein, with a predicted molecular weight of 26 kDa, was expressed in both the soluble supernatant and insoluble inclusion body precipitates. Notably, the highest soluble expression level was obeserved under 16℃ induction (Figure 1A). These results indicated that the target protein was successfully expressed after induction, while a portion of the expressed protein misfolded and aggregated into insoluble inclusion bodies. The His-tagged SBV-N expressed in the soluble supernatant of 16 ℃ induction was further purified using Ni-NTA affinity chromatography. After column equilibration and washing with low-concentration imidazole buffer to remove non-specifically bound impurities, the target protein was eluted using high-concentraion imidazole solution. The eluted fractions were dialyzed against PBS to eliminate imidazole and concentrated by ultrafiltraion. SDS PAGE and western blotting

analysis confirmed that highly purified SBV N protein with favorable antigenic activity was successfully obtained (Figure 1A and 1B).

## Preparation and characterization of mAbs 4D6 and 5D3

After cell fusion, primary screening and limiting dilution subcloning, two hybridoma cell lines stably secrete SBV-N specific monoclonal antibodies were obtained and designated 4D6 and 5D3, respectiviely. Isotype analysis revealed that both mAbs belong to the IgG1/κ subtype. Western blotting results demonstrated that mAbs 4D6 and 5D3 specifically bound recombinant SBV N protein without cross-reactivity to AKAV N protein (Figure 2A). Consistent IFA data showed strong specific fluorescent signals in SBV-N stablely expressed BHK-21 cells, further verifying that the two mAbs could recognize natively folded endogenous SBV N protein (Figure 2B).

## Epitope identification of mAbs 4D6 and 5D3

To precisely map the linear epitope recognized by mAbs 4D6 and 5D3, a total of nine overlapping truncated fragments were designed and constructed. First, three long overlapping fragments covering residues aa 1-84 , aa 69-162 and aa 139-233 were cloned into the pGEX-6p-1 vector and expressed in *E. coli* BL21 (DE3) competent cells (Figure 3). Western blotting results revealed that mAb 4D6 only bound the aa 69-162 fragment, whereas mAb 5D3 only bound the aa 139-233 fragment (Figure 4A). To further narrow down the minimal binding region, three additional shorter overlapping peptides were separately designed to cover aa 69-162 and aa 139-233. Indirect ELISA results yielded positive signal exclusively for the aa 105-146 fragment (recognized by mAb 4D6) and the aa 176-211 fragment (recognized by mAb 5D3) (Figure 4B). Furthermore, structural analysis of the SBV N tetramer (PDB ID: 00004idu) revealed that these two distinct polypeptide regions are prominently exposed on the outer surface of the protein complex, rather than buried inside the folded core (Figure 4C).

## Optimization and sensitivity of DAS-ELISA

To develop a DAS-ELISA for SBV antigen detection, mAb 4D6 was selected as the capture antibody, whereas HRP-conjugated mAb 5D3 was served as the detection antibody. A checkerboard titration assay with serial two-fold dilutions of both mAbs was performed to optimize the working concentrations. A high Positive/Negative (P/N) value means strong specific binding between paired antibodies and the target SBV N protein, and extremely low non-specific background absorbance in negative control wells. Accordingly, the optimal coating concentration for capture mAb 4D6 was 5.3 ng/well (dilution 1: 16,000), and the optimal working concentration of HRP-conjugated mAb 5D3 was 1.25 ng/well (dilution 1:80,000), yielding a maximum P/N ratio of 16.01 (Table 2).

Subsequently, serially diluted purified recombinant SBV N protein ranging from 125 ng/mL down to 3.9 ng/mL (125, 62.5, 31.25, 15.62, 7.81, 3.9 ng/mL) was used to establish the standard curve of the DAS-

ELISA. A linear regression equation (y=0.0224x+0.3896) was fitted to the standard curve of the recombinant N protein (Figure 5). Based on this standed curve, the developed DAS-ELISA had a detection limit of 3.9 ng/mL for SBV N protein.

## Cut-off value and specificity detection

Thirty-six SBV-negative bovine serum samples were used to calculate the cut-off value. The mean OD value ($\bar{x}$) of these negative samples was 0.161, with a standard deviation (SD) of 0.005. Therefore, the cut-off threshold was thus defined as $\bar{x}$+3SD= 0.176. This threshold served as the discriminative criterion: samples with OD values above 0.176 were classified as positive, and samples with OD values below 0.176 were considered negative (Figure 6).

The sepcificity of the developed DAS-ELISA was further evaluated, and the results showed that positive OD signals were exclusively observed in the SBV antigen group, whereas all AKAV, BVDV, and BTV groups yielded negative readings. These findings confirmed that the developed DAS-ELISA possessed high specificity and displayed no cross-reactivity with common ruminant arboviruses (Table 3).

## Discussion

SBV is a high-risk exotic Simbu serogroup arbovirus, which shares high genetic homology and antigen similarity with AKAV, Aino virus and other related orthobunyaviruses (Doceul et al. 2013). Serological cross-reaction is the main difficulty for differential diagnosis of Simbu serogroup viruses, especially for N protein-based immunological detection methods (Wernike, K et al. 2021). Most existing anti-SBV-N mAbs recognize conserved epitope shared by AKAV and SBV, which cannot realize independent SBV detection (Zhang et al, 2013). In the present study, the generated mAb 4D6 and 5D3 target an SBV-unique region that shares only 80% amino acid identity with the corresponding AKAV protein. Amino acid variation within two variable regions (residues 105-146 and 176-211) abolish cross-reactivity with AKAV, overcoming the critical limitation of conventional SBV diagnostic antibodies that exhibit broad cross-reactivity against other Simbu serogroup orthobunyaviruses.

SBV N protein is essential for viral RNA encapsulation, ribonucleoprotein assembly and the complete viral replication cycle (Dong et al 2013). Mapping B-cell epitopes on the N protein helps clarify viral antigenic variation mechanism, develop epitope-based diagnostic antigen and multi-epitope subunit vaccine (O'Connor et al. 2024; Wernike et al 2015). The epitope characterized herein lies on the exposed surface of the N protein tertiary structure, which explains the strong immunoreactivity observed in Western blotting and indirect ELISA detection, making it highly accessible to host immune cells and capable of triggering strong specific antibody responses. Owing to its favorable surface position and unique amino acid sequence signature, this epitope can serve as a core candidate motif, which greatly facilitates subsequent development of differential diagnostic kits and targeted epitope vaccines of SBV prevention.

Compared with nucleic acid aplification tests and VNT, DAS-ELISA has low requirement for experimental equipment and biosafety level, suitable for large-scale field sample screening and port batch detection (Bréard et al. 2013; Zhao et al. 2025). Unlike indirect ELISA platforms that target host serum antibodies, this newly established DAS-ELISA directly captures viral N antigens, which enables to identify early-infected animals prior to seroconversion, thus achieving rapid early diagnosis of SBV infection. Furthermore, the assay exhibits strict specificity solely for SBV, allowing effective discrimination of SBV monoinfection from concurrent AKAV infection in regions endemic for multiple Simbu serogroup orthobunyaviruses.

This study still has limitations: only common ruminant arboviruses were selected for specificity verification; more Simbu serogroup field strains need to be verified in follow-up research. In addition, whole SBV viral antigens are recommended for further validation of the analytical performance of this DAS-ELISA assay.

In conclusion, the obtained mAb 4D6 and 5D3 have high specificity for SBV N protein, and the developed DAS-ELISA is sensitive, stable and specific. This study expands the available tookit for SBV diagnostics, and lays technical reserves for the prevention and control of imported SBV, field epidemiological surveillance, and differential identification of co-circulating Simbu serogroup viruses within China.

# Declarations

### Author contribution statement

Wu SQ: Project administration, Resources, Supervision. Chen DJ:  Funding acquisition, Conceptualization, Methodology, Investigation, Validation, Writing—original draft. Wei F and Yu RY: Data curation, Investigation, Methodology, Validation. Wang D and Wang JJ: Formal analysis, Visualization, Writing—review & editing. All authors contributed to the article and approved the submitted version.

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### Conflict of interest

The authors declare that they have no competing interests.

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Ethical statement

This article does not contain any studies with animals performed by any of the authors.

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# Tables

Tables 1 to 3 are available in the Supplementary Files section.

# Figures

Figure 1 SDS-PAGE and WB

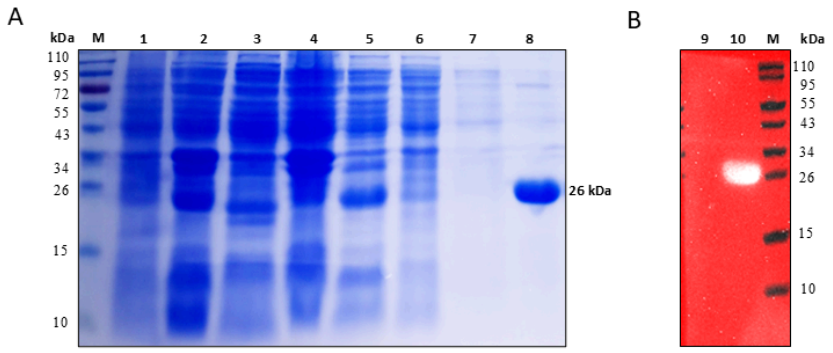
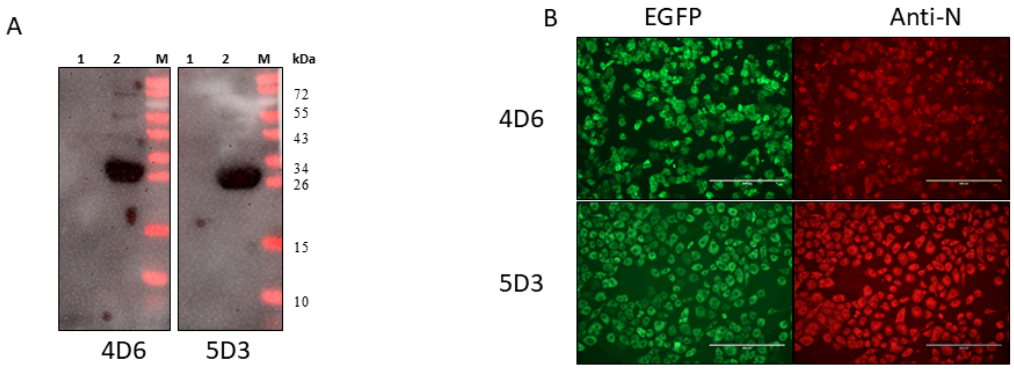



**Figure 1**

SDS-PAGE and Western blotting analysis of recombinant SBV N protein. (A) Prokaryotic expression and purification profiles of SBV N protein induced at 16℃ and 37℃; (B) Western blotting identification of recombinant SBV N protein. M: Protein molecular weight marker; 1: *E. coli* carrying empty pET-28a vector; 2: Insoluble precipitate of conicated recombinant bacteria induced at 37°C; 3: Soluble supernatant of sonicated recombinant bacteria induced at 37°C; 4: Insoluble precipitate of sonicated recombinant bacteria induced at 16°C; 5: Soluble supernatant of sonicated recombinant bacteria induced at 16°C; 6: Flow-through fraction; 7: Wash fraction eluted with 20 mM imidazole buffer; 8: Purified recombinant SBV N protein; 9: *E. coli* carrying empty pET-28a vector; 10: Purified SBV N protein.

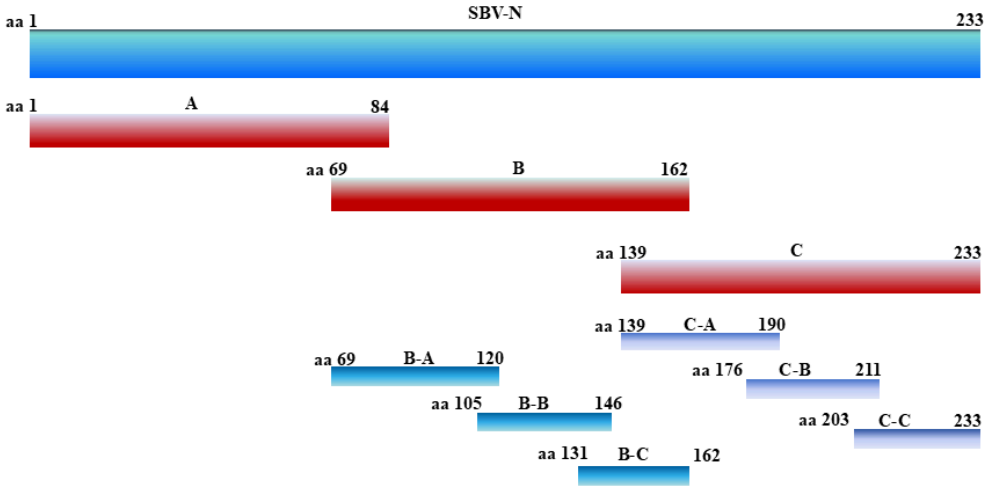
Figure 2




## Figure 2

**Specificity characterization of mAbs 4D6 and 5D3.** (A) Western blotting analysis for cross-reactivity of mAbs 4D6 and 5D3 with AKAV N protein; 1: AKAV N protein; 2: SBV N protein; (B) IFA detection of mAbs 4D6 and 5D3 with native SBV N protein in BHK-21 cells. Scale bars=400 μm.

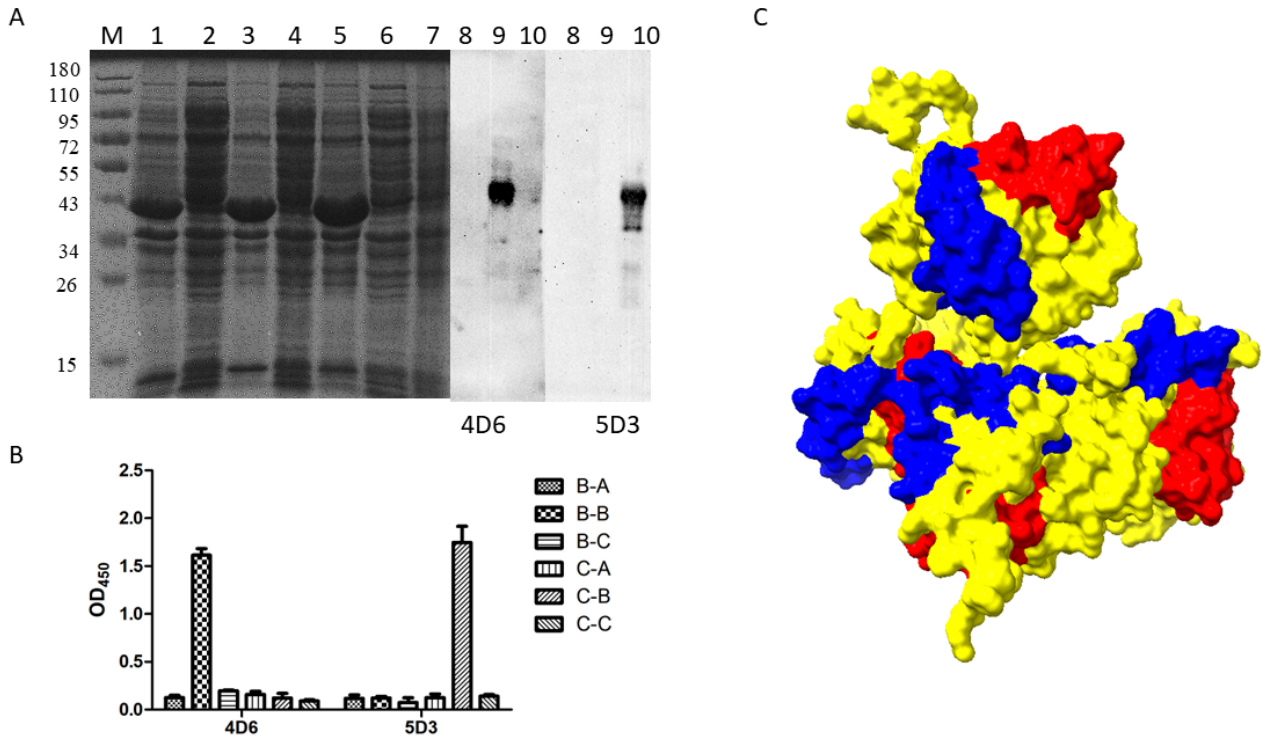
Figure 3 peptide




## Figure 3

**Schematic diagram of SBV N protein truncated fragments for epitope mapping.** aa, amino acid.

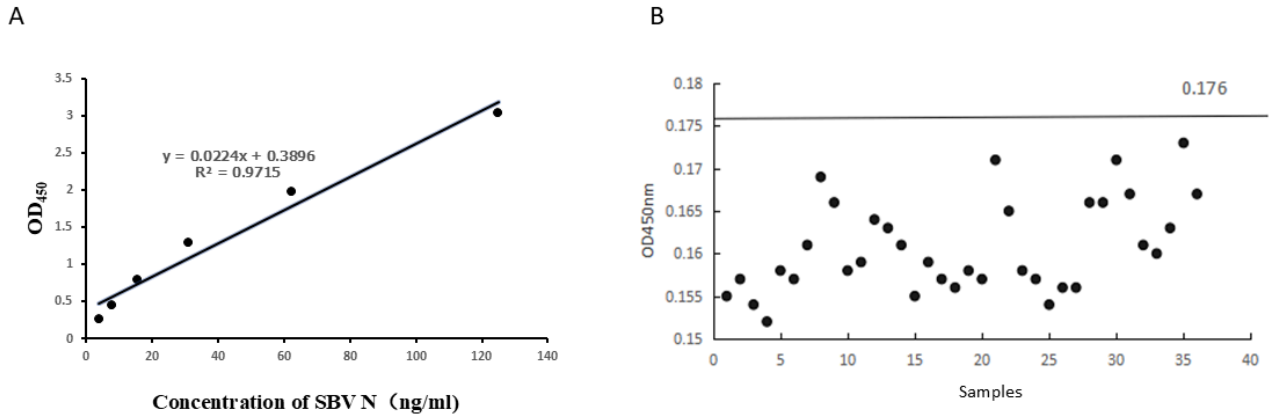
Figure 4



## Figure 4

**Epitope mapping of SBV N protein recognized by mAbs 4D6 and 5D3.** (A) SDS-PAGE and Western blotting analyses of truncated GST-N fusion proteins; M: Protein molecular weight marker; 1: Precipitate of SBV-N-A; 2: Supernatant of SBV-N-A; 3: Precipitate of SBV-N-B; 4: Supernatant of SBV-N-B; 5: Precipitate of SBV-N-C; 6: Supernatant of SBV-N-C; 7: BL21(DE3) control; 8-10:Western blotting detection of SBV-N-A, SBV-N-B, SBV-N-C precipitates using mAbs 4D6 and 5D3, respectively. (B): Indirect ELISA binding profiles of mAbs 4D6 and 5D3 against truncated SBV N fragments. (C): Structure model of SBV N tertramer. The SBV-N-B-B segment is shown in blue, and the SBV-N-C-B segment is shown in red.

Figure 5




## Figure 5

**Standard curve construction and cut-off value determination for the developed DAS-ELISA**

(A): Standard curve of the DAS-ELISA generated using serially diluted purified SBV N protein; (B): Determination of the Cut-off value for the DAS-ELISA.

Figure 5

```
AKAV_N_amino_aci MANQFIFNDVPQRNAATFNPDAGYVAFISKYGQQLNFTVA     40
SBV_N_amino_acid -ss----e------------ev------g--------g--     40

AKAV_N_amino_aci RVFFLNQKKAKMVLHKTPQPSVDLTFAGVKFTVVNNHFPQ     80
SBV_N_amino_acid -----------------a--------g-------------     80

AKAV_N_amino_aci YTANPVSDTAFTLHRISGYLARWVAEQCKANQIKLAEAAA    120
SBV_N_amino_acid -vs---p-n-i----m-------i-dt---svl-----s-    120

AKAV_N_amino_aci TIVMPLAEVKGCTWSDGYAMYLGFAPGAEMFLETFEFYPL    160
SBV_N_amino_acid q-------------a---t-------------da-d----    160

AKAV_N_amino_aci VIDMHRVIKDGMDVNFMRKVLRQRYGQLTAEEWMTSKLDA    200
SBV_N_amino_acid --e----l--n------k--------tm-------q-ite    200

AKAV_N_amino_aci VKAAFNSVAQISWAKSGFSPAARAFLAQFGIQI          233
SBV_N_amino_acid i-------g-la-----------t--q----n-          233
```

**Figure 6**

Legend not included with this version.

# Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.docx
- Table2.docx
- Table3.docx