Development and Application of a High Sensitivity Immunochromatographic Test Strip for Detecting Classical Swine Fever Virus Antibody

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Research Article

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Title:
Development and application of a high sensitivity immunochromatographic test strip for detecting classical swine fever virus antibody

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Running title:
A new test strip for detecting CSFV antibody

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Abstract:
Background: Classical swine fever (CSF) is caused by classical swine fever virus (CSFV) and has lead huge losses to the pig industry worldwide. Although vaccination and other control measures have been carried out, it is essential to establish a rapid valid method for CSF vaccination monitoring and clinical diagnosis. CSFV E2 protein has been well known as a major antigen for antibody detection. It is significant to improve affinity between E2 protein and CSFV antibody to result in a better performance of detection method.

Results: In this study, a recombinant E2 extracellular protein (aa 1-331), which was with the native homodimer conformation and had a high affinity with anti-CSFV-E2 monoclonal antibody WH303, was expressed using Bac-to-Bac baculovirus expression system. A novel immunochromatographic test strip based on the recombinant CSFV E2 protein was developed for CSFV antibody detection. The sensitivity of this strip for detecting CSFV standard positive serum was 1:102400, 4 times higher than that of the former developed CnC2 test strip. No cross reaction with other swine virus antibodies was observed. The detection of clinical swine serum
samples (n=138) demonstrated that the agreement of the new E2 test strip with three commercial ELISA kits was 88.40% (122/138), 86.23% (119/138), and 96.38% (133/138), respectively.

**Conclusion:** Our data indicate that a novel E2 test strip with higher sensitivity has been developed and can be applied for clinical sample detections, providing a new powerful and simple approach for future monitoring CSFV antibodies.

**Keywords:** Classical swine fever virus, Immunochromatographic strip, Colloidal gold, E2 protein, high affinity

**INTRODUCTION**

Classical swine fever (CSF) is a highly contagious and multisystemic disease of pigs, resulting in huge economic losses to the swine industry worldwide (1). CSF is caused by the classical swine fever virus (CSFV), which belongs to the genus *Pestivirus* within the *Flaviviridae* family (2). The CSFV genome consists of a single positive-stranded RNA encoding a polyprotein composed of approximately 3898 amino acids (aa). The polyprotein is processed into the mature forms of four structural proteins and eight nonstructural proteins, including the nucleocapsid protein C, Erns, E1, E2, Npro, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B (3). The envelope glycoprotein E2 represents as a major immunogen and induces neutralizing antibodies (4-5). Besides, previous studies have demonstrated that the Erns is also capable of inducing the production of neutralizing antibodies in the host (6-7).

Currently, only several countries, including the United States of America (USA), Canada, Australia, New Zealand, and some Member States of the European Union (EU), have successfully eradicated CSF. In these CSF-free zones, the main strategies for controlling CSF are non-vaccination and stamping-out policy. In contrast, CSF endemic countries have to use routine vaccination to prevent and control CSF. In China, the main reason for the frequent immunization failures is not vaccine itself but the interference of maternal antibodies, persistent infections, congenital infections and/or immune tolerance. As a result, the antibody against CSFV produced in vaccinated pigs is not sufficient to resist the infection of wild-type virus strain, which
leads to the distribution and epidemic of CSF in the herd (8).

In order to evaluate the efficacy of CSF vaccines, develop better immune procedure and eliminate pigs with low immune responses, a practical and rapid test approach is needed for monitoring CSFV antibodies in routine field practice. At present, the indirect fluorescent assay (IFA), immunoperoxidase monolayer assay (IPMA), virus neutralization test (VNT), enzyme-linked immunosorbent assay (EILSA), and the positive indirect hemagglutination assay (IHA) are the commonly used methods to detect CSFV antibodies (9-12). These methods are all highly sensitive and accurate but still have certain limitations, such as complicated experimental procedure, time-consuming, and necessary expensive equipment and maintenance cost.

The immunochromatographic strip is one of the most rapid detection methods utilized in numerous fields, including in clinical diagnosis, environmental analysis, and food safety. This method has several advantages over traditional immunoassays, such as rapid operation, low cost, immediate results, simplicity of the procedure, no requirements for skilled technicians or expensive equipment, and high-throughput with sufficient sensitivity and specificity. In a previous study, the chimera protein CnC2 containing E\textsuperscript{ms} epitope (aa 109–160, designated Cn) and E2 epitope (aa 1–176, designated C2) was prepared from the \textit{Escherichia coli (E. coli)} expression system and was used as a detection antigen to make the CSFV antibody test strip (13). The CnC2 antigen was produced as inclusion bodies in \textit{E. coli}, which needs further dialysis and refolding. It is extremely difficult to fully restore the native conformation of the protein by refolding, leading to a semi-folded or misfolded protein which may reduce the antibody detection efficacy. In the present study, the baculovirus expression system has been used to produce the CSFV E2 protein that maintains the native dimeric conformation and could be glycosylated efficiently. Based on the newly prepared E2 protein, a novel rapid test strip was subsequently developed for CSFV antibody detection. Furthermore, a comparison between the new E2 test strip, the former CnC2 test strip and commercial ELISA kit for CSFV antibody detection was performed, which has demonstrated us a powerful and simple tool for monitoring
CSFV antibodies in the future.

MATERIALS AND METHODS

Serum and animals
CSFV positive serum was purchased from Institute of Veterinary Drug Supervision of China (Cat. No. S0760905-2). The clinical serum samples, including CSFV positive serum (n=20), CSFV negative serum (n=10), porcine circovirus type 2 (PCV2) positive serum (n=5), porcine reproductive and respiratory syndrome virus (PRRSV) positive serum (n=5), foot-and-mouth disease virus (FMDV) positive serum (n=5), porcine pseudorabies virus (PRV) positive serum (n=4), Japanese encephalitis virus (JEV) positive serum (n=3) and bovine viral diarrhea virus (BVDV) positive serum (n=3), were provided by Key Laboratory of Animal Immunology (KLAI) of Henan Academy of Agricultural Sciences (HAAS). The African swine fever virus (ASFV) positive serum (n=3) was provided by Henan Animal Husbandry Bureau and pig farms. A total of 138 clinical serum samples were collected from pig farms distributed in Henan province in China. Rabbit anti-E2 polyclonal antibodies and mouse anti-CSFV-E2 mAb WH303 was provided by KLAI of HAAS.

Preparation of CSFV E2 truncated protein
Recombinant CSFV E2 truncated protein was prepared using Bac-to-Bac baculovirus expression vector system (Invitrogen, US). Briefly, the gp67 signal peptide was inserted into pFastBacI vector to obtain the plasmid pFastBacI-gp67 for secretory expression of the protein. The coding sequence for the residues 1-331 of E2 were cloned into the pFastBacI-gp67 to generate a C-terminal 6×His tag fusing E2 protein. Then, recombinant baculovirus and large-scale protein were generated by infecting Sf21 cells using the Bac-to-Bac baculovirus expression vector system. The cell culture supernatant was harvested at 72 hours post-infection (hpi). The proteins were purified from cell culture supernatant using the nickel-affinity chromatography and verified by SDS-PAGE and western blot analysis using mouse anti-CSFV monoclonal antibody WH303 or by indirect ELISA using CSFV standard positive serum. Finally,
the recombinant CSFV E2 protein was collected after dialysis in saline at 4°C for 48 h by centrifuging at 10,000 r/min for 5 min and stored at -20°C.

Preparation of CSFV E2 protein labeled with gold nanoparticles
An aliquot of 15 μg recombinant CSFV E2 protein was slowly added into 1 ml pH7.5 gold nanoparticles (AuNP) solution that was obtained as previously described (14), and the solution was kept at room temperature for 30 min. Then 100 μL sodium tetraborate solution A (20 mmol/L Na₂B₄O₇, 3% BSA Casein protein) was added, and the solution was stand at room temperature for 10 min and centrifuged with 12,000 r/min at 4°C for 25 min. The supernatant was discarded and 1 ml sodium tetraborate solution B (20 mmol/L Na₂B₄O₇, 1% BSA) was added, and the supernatant was centrifuged again. The supernatant was discarded and 100 μL of sodium tetraborate solution C (20 mmol/L Na₂B₄O₇, 3% BSA) was added to resuspend the conjugated antigen E2-AuNPs.

Assembly of the test strips
The test strip was composed of one nitrocellulose (NC) membrane and three pads (sample pad, conjugate pad and absorbent pad). The NC membrane (300 mm × 60 mm) was spotted with a test line (T line) with Staphylococal Protein A (SPA) and a control line (C line) with pig polyclonal anti-CSFV sera. The T line and C line were situated at the center of the NC membrane with a distance of 0.5 cm. After drying at 45 °C for 1 h, the membrane was vacuum packaged and stored at 4°C. The conjugate pad (300 mm × 8 mm) was prepared by dispensing the colloidal gold-labelled E2 or CnC2 recombinant proteins onto the glass fiber at a jetting rate of 5 µL/cm using the same dispenser, followed by drying at 45 °C for 1 h and storing at 4 °C. The sample pad (300 mm × 15 mm) was saturated with the treatment buffer (20 mmol/L Na₂B₄O₇, 1% BSA), then dried at 56 °C for 1 h. The adsorbent pad was cut into dimensions of 300 mm × 18 mm. The assembly of strip was performed as shown in Figure 1A. Briefly, the NC membrane was first attached to the backing card, then the absorbent pad and the conjugate pad were pasted on both sides of the NC membrane with a 2
mm overlap, and finally the sample pad was attached to the card with a 2 mm overlap on the conjugate pad. Consequently, the assembled card was cut into 2.8 mm wide strips, and then the strips were sealed in plastic bags and stored at 4 °C (Fig. 1B).

**Principle and method of test strip**

After added to the sample pad, serum samples diluted in saline solution were flowed from the end of sample pad to the absorbent pad by capillary action. When the sample contains anti-CSFV IgG, the serum would conjugate with the conjugated antigen E2-AuNPs on the conjugate pad to form antigen-antibody-AuNP compound. Then the IgG Fc domain of the compound will combine the SPA on T line, as a result T line turns red (Fig. 2A). For the negative sample without anti-CSFV IgG, T line will not turn red (Fig. 2B). Additionally, rabbit anti-E2 polyclonal antibody on the C line intercepts the conjugated antigen E2-AuNPs to serve as quality controls.

**Performance analysis of the strips**

The sensitivity of the strips was determined by a 2-fold diluted series of CSFV standard positive serum. The dilution was from 1:100 to 1:204800. Each serum sample was triplicated tested and the sensitivity of the new E2 strip was compared to that of the previously developed CnC2 strip (13). The specificity and cross reaction of the strips was evaluated with 58 serum samples, including CSFV positive serum (n=20), CSFV negative serum (n=10), PCV2 positive serum (n=5), PRRSV positive serum (n=5), FMDV positive serum (n=5), PRV positive serum (n=4), JEV positive serum (n=3), BVDV positive serum (n=3) and ASFV positive serum (n=3). Three batches of test strips, namely CSFV001, CSFV002 and CSFV003, were prepared to for determing the repeatability of the strips according to the coefficients of variability (CV) in the rate of indicators. For this experiment, a total of 10 CSFV positive serum samples including 5 weak positive samples and 5 strong positive samples were used. For the stability analysis, different batches of test strips were stored at room temperature for 12 months and tested every three months. The appearance, sensitivity and specificity of the strips were observed and recorded at different testing time. Each
test was performed in triplicate. According to the test results, the shelf life was determined.

**Clinical application of the test strips**

The clinical serum samples (n=138) collected from pig farms from Henan province in China were tested in triplicate using different batches of the strips. Simultaneously, these samples were detected for antibodies against CSFV using three commercial ELISA kits, including the IDEXX CSFV Ab Test Kit (IDEXX GmbH, Switzerland), CIVTEST SUIS HC/PPC (HIPRA, Spain), and VDPro® CSFV AB C-ELISA (MEDIAN, Korea). The strip test was performed as described above and the ELISA was conducted according to the manufacturer's descriptions.

**RESULTS**

**Expression and purification of recombinant CSFV E2 protein**

The recombinant CSFV E2 was successfully expressed and purified from the Bac-to-Bac baculovirus expression system. As demonstrated in Figure 3A, western blot analysis with anti-his mAb showed that the molecular size of recombinant E2 was about 45 kDa although its predicted size was about 36 kDa. It was suggested that the practical size of E2 protein was bigger than the theoretical size due to glycosylation. The analysis of SDS-PAGE showed that the nondenatured E2 protein was about 90 kDa while the denatured E2 protein was about 45 kDa (Figure 3B). It has been previously confirmed that the native conformation of CSFV E2 protein was homodimer (15), which implies that the recombinant E2 protein presently expressed using baculovirus expression system has the native conformation. The immunoreactivity of both denatured and nondenatured E2 protein was identified by western blot with mouse anti-CSFV-E2 mAb WH303. As shown in Figure 3C, the E2 homodimer had a much higher affinity to WH303 than the E2 monomer. An indirect ELISA was performed using the purified recombinant E2 protein as coating antigen and has shown that the titer of CSFV standard positive serum was 1:409600 (Figure 3D). These data indicate that the present developed recombinant CSFV E2 was a good
Conjugation of CSFV E2 protein with AuNPs

After the mixing of 100 μL colloidal gold solution with the CSFV E2 protein solution diluted at different concentrations and adding 10% sodium chloride solution, the color of mixed solution will change from red to grey purple (Figure 4). The minimum concentration of protein which keeps the color of mixed solution red is considered to be the optimal concentration of protein for stabilizing colloidal gold. Thus, the optimal concentration of protein for making the E2-AuNPs conjugate was 4 ng/mL. As demonstrated in Figure 5, the mean size of CSFV E2 and AuNPs was about 10 nm (Figure 5A and 5B) and 30 nm (Figure 5C and 5D), respectively, which were observed by transmission electron microscope (TEM) (JEM-1400, Japan) and particle size analyzer (ZETA SIZER Nano series, Malvern, UK). AuNPs was almost same size and spaced regularly. After conjugating, the particles were gathering to form E2-AuNPs and its size was near 100 nm (Figure 5E and 5F). As shown in Figure 5G, two ultraviolet (UV) absorption peaks of E2 protein were 210 nm and 280 nm in UV scanning curve while two ultraviolet (UV) absorption peaks of E2-AuNPs shifted to 220 nm and 275 nm. It indicated that coupling occurred between E2 protein and AuNPs to form complexes E2-AuNPs, which can be used for the preparation and assemble of CSFV test strip.

Sensitivity of CSFV E2 test strips

As described in the material and methods, the E2 test strips were prepared and assembled. Sensitivity of test strip was evaluated using 2-fold diluted CSFV strong positive serum and was compared to that of the CnC2 test strip developed previously. As shown in Figure 6A and 6B, the T lines of strips were scanned with a membrane strip reader (Bio-Dot, Richmond, CA, USA) and the G/D × A (area) value was decreasing as the dilution increased for both E2 test strip (R²=0.9685) and CnC2 test strip (R²=0.9834). When the value of G/D × A is less than 15, the test line is almost
invisible to the naked eyes. The curve of Dens-ROD for E2 test strip was smoother than that of CnC2 test strip (Figure 6C and 6D). As Figure 6E shown, the sensitivity of E2 and CnC2 test strip was 1:102400 and 1:25600, respectively. The data demonstrated that the E2 test strip had a more favourable sensitivity than CnC2 test strip.

**Specificity of CSFV E2 test strips**

Using the E2 test strips, a total of 20 CSFV positive serum samples were detected to be 100% positive due to that both of the T and C lines were colored as red (Figure 7). On the contrary, CSFV negative serum (n=10) and all the other samples (n=28) including PCV2, FMDV, PRRSV, PRV, JEV, BVDV and ASFV serum (shown in Table 1) were tested as negative because only the C lines turned red, suggesting that there is no cross reaction with other swine virus antibodies.

**Repeatability and reproducibility of CSFV E2 test strips**

Serum samples (n=10) were determined by three batches of E2 strips (CSFV001, CSFV002 and CSFV003) and the G/Dens values were measured to calculate the CV% and analyze the repeatability and reproducibility. For the repeatability analysis, different serum samples (n=10) were tested by E2 strip in triplicate in one batch. For the reproducibility analysis, different serum samples (n=10) were tested by E2 strip in three independent batches on different days. The intra-assay CV for CSFV001, CSFV002 and CSFV003 ranged from 2.31% to 13.55%, the inter-assay CV was 1.77 % to 10.62 %, indicating that the E2 strip had a good repeatability and reproducibility.

**Stability analysis of CSFV E2 test strips**

The E2 strips were stored in a dry place at room temperature and serial diluted serum samples were used for the determination of strip sensitivity and specificity every three months. As shown in Table 2, the sensitivity of the E2 strips was stably observed to be 1:102400. The outward appearance of the strips was fine and there was
no cross reaction with negative serum in 12 months.

**Application of CSFV E2 test strips in detecting clinical samples**

A total of 138 swine serum samples collected from pig farms distributed in Henan province of China were separately diluted to 1:200 using saline solution and were tested by the E2 strips as well as three commercial ELISA kits, such as IDEXX CSFV AbTest Kit, CIVTEST SUIS HC/PPC and VDPro® CSFV AB C-ELISA. As shown in Table 3, the positive rate of E2 strip corresponding to IDEXX CSFV AbTest Kit, CIVTEST SUIS HC/PPC and VDPro® CSFV AB C-ELISA were 97.39% (112/115), 93.04% (107/115), and 97.39% (112/115), respectively. The sensitivity of E2 strip corresponding to IDEXX CSFV AbTest Kit, CIVTEST SUIS HC/PPC and VDPro® CSFV AB C-ELISA were 89.60% (112/125), 83.59% (107/128), 98.25% (112/114), respectively. The corresponding specificities were 76.92% (10/13), 60.00% (12/20), and 87.50% (21/24), respectively. The coincidence rates of E2 strip corresponding to IDEXX CSFV AbTest Kit, CIVTEST SUIS HC/PPC and VDPro® CSFV AB C-ELISA were 88.40% (122/138), 86.23% (119/138), and 96.38% (133/138), respectively. A kappa value of E2 strip corresponding to IDEXX CSFV AbTest Kit, CIVTEST SUIS HC/PPC and VDPro® CSFV AB C-ELISA were 0.495 (P <0.0001), 0.342 (P <0.0001), and 0.872 (P <0.0001) by SPSS analysis. It showed that E2 strips had good consistency with VDPro® CSFV AB C-ELISA kit.

The positive rate of the CnC2 strip corresponding to IDEXX CSFV AbTest Kit, CIVTEST SUIS HC/PPC and VDPro® CSFV AB C-ELISA were 97.30% (108/111), 93.69% (104/111), and 90.99% (101/111), respectively. The sensitivity of CnC2 strip corresponding to IDEXX CSFV AbTest Kit, CIVTEST SUIS HC/PPC and VDPro® CSFV AB C-ELISA were 86.4% (108/125), 81.25% (104/128), and 88.59% (101/114), respectively. The specificity were 76.92% (10/13), 65.00% (13/20), and 58.33% (14/24), respectively. The coincidence rates of CnC2 strip corresponding to IDEXX CSFV AbTest Kit, CIVTEST SUIS HC/PPC and VDPro® CSFV AB C-ELISA were 85.51% (118/138), 84.78% (117/138), and 83.33% (115/138). The kappa value of CnC2 strip corresponding to IDEXX CSFV AbTest Kit, CIVTEST SUIS HC/PPC and
VDPro® CSFV AB C-ELISA were 0.427 (P <0.0001), 0.340 (P <0.0001), and 0.447 (P <0.0001) by SPSS analysis. These results indicated that the E2 strip was in accordance with the commercial ELISA kits and is more suitable for the detection of clinical swine serum samples, which shows a better performance than the former developed CnC2 strip.

Discussion
In the present study, a rapid immunochromatographic strip for the detection of CSFV antibody was developed based on the eukaryotic expressed E2 protein, which was efficiently glycosylated and mainly present as native homodimer. The comparative evaluation showed that the sensitivity and stability of new E2 test strip were both higher than that of the former developed CnC2 strip. Envelope glycoprotein E2 of CSFV has been proved to be the major antigen that induces neutralizing antibodies with multiple identified epitopes and makes it to be the focus for CSF subunit vaccine development and serological diagnosis. Indeed, routinely applied commercial ELISA kits for E2 antibody detection produced by different manufacturers have been available on the market for several years, including the presently used IDEXX CSFV Ab Test Kit and the VDPro® CSFV AB C-ELISA. These E2-based ELISA kits usually show a similar sensitivity compared to virus neutralization test (VNT) combined with higher specificity. The preference of E2 over E\textsubscript{map} for diagnostic formulation against CSFV has also been confirmed and reported in other studies (15). Taken together, the E2 protein is really a suitable antigen to be chose for the development of immunochromatographic assay.

Utilizing the Bac-to-Bac baculovirus expression system, the E2 protein has been presently expressed and the purified E2 protein mainly present as homodimer. It is consistent with the native glycosylated E2 protein as previously reported (17,19-21). The results of western blot have demonstrated that compared to the E2 monomer, the homodimer has higher affinity to anti-CSFV-E2 mAb WH303, revealing that the dimer conformation and glycosylation of E2 protein are important for the induction of neutralizing antibodies. It has been confirmed that the glycosylation of E2 protein
plays an essential role in its immunogenicity, which may be achieved by influencing its conformation (7). Thus, the E2 protein maintains a better immunogenicity than the CnC2 protein, which was expressed in *E. coli* without the post-translational modifications and composed of N-terminal 176 amino acids. The recombinant E2 protein produced in this study is composed of the entire extracellular region of E2, which contains more conformational and linear epitopes than the CnC2 protein. Consequently, it has been shown that the sensitivity and stability of the new E2 strip are better than that of the former CnC2 strip, indicating the feasibility of the new developed immunochromatographic strip has been improved. Besides, the CnC2 protein was produced as inclusion bodies in *E. coli*, of which the preparation of the protein requires dialysis and refolding. The new E2 protein was soluble and the purified protein could be used for the conjugation with colloidal gold directly. Hence, the simplification of protein preparation facilitates the development of test strips and enhances their availability.

Although the new label materials such as colloidal selenium, up-converting phosphors nanoparticles and so on were risen, we still chose the conventional colloidal gold in this study due to that colloidal gold is more stable, sensitive and specific. The displayed results of E2 strip can be checked by naked eyes in 5-10 min. However, we found that the value of $G/D \times A$ was lower when the dilution factor was less than 1:1600 in sensitivity assays. This phenomenon was called “hook effect” - a false-negative result from immunoassays due to very high concentrations of the analyte (23). Thus, to avoid false-negative result caused by “hook effect”, we have diluted the sera to 1:200 prior to the detection.

At present in China, ELISA is the golden standard for CSFV antibody determination. For using the ELISA kits, the serum samples always need to be sent to professional laboratories with extra costs, labor and time. For the test strip, it is more suitable for field utilization to monitor the level of CSFV antibodies in vaccinated pig populations. In summary, the strip assay is portable, rapid, low cost and easy to use. The weakness of the E2 strip developed in this study is that it can not differentiate the virus-infected animals from vaccine immunized animals. Thus, differentiation
diagnosis between infected and vaccinated animals needs further investigation.

**Author Contributions:** Conceptualization, Yilin Bai and Gaiping Zhang; Data
curation, Yilin Bai, Rui Jia, Jun Luo, Yaning Sun and Gaiping Zhang; Formal analysis,
Yilin Bai and Yaning Sun; Investigation, Yilin Bai, Rui Jia, Qiang Wei and Li Wang;
Writing – original draft, Rui Jia; Writing – review and editing, Rui Jia, Yilin Bai,
Yiwei Li and Jun Luo.

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**ETHICS APPROVAL AND CONSENT TO PARTICIPATE**
Ethics approval and consent to participate is not applicable because sample collection
has been gathered.

**AVAILABILITY OF DATA AND MATERIALS**
All data and materials presented in this study are available on request from the
corresponding authors.

**COMPETING INTERESTS**
The authors declare no competing interest.

**CONSENT FOR PUBLICATION**
The authors declare consent for publication

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Tables

Table 1 The specificity of the E2 test strip for detecting reference swine serum samples.

<table>
<thead>
<tr>
<th>Serum samples</th>
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<td>ASFV positive</td>
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Table 2 Comparison of the stability between the E2 strips

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Table 3 Comparison of the test strips with three commercial ELISA kits.

<table>
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<th>Strip</th>
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<th>CIVTEST</th>
<th>VDPro®</th>
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<td>10</td>
<td>24</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>125</td>
<td>13</td>
<td>128</td>
<td>20</td>
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Figure 1

The structure and packaging of the strips. A. Schematic of the strip structure composed of sample pad, conjugated pad, back plate, absorbent pad, NC membrane, T line and C line. B. The steps of packaging after assembly of the test strips.
Figure 2

Procedure and principle of test strips. A. When the sample containing the anti-CSFV IgG, both T line and C line turn red. B. When the sample does not contain anti-CSFV IgG, T line shows no visible color change and C line changes red.
Figure 3

Characterization of the recombinant CSFV E2 protein. A. Expression analysis of denatured CSFV E2 by western blot with anti-his mAb. B. SDS-PAGE analysis of the denatured E2 protein and nondenatured E2 protein after being purified. C. Western blot analysis of the denatured E2 protein and nondenatured E2 protein after being purified with anti-CSFV-E2 mAb WH303. D. The titer of CSFV standard positive serum determined by indirect ELISA using the purified E2 protein as coating antigen.

Figure 4

Concentration of E2 protein

Color change of coupling solution

Solubleness

Dissolution

Sedimentation

Color change

Red

Gray purple
Determination of the optimal concentration of protein for gold nanoparticle labeling. The optimal concentration of protein for making E2-AuNPs conjugate was 4 ng/mL, which was the minimum concentration of protein to keep the color of mixed solution red for stabilizing colloidal gold.

Figure 5

TEM images and particle size distribution of CSFV E2 protein with AuNPs. A, B & C. TEM images of E2 protein AuNPs and E2-AuNPs complex, respectively. Distribution of the particle sizes of E2 protein (D),
gold nanoparticles (E) and E2-AuNPs complex (F) were about 10 nm, 30 nm and 100 nm, respectively. In addition, a part of E2 protein was near to 800 nm due to aggregation.

**Figure 6**

Determination of the sensitivity of E2 test strips. A and B. The standard curve between G/D ×A value and the dilution of standard E2 positive serum. C and D. Relative optical density (ROD) curve of chromogenic

Figure 7

Specificity of E2 test strips. No cross reaction with other virus antibodies was observed by E2 test strip detecting.