Short Communication

Immunochromatographic test (ICT) for the detection of antibody against ALV-J

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Abstract

ALV-J is an important pathogen in the poultry industry in China. In this study, we developed an immunochromatographic test (ICT) assay for the detection of antibody against of ALV-J to evaluate ALV-J infection. Gp85 protein of ALV-J was employed as a capture antigen. Antigen-antibody reaction was detected by either colloidal-gold conjugated gp85 protein or monoclonal antibody of ALV-J. The high consistency of results in ICT and ELISA indicated that the ICT is reliable. This suggests that the gp85-based ICT can be used as a rapid on-site diagnostic method of ALV-J.

Keywords: immunochromatographic test, antibody, ALV-J, rapid diagnosis

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Introduction

ALV-J is one of the pathogens causing serious harm to the poultry industry in China. Eliminating infected chickens is an effective method to control the spread of ALV-J (Feng and Zhang, 2016). However, coinfection and recombination of ALV-J with other pathogens have been reported (Li *et al*., 2021; Li *et al*., 2020; Sun *et al*., 2020). Therefore, early rapid and accurate detection and diagnosis is very important. Enzyme-linked immunosorbent assay (ELISA) (Yun *et al*., 2013), indirect fluorescence assay (IFA) and polymerase chain reaction (PCR) (Smith *et al*., 1998) are usually used in diagnosis. ELISA is a simple, specific but expensive method. IFA is a sensitive and specific method however, it is time consuming. DNA based detection methods are highly sensitive but require specific instruments and trained operators. Thus, a rapid, cheap and simple test is demanded in the detection of clinical ALV-J infection. In this study, a colloidal gold test strip was developed for simple and rapid detection of ALV-J antibody to evaluate ALV-J infection.

Materials and Methods

Protein expression: We compared isolated wild ALV-J strains published on NCBI in the last ten years, and selected 504 bp relatively conservative and higher antigenicity of ALV-J gp85 protein as antigen. The selected gp85 gene sequences were expressed with forward primer (5'-GCGGATCCATCAAGAACGGAA CAACACG-3'), and reverse primer (5'-GCGCGCAAG CTTGTCCCCACAAATCAAGAAAATA-3'). A 504 bp target fragment was obtained by PCR amplification and PCR products were transfected into the DH5α receptor cells. The objective fragment was induced by expression in Escherichia coli, then, the thallus was cleaved by ultrasound. The expressed proteins were purified by dialysis. The gp85 antibodies in ALV-J positive serum were used to detect the reactogenicity and specificity of antigen by Western blot.

Preparation of monoclonal antibody against ALV-J: BALB/c mice (6 weeks old) were immunized subcutaneously by purified protein. The mouse with the highest serum titer was sacrificed and spleen cells were fused with SP2/0 myeloma cells. Positive hybrioma cells were cultured for monoclonal antibody (mAb) production. The animal experiments were approved by the Shandong Agricultural University Animal Care Committee.

Preparation of colloidal gold particles: Colloidal gold particles were prepared as reported (Liu *et al*., 2012). Briefly, 1% chloroauric acid solution was boiled, mixed with an equal volume of 1% sodium citrate solution and stirred for 10 mins until the color of solution to be stable. Transmission election microscopy observation revealed that the diameter of colloidal gold particles was about 25 nm.

Preparation of the colloidal gold conjugate: Different amounts of 25 mM K_2CO_3 were used to adjust pH gradient of colloidal gold solution from 7.1 to 7.9. Different concentrations of purified gp85 protein were added to colloidal gold solution (10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20 µg/mL) with gentle stirring at room temperature for 20 mins. The mixture was blocked by different concentrations of BSA (0.5%, 0.8%, 1.0%, 2.0%, and 3.0%) and then centrifuged at 5 000 g for 20 mins. The precipitate was redissolved in 0.01M PBS (pH 7.4) containing 1% BSA and 2.5% sucrose and stored at 4℃.

Preparation of the immunochromatographic strip: The antigens and prepared mAb were diluted to 1.0 mg/mL, 1.5 mg/mL and 2.0 mg/mL with 0.01 M sodium PBS (Ph 7.4). The diluted gp85 protein and mAb were transferred on to the Nitrocellulose membrane to form the test (T) and the control (C) line. Polyester cellulose membrane was soaked in immune colloidal gold solution for 10 minutes and then was dried at 37℃. The absorbent paper and sample pad were cut to an appropriate size for assembling according to Figure 1A. The test strips were stored in a desiccator at room temperature.

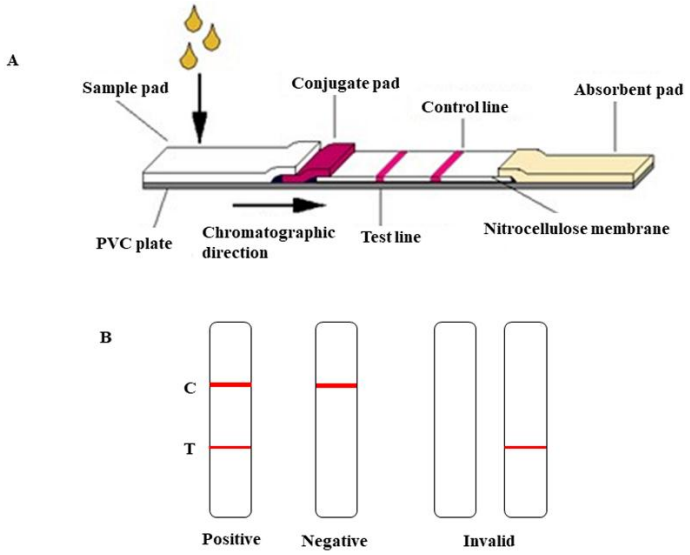

Figure 1 The immunochromatographic test strip. (A) Schematic diagram of the immunochromatographic strip. (B) The results of immunochromatographic test.

Sensitivity, specificity and accuracy of the immunochromatographic strip: Serial diluted (1 mg/mL, 100 µg/mL, 10 µg/mL, 1 µg/mL, 100 ng/mL, and 10 ng/mL) ALV-J antibody positive and negative sera were dropped on the sample pad, respectively, to evaluate sensitivity. ALV-J infected chicken serum was used as a positive. Un-infected chicken serum was used as a negative. The specificity was determined using different antibody of Marek's disease virus (MDV), reticuloendotheliosis virus (REV) and avian influenza virus (AIV). To evaluate the accuracy, we compared the detection results of clinical 278 serum samples tested with the test strips and IDEXX commercial ELISA kits.

Results and Discussion

To detect the purified gp85 protein, chicken positive serum which was detected by ALV-J antibody detection kit (IDEXX, America) was used as the primary antibody for gp85, followed by HRP-labeled goat anti-chicken IgG (Bioss, China) as the secondary antibody. Western Blot result showed that the purified protein was capable of binding with gp85 antibody with a good reactogenicity and specificity (Figure 2A).

Our results demonstrate that the optimal pH of colloidal gold solution was 7.5. The optimal concentration of purified gp85 labelled by colloidal gold was 20 µg/mL. One percent BSA was the optimal concentration of blocking buffer. Following the experimental procedure, the optimal concentrations of coating antigen and antibody were both 2 mg/mL.

ALV-J antibody positive and negative sera were used to determine the validity and sensitivity of the immunochromatographic strip. As shown in Figure 1B, the appearance of both test and control lines was judged to positive results. The negative result was judged by the presence of only the control line. The test was invalid if only the test line appeared or no control line appeared. The antibodies of different antigens were used to determine the specificity of the immunochromatographic strip. We found that the immunochromatographic strip was specific for ALV-J antibody (Figure 2B).

A total of 278 clinical sera samples were detected by the developed test strips and ELISA kit, respectively. The result showed that the positive rate of test strips was 57.6% (Figure 2C) and ELISA was 58.7%. The rate of matching was 98.12%, indicating that the test strip had good specificity, sensitivity and accuracy.

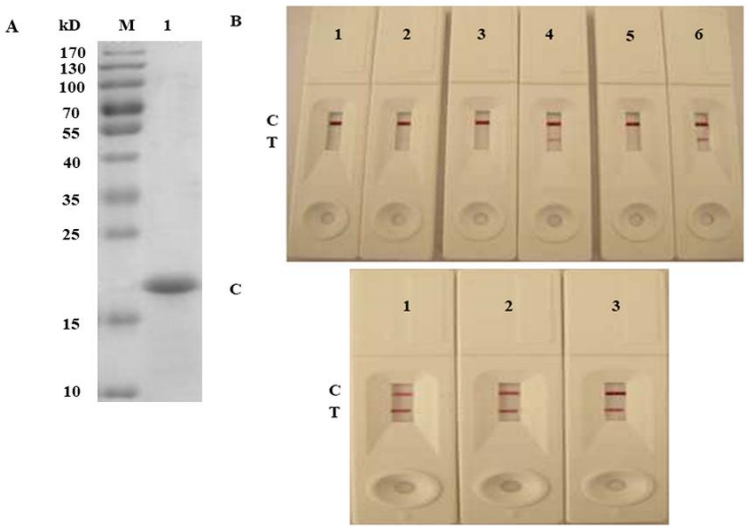

Figure 2 A) The reactogenicity and specificity of recombinant protein were analyzed by Western blot. (B) Specificity test of immunochromatographic test strip. 1: MDV infection sera sample; 2: REV infection sera sample; 3: AIV infection sera sample; 4: ALV-J infection sera sample; 5: negative sera sample; 6: positive sera sample; (C) Some of the results of clinical sera samples. 1, 2, and 3 from different farms.

ALV-J infection causes enormous economic loss to the poultry industry in China (Qu *et al*., 2016; Zhou *et al*., 2019). ELISA, immunofluorescence assays, PCR and virus isolation, which require specialized technologies and equipment, are usually applied to detect ALV-J infection. Immunochromatographic test (ICT) assays are rapid, simple, cheap and have no need of special equipment for detection (Feng *et al*., 2015), thus they is convenient for primary level and field use.

Conflict of Interest: The authors declare no conflicts of interest.

Acknowledgements

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