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Development of a convenient immunochromatographic strip for the diagnosis of vesicular stomatitis virus serotype Indiana infections

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A rapid and simple immunochromatography strip (ICS) test for the specific detection of vesicular stomatitis virus serotype Indiana (VSV-IND) using two distinct monoclonal antibodies MAbs (1A2 and 4C3) against the G protein of VSV-IND was developed. The MAb 1A2 was conjugated with colloidal gold, and the MAb 4C3 and goat anti-mouse IgG were sprayed onto a nitrocellulose membrane in strips at positions designated T and C, respectively. The results showed that samples of VSV-IND combined with CG-MAb 1A2, and that these complexes were captured by MAb 4C3 at the test line (T), resulting in the appearance of a purple band. When samples did not contain VSV-IND or when they contained a quantity of VSV-IND below the detection limit of the test, only the control line (C) was visible. The analysis of the sensitivity of the test demonstrated that the lowest detected quantity of VSV-IND was $1.85 \times 10^{3.0}$ $TCID_{50}$/ml. Storage of the ICS test at room temperature for 6 months or at 4 °C for 12 months did not change their sensitivity and specificity. In clinical trials using RT-PCR as a reference test, the relative specificity and sensitivity of the ICS were determined to be 98.9% and 91.4%, respectively. Based on these results, the ICS test developed may be a suitable tool for rapid on-site testing for VSV-IND infection.

1. Introduction

Vesicular stomatitis (VS) is an acute highly contagious human zoonotic infectious disease in cattle, horses, pigs and other mammals that is caused by the vesicular stomatitis virus (VSV) (Letchworth et al., 1999). VSV is a member of the genus Vesiculovirus in the family Rhabdoviridae. The VSV genome is an 11 kb nonsegmented single-stranded RNA of negative polarity that encodes five mainly structural proteins: nucleocapsid (NC), phosphoprotein (P), matrix (M), glycoprotein (GP) and large polymerase (L) (Rainwater-Lovett et al., 2007). Among these proteins, the G protein is the only envelope protein of VSV that induces neutralizing antibodies and is thus recognized as a protein candidate for the development of diagnostic methods.

At present, the classical detection methods for VSV antibodies or antigens in clinical samples include virus isolation, complement fixation (Jenney et al., 1958) and neutralization tests (Alvarado et al., 2002), ELISA (Ferris and Donaldson, 1988; Vernon and Webb, 1985; Zhou et al., 2001), RT-PCR (Nunez et al., 1998; Rodriguez et al., 1993), hemi-nested PCR (Hofner et al., 1994), and real-time PCR (Hole et al., 2006; Wilson et al., 2009). However, the application of these methods is limited due to the lengthy running time, the required technical expertise and the necessity of specialized laboratory equipment or facilities. Therefore, these methods are unsuitable for the analysis of clinical samples, and it is necessary to develop a rapid, specific and easily performed assay for the detection of VSV.

The immunochromatographic assay is a new technique in which a colloidal gold-labeled antigen or antibody is used as a tracer to detect antibody or antigen, respectively. This assay has been widely applied for the diagnosis of many contagious human diseases. Recently, it has been effectively used to detect some animal viruses, such as porcine rotavirus (PRV) (Kang et al., 2007), avian influenza virus (AIV) (Peng et al., 2008), Japanese encephalitis virus

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(JEV) (Li et al., 2010), white spot syndrome virus yellow head virus (Sithigorngul et al., 2011), porcine reproductive and respiratory syndrome virus (PRRSV) (Li et al., 2011) and foot-and-mouth disease virus (FMDV) (Jiang et al., 2011).

The aim of this study was to develop an immunochromatographic strip for the rapid detection of VSV-IND using two high-affinity monoclonal MAbs (1A2 and 4C3) that bind to different epitopes in the G protein of VSV-IND. The sensitivity and specificity of the immunochromatographic strip were evaluated using conventional RT-PCR as a reference test. In clinical trials, 125 clinical specimens from animals with suspected VSV-IND infections were analyzed by both the RT-PCR and the ICS test. The results indicated that there was a high level of agreement between the RT-PCR and ICS assays ($\kappa = 0.92$).

2. Materials and methods

2.1. Virus strain

Vesicular stomatitis virus serotype Indiana (VSV-IND) was stored at the College of Animal Science and Veterinary Medicine, Jilin University (Changchun, China). The viruses were produced and passaged in Vero (African green monkey kidney) cells, as reported previously, and purified by sucrose density gradient centrifugation. The viruses were stored at −80 °C until needed. Several other viruses, including foot-and-mouth disease virus (FMDV), swine vesicular disease virus (SVDV), vesicular exanthema of swine virus (VESV), porcine reproductive and respiratory syndrome (PRRSV) and classical swine fever virus (CSFV) were kindly provided by the State Key Laboratory of Zoonosis, Ministry of Education, Institute of Zoonosis and Animal Research Center, Jilin University (Changchun, China). Nitrocellulose membranes were purchased from Millipore. Goat anti-mouse antibody and chloroauric acid were purchased from Sigma.

2.2. Preparation of the G protein

The G gene of VSV-IND was amplified by revere transcription (RT)-polymerase chain reaction (PCR), with specific primers (forward primer: 5′-CGGATCCGTCAAAATGCCCAAGAGTCACAA-3′, reverse primer: 5′-GGAATTCGGTTCCTGGGTTTTTAGGAGC-3′). The target region for RT-PCR spanned nucleotides 3252–4061 of the G protein of VSV-IND (GenBank accession no.: AF473864). Then, the PCR products were cloned into the pGEX-4T-1 vector. The positive recombinant plasmid was transformed into *Escherichia coli* BL21 cells, and protein expression was induced with isopropyl-thiogalacto-pyranoside (IPTG). The bacterial culture was collected and centrifuged at 4000 × *g* for 10 min. The bacterial pellet was resuspended and sonicated to obtain a clear lysate. The target protein (GST-G) was purified using a commercial protein purification product (Glutathione Sepharose 4B, Sweden). Then, the purified protein was divided into small aliquots of 2 mg/ml prior to storage at −80 °C.

2.3. Preparation of monoclonal antibodies against the G protein

The MAbs against the G protein were produced based on the protocol of Kohler and Milstein (1975). Briefly, 6-week-old female BALB/c mice were immunized subcutaneously with 100 μg of the recombinant GST-G protein at 2-week intervals. The splenic cells of selected mice were isolated the 3rd day after the third immunization, and fused with SP2/0 cells in the log phase. Fusion was promoted with 50% polyethylene glycol (PEG)-4000 (Sigma Aldrich, MO, St. Louis, USA). Hybridoma culture supernatants were screened using ELISA for MAbs that bound G protein but not GST protein. The positive cells were cloned continually by the method of limiting dilution and screened according to the specificity of the MAbs that they produced. Then, the selected hybridoma clones were propagated in vivo by intraperitoneal injection into liquid paraffin-pretreated abdominal cavities of BALB/c mice. Subsequently, the ascitic fluids were harvested, and the MAbs were purified using the ImmunoPure IgG Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Finally, the activities of the MAbs were evaluated by Western blot analysis and indirect immunofluorescence assays (IFAs).

2.4. Western blot and indirect immunofluorescence assay (IFA)

Western blot analysis was performed as previously described (Kotani et al., 2010). Purified GST-G or GST protein (100 μg) was mixed with sample buffer and resolved by 10% SDS-PAGE. The transfer membranes were probed with MAbs (1A2 and 4C3) and an HRP-conjugated anti-mouse IgG antibody (Sigma–Aldrich, MO, St. Louis, USA).

Cytopathic Vero cells grown in a cell culture box (Thermo, Germany) were washed with PBS and fixed at room temperature for 30 min with 4% formaldehyde that was freshly prepared from paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.2). Immunofluorescence staining was performed as described previously (Saarikettu et al., 2010). The plated cells were mounted and observed under an inverted confocal microscope.

2.5. ELISA additive tests

Additive ELISA was performed as previously described (Friguet et al., 1983; Li et al., 2010). The correlation of the epitopes recognized by the two MAbs was measured with the additive index (AI). To estimate the epitope correlation, the lowest AI reported was used as the threshold for determining whether the MAbs recognize different epitopes on VSV-IND.

2.6. Preparation of colloidal gold and colloidal gold-MAb conjugates

Colloidal gold particles with a mean diameter of 30 nm were prepared according to the literature protocol (Frens, 1973; Hermanson, 2008). Under rapid magnetic stirring, 1.4 ml of 1% trisodium citrate solution (w/v) was rapidly added to 100 ml of 0.01% aqueous chloroauric acid ($HAuCl_4$) solution (w/v) at 100 °C and boiled for 5 min. After boiling for 10 min, the colloidal gold was gradually cooled. The pH of the solution was adjusted to 8.4 using 1% potassium carbonate (wt/vol), and then the solution was stored at 4 °C in a dark-colored glass bottle. The MAb 1A2 (200 μl; 1 mg/ml) was added dropwise into 10 ml of pH-adjusted colloid gold solution. The mixture was vigorously stirred for 30 min, and then 2.5 ml of 10% (wt/vol) BSA was added to block excess reactivity of the gold colloid. The mixture was then stirred for an additional 30 min. After the mixture was centrifuged at 10,000 × *g* at 4 °C for 30 min, the gold pellets were suspended in 1 ml dilution buffer (20 mM Tris/HCl buffer (pH 8.2) containing 1% (w/v) BSA, 3% (w/v) sucrose and 0.02% sodium azide). The sizes and shapes of the unconjugated colloidal gold and the colloidal gold-conjugated antibodies were characterized using transmission electron microscopy.

2.7. Preparation of the immunochromatographic strip (ICS)

The immunochromatographic strip includes four components: a sample pad, a conjugate pad, a nitrocellulose membrane, and an absorbent pad. MAb 4C3 (2 mg/ml) and goat anti-mouse IgG (2 mg/ml) dissolved in PBS were loaded onto the test and control lines on the nitrocellulose membrane using an XYZ3050 Dispense