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Development of a multiplex lateral flow strip test for foot-and-mouth disease virus detection using monoclonal antibodies

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A B S T R A C T

Foot-and-mouth disease (FMD) is one of the world's most highly contagious animal diseases with tremendous economic consequences. A rapid and specific test for FMD diagnosis at the site of a suspected outbreak is crucial for the implementation of control measures. This project developed a multiplex lateral flow immunochromatographic strip test (multiplex-LFI) for the rapid detection and serotyping of FMD viruses.

The monoclonal antibodies (mAbs) against serotypes O, A, and Asia 1 were used as capture mAbs. The mAbs were conjugated with fluorescein, rhodamine or biotin for serotype O, A and Asia 1, respectively. The detection mAbs which consisted of a serotype-independent mAb in combination with one serotype A-specific mAb and one Asia 1-specific mAb, were each colloidal gold-conjugated. The strips used in this study contained one control line and three test lines, which corresponded to one of the three serotypes, O, A or Asia 1.

The newly developed multiplex-LFI strip test specifically identified serotype O ($n = 46$), A ($n = 45$) and Asia 1 ($n = 17$) in all tested field isolates. The sensitivity of this strip test was comparable to the double antibody sandwich ELISA for serotypes O and A, but lower than the ELISA for serotype Asia 1. The multiplex-LFI strip test identified all tissue suspensions from animals that were experimentally inoculated with serotypes O, A or Asia 1. FMD viruses were detected in 38% and 50% of the swab samples from the lesion areas of experimentally inoculated sheep for serotypes O and A, respectively.

The capability of the multiplex-LFI strip tests to produce rapid results with high specificity for FMD viruses of multiple serotypes makes this test a valuable tool to detect FMD viruses at outbreak sites.

1. Introduction

Foot-and-mouth disease (FMD) remains one of the world's most widespread epizootic and a highly contagious animal disease that affects a wide host range of cloven-hoofed farm animals, such as cattle, buffaloes, pigs, sheep, and goats, and approximately 70 wildlife species (Coetzer et al., 1994). The rapid spread of this disease, especially in pigs and dairy cattle, can cause major economic losses in countries that were previously FMD free. Over 100 countries around world are not considered FMD free according to the report by the World Organisation for Animal Health (OIE) (World Organisation for Animal Health, 2008). FMD is caused by a single-stranded RNA virus belonging to the family *Picornaviridae*. There are seven serotypes of the FMD virus (FMDV), including O, A, C, Asia 1, SAT 1, SAT 2 and SAT 3. There are a large number of subtypes within each serotype as a result of extensive genetic and antigenic variations (Domingo et al., 2003; Knowles and Samuel, 2003). The features that make FMD difficult to control and eradicate include the wide range of hosts, ability of small doses to infect, rapid replication, high levels of viral excretion and multiple forms of transmission (Alexandersen et al., 2003). Frequent outbreaks and fast spreading of FMD indicate the need for rapid tests capable of performing on-site diagnosis and serotyping simultaneously.

FMD is clinically indistinguishable from other vesicular diseases such as vesicular stomatitis (VS) and swine vesicular disease (SVD) (Alexandersen et al., 2003; Jamal and Belsham, 2013). Therefore, laboratory-based diagnoses are necessary. Various laboratory methods are presently used for FMDV detection, including virus isolation, real-time reverse-transcription (RRT) PCR and double antibody sandwich (DAS) enzyme-linked immunosorbent assay

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(ELISA). However, these tests are difficult to perform on-site because they require laboratory operations, well-trained personnel, and special equipment/facilities. Virus isolation generally requires up to four days before the results can be obtained, and antigen ELISA and RRT-PCR require four to five hours. Moreover, epithelial or vesicular fluids need to be shipped from the scene of the suspected outbreak to the diagnostic laboratory (World Organisation for Animal Health, 2008). Many factors, such as transportation delays, incorrect transportation media and temperatures during shipment, may affect diagnostic accuracy.

A rapid and specific test for FMD diagnosis at the site of a suspected outbreak may permit the suitable implementation of control measures (Jamal and Belsham, 2013). Lateral flow immunochromatographic (LFI) strip tests are widely used for the diagnosis of infectious diseases because these tests are quick and simple to perform on site during a major epidemic (Kang et al., 2014). The LFI strip test was used to rapidly (<10 min) confirm FMD in the field during the 2007 FMD outbreak in UK. Strip tests are efficiently applied for the detection of specific antibodies against FMDV non-structural proteins (Wu et al., 2011) and structural proteins of serotypes A, Asia 1 and O (Yang et al., 2010a; Jiang et al., 2011a,b; Lin et al., 2011). However, polyclonal sera from rabbits and guinea pigs were used as the capture and detection antibodies in these tests.

Monoclonal Ab-based chromatographic strip tests for FMD diagnosis were developed and reported for the serotype-independent detection of FMDV (Reid et al., 2001; Ferris et al., 2009). The strip test was as sensitive as the conventional antigen ELISA for FMDV detection in the epithelial suspensions tested, and it exhibited an equivalent sensitivity for the detection of FMDV serotypes O, A, C and Asia 1 in cell culture supernatants. Only one specific antigen can be detected, and the specific capture antibody is immobilized on the membrane surface of the strip in a general LFI strip test. In this case, the strips must be custom-made specifically for each test. SVANOVA Biotech AB has also marketed the Pan-serotypic LFI strip test. One limiting factor for the serotype-independent strip tests is that they cannot identify the FMDV serotype, which reduces their potential benefit in endemic countries. Determinations of the serotype involved in outbreaks are important because there is no cross protection and specific clinical signs of each serotype, which would allow the implementation of appropriate control/vaccination programs (Jamal and Belsham, 2013).

FMDV A, O and Asia 1 are the most widespread serotypes, and they are found in Africa, the Middle East, Asia, limited areas of South America and occasionally Europe. We previously developed a single serotype LFI strip test for FMDV (O, A, and Asia 1) detection using serotype-specific mAbs as the capture mAb and a serotype-independent mAb as the detection mAb (Yang et al., 2013). However, a single test is specific for only one serotype. The strip test for serotype O cannot detect the isolate, ECU4/10, which was isolated in Ecuador in 2009 and 2010 (Maradei et al., 2011). Amada et al. (2014) developed a multiplex strip test for the serological survey of hantavirus infections in humans, but there was no test available for multiplex FMDV detection using a single LFI strip test, until now. Development of a multiplex-LFI strip test that is capable of detecting several FMDV serotypes will have great potential to diagnose and serotype FMD in the field to confirm the clinical findings. The early detection and typing of FMDV infections will provide important information for vaccine selection and facilitate disease control.

The current study developed a rapid multiplex-LFI strip test for the detection of FMDV serotypes O, A and Asia 1 using commercially available strips. The test lines of each strip in this study are sprayed with antibodies against commonly used conjugates or binding proteins, such as anti-rhodamine, anti-fluorescein and biotin-binding protein. To our knowledge, this study is the first report to use this technique in an LFI strip test. The performance of the newly developed multiplex-LFI strip tests for the detection of multiple FMDV serotypes was compared to other methodologies. This test can produce rapid results, and it would be useful for the early diagnosis and serotyping of FMDV O, A and Asia 1 on-site during FMD outbreaks.

2. Materials and methods

2.1. Ethics statement

All animal work was performed in compliance with the Canadian Council on Animal Care guidelines. The Animal Care Committee at the Canadian Science Centre for Animal and Human Health approved all studies.

2.2. Preparation of FMDV and plaque assay

All viruses used in the study were provided by the FAO/OIE World Reference Laboratory for FMD (Pirbright Institute, Pirbright, UK). The procedures for the preparation of FMDV were performed as previously described (Yang et al., 2007). For plaque assay, Mengeling–Vaughn Porcine Kidney (MVPK) cells were cultured in Alpha Modification of Eagle's medium (AMEM; WISENT Inc., Canada) supplemented with 10% fetal bovine serum in 6-well plates. FMDV serotypes O1/BFS, A24/Cruzeiro and Asia 1/Shamir were serially 10-fold diluted with the culture medium. Subconfluent MVPK monolayers in 6-well tissue culture plates were infected with virus and overlaid with AMEM/1% SeaPlaque agarose. Overlaid plates were incubated at 37 °C in a CO_2 incubator overnight. Plaques were counted after the plates were fixed using 10% phosphate-buffered formalin (Fisher Scientific) and stained using 0.05% crystal violet.

2.3. Monoclonal antibody production, purification and conjugation

The procedures for mAb production, purification, biotinylation and colloidal-gold conjugation of mAbs were performed as described in detail previously (Yang et al., 2007, 2013). For fluorescein and rhodamine conjugation, purified mAbs were buffer exchanged against 100 mM carbonate buffer, pH 9.6, using desalting columns (GE col G-25DNA grade, Cat# 17-0853-02). Fluorescein isothiocyanate (FITC) (MW 389.38, Sigma F4247) and rhodamine B isothiocyanate (RITC) (MW 536.08, Sigma R1755) were dissolved in anhydrous DMSO (1 mg/ml) immediately before use. FITC or RITC were added to purified mAbs at a molar excess of a ratio of approximately 20:1 and mixed immediately. The mixtures were incubated at room temperature for 2 h. Unconjugated chemicals were removed using a desalting column. Reagents were stored at 4 °C in PBS with 0.01% NaN_3.

2.4. Lateral flow immunochromatographic strip test for multiplex FMDV detection

The strips that were used for this study were purchased from RapidAssays ApS, (Copenhagen S, Denmark). Each strip contained one control line and three test lines. The control line contained antibodies that bound the mouse antibody that was responsible for the immobilization of the detection conjugate at the control line. The three test lines contained biotin-binding protein, goat-anti-rhodamine and goat anti-fluorescein, which captured antibody–antigen complexes and free conjugated capture mAbs. Viruses in test samples (50 μl) were mixed with the mAb cocktail (50 μl), which contained capture and detection mAbs in the running buffer (RapidAssays ApS, Copenhagen, Denmark) and formed complexes. Each strip was dipped into a tube that contained the