Development, Evaluation, and Application of Lateral-Flow Immunoassay (Immunochromatography) for Detection of Rotavirus in Bovine Fecal Samples†

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A lateral-flow immunoassay (LFT) was developed to detect bovine rotavirus in fecal samples. Using samples (n = 74) from diarrheic calves, a comparison of the LFT with a commercial latex agglutination test (LAT) and transmission electron microscopy (EM) was conducted. When EM was used as the reference method, initial studies of 29 samples indicated 70 and 80% sensitivities of the LFT and LAT, respectively, with both being 100% specific. When the LAT was the reference test, the LFT was 75% sensitive and 91% specific. Additional specimens (n = 45) were tested by the LFT and LAT alone, and results were identical for both methods.

Rotaviruses are the major cause of severe gastroenteritis in human infants and animals worldwide (2, 5–9). Bovine rotavirus (BRV) is responsible for approximately 30% of calf enteritis (3); thus, accurate and rapid diagnostic tests are important for proper treatment and prevention. The objective of this study was to develop and evaluate a lateral-flow immunoassay (LFT) for rapid detection of BRV in fecal samples. Evaluation of the LFT was conducted by comparing its performance to that of transmission electron microscopy (EM) and the latex agglutination test (LAT).

Seventy-four fecal samples from calves with acute diarrhea were tested. Fecal samples and supernatants of BRV-infected cell cultures previously tested by enzyme-linked immunosorbent assay (ELISA) with the Rotazyme II kit (Abbott Laboratories, Abbott Park, Ill.) were used as positive and negative controls for this study (1).

For examination by EM, 10% (wt/vol) suspensions of calf feces were homogenized in 0.01 M phosphate-buffered saline (PBS) (pH 7) and were centrifuged at 1,500 g for 5 min. The supernatant was removed and centrifuged at 28,000 g for 1 h. A 35-μl aliquot of pelleted material was treated with 0.7 ml of double-distilled water, 140 μl of phosphate-stungic acid (pH 7), and 35 μl of 1% bovine serum albumin for 5 min and then was sprayed onto carbon-coated grids for viewing at ×30,000 magnification. The manufacturer’s instructions for testing of human fecal samples were followed to test the bovine feces using the Virogen Rotatest (Wampole Laboratories, Cranbury, N.J.), a rapid LAT which utilizes latex particles coated with antibodies specific for group A rotavirus antigens.

All components of the LFT system, including BRV anti-serum, anti-mouse immunoglobulin G (IgG), antirotavirus antibody-gold conjugated, membrane-blocking reagent, and known positive and negative controls, were evaluated in a series of titration experiments to determine optimum concentrations for the assay. Criteria used in these experiments were the attainment of maximum signal strength for specific line formation (true-positive/true-negative result) with an absence of nonspecific line formation (false-positive/false-negative result) and low background staining. The colloidal gold particles (2 nm; BBI International, Cardiff, United Kingdom) and monoclonal antibody used to prepare the LFT indicator conjugate were handled following the manufacturer’s instructions. Monoclonal antibody clone 3C10 (Research Diagnostics, Inc., Flanders, N.J.) specific to the BRV inner capsid protein p42 was titrated to determine the appropriate concentration needed to stabilize the colloidal gold prior to conjugation. The sensitivity of the resulting antibody-gold conjugate was assayed by immunoblotting onto nitrocellulose. Fecal samples were initially prepared as either 10% (wt/vol) suspensions from solid or semisolid feces or as 20% (vol/vol) suspensions from liquid feces in 0.01 M PBS (pH 7) that were centrifuged at 1,500 g for 10 min, and the supernatants were stored in sterile vials at −80°C until used. After the LFT was optimized, the centrifugation step was eliminated on fresh samples.

Hi-flow Plus Mylar-backed nitrocellulose membranes (Millipore Corp., Bedford, Mass.) were cut into 6-cm by 6-mm strips and were etched at 2.1 and 3.3 cm from the end designated zone 1, the sample application site. Ten microliters of anti-BRV polyclonal antiserum (1:1,000) was spotted at the application step was eliminated on fresh samples.

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which, respectively, are saturated with anti-BRV and anti-mouse IgG antibodies. Following incubation for 30 min at RT, the membrane was evaluated visually. A single colored line appearing in zone 3 (Fig. 1A) indicates the absence of BRV. The concurrent presence of colored lines in zones 2 and 3 (Fig. 1B) indicates the presence of BRV. The absence of line formation indicates an invalid test that must be repeated. Once the test result has developed, the reaction lines, which form within 2 min of sample application, are permanent.

Rapid, accurate diagnosis of BRV as a cause of diarrhea is required to manage disease and to prevent loss of animals. Virus isolation (VI), fluorescent antibody (FA) testing, EM, ELISA, and the LFT are presently used by diagnostic laboratories for detection of BRV in feces (1, 3, 4), but EM, VI, and FA testing require expensive equipment and skilled personnel. Transmission EM is often used as the “gold standard” for virus detection, but it achieves definitive results only when greater than \(10^6\) to \(10^8\) virus particles are present per ml of feces (3). VI and follow-up FA testing are more sensitive than EM because virus is rapidly amplified to detectable numbers in culture, but it takes 3 to 8 days before the BRV cytopathic effect develops in the cells (1). FA testing of fresh intestinal tissue is rapid, but the tissues must be retrieved 4 to 6 h after the onset of diarrhea because the infected villous epithelial cells are rapidly destroyed. ELISA is commonly performed because even small amounts of BRV can be detected in feces 4 to 9 days after the onset of diarrhea and because the test can be completed in less than 4 h (4). The LFT is rapid and sensitive for detection of BRV in feces, but due to rapid dissociation of the latticed latex-virus precipitate, LAT slides must be read without delay. This is the first report on the development of an LFT for the detection of BRV in fecal samples and evaluation of the LFT in comparison to the LAT and EM.

Twenty-nine specimens were evaluated by comparing EM (reference method), the LAT, and the LFT (Table 1). When EM was the reference method, initial studies of 29 samples indicated 70 and 80% sensitivities of the LFT and LAT, respectively, with both being 100% specific. When the LAT was the reference test, the LFT was 75% sensitive and 91% specific. Forty-five additional specimens were evaluated by the LFT (Table 2) with the Virotone Rotatest LAT as the reference test, and there was complete agreement between the test results.

Major advantages of the LFT over other testing formats include rapidity, simplicity, and the need for minimal training of personnel. Unlike the LAT, the formation of permanent lines allows the results to be read at times convenient for the technician. The LFT can be performed virtually anywhere, including laboratories, the office, or the field. Because the LFT is simple to perform, it can be adopted in underdeveloped countries where diagnostic facilities are limited.

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REFERENCES

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\(\pi^n = \text{frequency}/n\), where \(n\) is the portion of samples having a particular outcome.