Evaluation of a Rapid Lateral Flow Point-of-Care Test for Detection of Cryptosporidium

Molly E. Fleece,1 Jack Heptinstall,2 Shaila S. Khan,3 Mamum Kabir,3 Joel Herbein,2 Rashidul Haque,3 and William A. Petri Jr.1*

1Department of Medicine, University of Virginia, Charlottesville, Virginia; 2TechLab, Inc., Blacksburg, Virginia; 3International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh

Abstract. A new rapid lateral flow fecal antigen detection test for Cryptosporidium was evaluated using diarrheal stool samples from a cohort of children in Bangladesh. The test had a sensitivity of 100% and a specificity of 94% when compared with enzyme-linked immunosorbent assay antigen detection.

Diarrheal diseases are a major cause of morbidity and mortality in the world.1,2 Cryptosporidium is an enteric protozoan parasite that is transmitted through the fecal-oral route, typically by consumption of contaminated food and water.3,4 The parasite has a low infectious dose resulting in diarrhea and abdominal pain.3,5 Cryptosporidium is a common cause of waterborne diarrheal disease worldwide, both in developing and developed countries as well as urban and rural areas; however, due to poor sanitation and urban crowding, Cryptosporidium infections are more prevalent in underdeveloped areas.1,4 Although infections do occur in immunocompetent hosts, immunocompromised hosts and children tend to have a more severe and prolonged disease course.4,5 There is need for a practical point-of-care diagnostic test that is rapid, reliable, and feasible for use in the field.

Cryptosporidium lateral flow (TechLab, Inc., Blacksburg, VA) is a newly developed immunochromatographic assay that qualitatively detects Cryptosporidium antigen in fecal specimens. It is a dipstick that uses a monoclonal antibody sandwich design to detect Cryptosporidium oocyst wall antigen. The assay flow begins with a diluted specimen that is drawn up via capillary action, the liquid fraction of which liberates membrane-embedded gold particles conjugated with anti-Cryptosporidium antigens. This mixture then flows to the visible reaction window where additional anti-Cryptosporidium antibodies are immobilized and capture antigen–gold complexes for a visual positive result.

The data presented here are of the first field test of the Cryptosporidium lateral flow focusing on the sensitivity and specificity of this rapid dipstick test. All diarrheal stool samples were collected from a cohort of children living in an urban slum in Bangladesh where Cryptosporidium is prevalent.7 The specimens were tested at the International Centre for Diarrhoeal Disease Research, Bangladesh. The samples were stored on average for 2 years at −20°C until testing in batches. Real-time polymerase chain reaction (PCR) testing had been performed on all diarrheal stool samples before this study.8 As a comparison of measurement of the presence/absence of Cryptosporidium antigen, enzyme-linked immunosorbent assays (ELISAs) were performed using the Cryptosporidium II test (TechLab, Inc.). The lateral flow was tested on 50 diarrheal stool samples known to be Cryptosporidium positive by PCR and 50 negative diarrheal stool samples. In addition, 100 randomly selected diarrheal stool specimens from children 6–12 months of age were tested using Cryptosporidium lateral flow and compared with the results of PCR testing.

Fecal samples were brought to room temperature and mixed thoroughly before beginning the test. Fifty microliters of specimen were transferred via pipette into the specimen dilution tube containing diluent (buffered protein solution). The sample end of a test strip was inserted into the specimen dilution tube. Results were read visually after 10 minutes. A sample was interpreted as positive if both test and control lines were present (Figure 1). The color of the lines ranged from dark red to light pink, recognizing that color intensity did not correlate with strength of positivity. A sample was interpreted as negative if only the control line was visible. The test was considered invalid if the control line was absent.

We first tested 50 diarrheal stool samples known to contain Cryptosporidium DNA by PCR and 50 negative controls. Using ELISA as the reference standard for antigen detection, the Cryptosporidium lateral flow had a sensitivity of 100%, 94% specificity, 89% positive predictive value, and 100% negative predictive value.

*Address correspondence to William A. Petri Jr., Infectious Diseases and International Health, 1709A Carter-Harrison Bldg., University of Virginia, Charlottesville, VA 22908-1340. E-mail: wap3g@virginia.edu

FIGURE 1. Lateral flow test for the detection of Cryptosporidium in stool specimens. The lateral flow on the left is an example of a negative test result where only the control line (upper) is positive. A positive test result is shown on the right with both the control and test lines visible.
negative predictive value (Table 1). Three of the four discrepant specimens (i.e., that were positive by Cryptosporidium lateral flow and negative by Cryptosporidium II test) were confirmed negative via PCR (with the fourth PCR positive).

We also evaluated the field adaptability of the lateral flow by testing 100 randomly selected diarrheal stool samples from the same cohort in Bangladesh, the vast majority of which did not have Cryptosporidium. There were no false positives: none of the 96 Cryptosporidium-negative samples had a positive lateral flow result. Of the four diarrheal samples with detectable Cryptosporidium DNA by PCR, the Cryptosporidium lateral flow detected one true positive sample with a Ct value 31.5. The three PCR (+) samples that were not lateral flow detected one true positive sample with positives: none of the 96 Cryptosporidium results and easy visual result interpretation; however, the other Cryptosporidium II lateral flow has a comparable sensitivity and specificity to the lateral flow test. We concluded that the diarrheal episode increases at higher pathogen loads.

Table 1

| Lateral flow (+) | Cryptosporidium II ELISA (+) | Cryptosporidium II ELISA (−) |
|------------------|-----------------------------|-----------------------------|
| 34               | 4                           | 62                          |

ELISA = enzyme-linked immunosorbent assay.

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