Short Report: Evaluation of a Rapid Point-of-Care Fecal Antigen Detection Test for *Entamoeba histolytica*

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Abstract. Amebiasis is a major cause of morbidity and mortality in the developing world. A reliable point-of-care test would help to improve diagnosis and early treatment. We evaluated a novel rapid fecal antigen detection test for *E. histolytica* (*E. HISTOLYTICA QUIK CHEK*; TechLab, Inc., Blacksburg, VA), in a cohort of children in Bangladesh where amebiasis is endemic. This point-of-care test had a sensitivity of 100% and a specificity of 100% when compared with enzyme-linked immunosorbent assay antigen detection.

Amebiasis is a major cause of parasitic dysentery in developing countries and is estimated to be second only to malaria as the leading cause of protozoan-related mortality worldwide.¹ Infection with *Entamoeba histolytica* can cause a wide range of manifestations ranging from asymptomatic carriage to amebic dysentery and amebic liver abscess.²

Current modes of diagnosis include identification of cysts or trophozoites by microscopy, antigen detection, DNA detection by polymerase chain reaction (PCR), and serologic antibody detection. Stool microscopy has low sensitivity and is unable to differentiate *E. histolytica* from the morphologically similar amoeba *E. dispar*, which is not believed to cause intestinal disease.³ Serologic antibody detection is insensitive early in disease and unable to distinguish active infection from previous exposure.⁴ Real-time PCR is the most sensitive method, but it is expensive and requires skilled personnel, which limits its use.⁵ Fecal antigen detection is becoming more widely used, but requires technical expertise.⁶ Development of a rapid fecal antigen detection test would provide a simple method for diagnosis that may be easily applied in resource-limited settings.

Accurate diagnosis of amebiasis is important for prompt treatment and prevention of progression to invasive disease. We evaluated a point-of-care fecal antigen detection test based on immunochromatographic technology.⁷

This study was approved by the University of Virginia Institutional Review Board and the Ethical Review Committee at the International Centre for Diarrhoeal Disease Research, Bangladesh. Diarrheal and surveillance fecal samples were obtained from a cohort of children living in an urban slum of Mirpur, Bangladesh. Informed consent was obtained from parents or guardians. Children ranged in age from three months to eleven years.

Fecal specimens were analyzed by using microscopy and then screened by using a multiplex enzyme-linked immunosorbent assay (ELISA) for *E. histolytica*, *Giardia*, and *Cryptosporidium* (*TRI-COMBO PARASITE SCREEN*, TechLab, Inc., Blacksburg, VA). Positive samples were individually tested by using a commercially available ELISA for *E. histolytica* (*E. HISTOLYTICA II*, TechLab). All ELISAs were performed according to their package insert. Fresh and frozen fecal samples were tested.

We tested a third-generation fecal rapid antigen detection test (*E. HISTOLYTICA QUIK CHEK*; TechLab), which is specific for *E. histolytica* adherence lectin.⁸ The test consists of a membrane device containing two antibody-lined strips. One strip is a control line that binds conjugate, and the other strip contains monoclonal antibody against *E. histolytica* lectin and binds to antigen-conjugate complexes in the fecal sample (Figure 1).

Specimens were prepared by adding 25 μ L of feces to 500 μ L of diluent in prelabeled test tubes. Tubes were then

FIGURE 1. Positive (**top**) and negative (**bottom**) results in the *E. HISTOLYTICA QUIK CHEK* test. The line on the left in both tests is an internal control. The line on the right visible in the positive test result indicates presence of *Entamoeba histolytica* antigen in the fecal specimen. The absence of a second line in negative (bottom) test result indicates that the fecal specimen is negative for *E. histolytica* antigen.

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TABLE 1

Comparison of results of the *E. HISTOLYTICA QUIK CHEK* test and an *Entamoeba histolytica* enzyme-linked immunosorbent assay*

No. specimens with the following <i>E. histolytica</i> Quik Chek test results	No. specimens with the following <i>Entamoeba histolytica</i> enzyme-linked immunosorbent assay results		
	Positive	Negative	Total
Positive	56	0	56
Negative	0	172	172
Total	56	172	228

*The total number of stool samples tested was 228. Fifty-six samples showed positive results by both tests. The remaining 172 samples showed negative results by both tests.

inverted and vortexed to ensure adequate suspension. A positive control was prepared by adding 40 μ L of positive control solution to 500 μ L of diluent. A negative control was prepared by adding 40 μ L of diluent (in place of sample) to a tube containing 500 μ L of diluent. One drop (40 μ L) of conjugate was added to each test tube. Five-hundred microliters of sample-conjugate mixture was transferred into the sample port of the membrane device and the device was incubated for 15 minutes at room temperature. After incubation, 300 μ L of wash buffer was added to the reaction window of the membrane device. Subsequently, 60 μ L of substrate was added to the reaction window and the device was incubated for 10 minutes at room temperature. Test results were read immediately after the final incubation.

Two-hundred twenty-eight samples were tested with the rapid antigen detection test. Of the 228 stool specimens tested, 56 were positive by ELISA for *E. histolytica*, which was defined as an optical density value > 0.05 after subtraction of a negative control value. Microscopy was 16.1% sensitive and 98.8% specific for detecting *E. histolytica* cysts and trophozoites when compared with the ELISA. The same 56 samples were positive by the *E. HISTOLYTICA QUIK CHEK* as by the ELISA. Also, the same 172 samples were negative by the ELISA and the *E. HISTOLYTICA QUIK CHEK* (Table 1). Thus, the *E. HISTOLYTICA QUIK CHEK* had a sensitivity of 100% and a specificity of 100% when compared to ELISA. The positive predictive value and negative predictive value of the rapid antigen test were 100%.

Microscopy is often the only means for diagnosis of amebiasis in resource-limited settings and is highly dependent on the skill of the microscopist.⁹ Our findings, consistent with those of previous reports, show that microscopy has poor sensitivity for diagnosis of amebiasis.² The *E. HISTOLYTICA QUIK CHEK*, a rapid antigen detection test, appears to be just as sensitive and specific as the commercially available *E. HISTOLYTICA II* ELISA.

The *E. HISTOLYTICA QUIK CHEK* is the first rapid antigen test available that can distinguish between *E. histolytica* and the non-pathogenic *E. dispar*. The *E. HISTOLYTICA QUIK CHEK* can be used for quick and reliable diagnosis of amebiasis, and as an alternative to labor-intensive methods such as ELISA and PCR. The *E. HISTOLYTICA QUIK*

CHEK may be more practical for use in the developing world than the commercially available *E. HISTOLYTICA II* ELISA because the latter requires expensive equipment, skilled personnel, and takes more than two hours to perform. The *E. HISTOLYTICA QUIK CHEK* can be performed and interpreted within 30 minutes, without any additional equipment, and with minimal technical training. Thus, the *E. HISTOLYTICA QUIK CHEK* is an ideal point-of-care test that can be performed at the bedside and in resource-limited settings for rapid and accurate diagnosis of amebiasis.

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