Comparison of Two Immunoassays for Detection of *Entamoeba histolytica*

Sarah Buss,1* Mamun Kabir,2 William A. Petri, Jr.,1,3 and Rashidul Haque2

Department of Microbiology1 and Department of Medicine,3 University of Virginia, Charlottesville, Virginia, and International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh2

Received 7 April 2008/Returned for modification 17 May 2008/Accepted 3 June 2008

Effective diagnostic tools are essential in order to combat disease caused by the parasite *Entamoeba histolytica*. In this study, we compared the commercially available Ridascreen *Entamoeba histolytica* test (R-Biopharm) and the *E. histolytica* II test (Techlab), and we found that the *E. histolytica* II test detects *E. histolytica* infections more accurately.

*Entamoeba histolytica*, the causative agent of amoebiasis, is currently the only species within the genus *Entamoeba* known to cause disease in humans (1, 2, 4, 5). Although 90% of *E. histolytica* infections remain asymptomatic, approximately 50 million cases of amoebic dysentery and 40,000 to 100,000 *E. histolytica*-induced deaths occur each year (11, 20). Because asymptomatic individuals shed infectious cysts and occasionally develop symptoms over time, both symptomatic and asymptomatic infections necessitate treatment (8). Amoebiasis can be successfully treated with 5-nitroimidazoles and luminal amoebicides, but treatment depends on accurate diagnosis (12, 14).

Microscopy has been used to detect *E. histolytica* cysts and trophozoites in the stool of infected individuals (6). However, nonpathogenic *Entamoeba* species, such as *E. dispar* and *E. moshkovskii*, confound the issue of diagnostics. *E. histolytica* and nonpathogenic *Entamoeba* species are morphologically identical and cannot be differentially detected by microscopy (1, 2, 4). Techniques such as isoenzyme analysis (15, 17) and PCR (7, 14, 18) accurately distinguish between the species, but such techniques are not practical for routine use in developing nations where *E. histolytica* is prevalent (5, 10, 19).

Enzyme-linked immunosorbent assays (ELISAs) are commonly used in diagnostic laboratories, including those in the developing world. Although there are a few FDA-approved ELISAs for the detection of *Entamoeba* species, only the Techlab (Blacksburg, VA) *E. histolytica* II test specifically identifies *E. histolytica*. The *E. histolytica* II test has been reported to be more sensitive than the combination of culture and microscopy (9) but only 79% sensitive and 96% specific compared to real-time PCR (14).

The R-Biopharm Ridascreen *Entamoeba* test (Darmstadt, Germany) detects *E. histolytica* sensu lato. In one study, the Ridascreen *Entamoeba* test was found to be 81.8% sensitive and 99.2% specific compared to microscopy (16). However, another study found microscopy 53.8% sensitive and 94% specific compared to the Ridascreen *Entamoeba* test (3). Although these studies have demonstrated the efficacy of ELISAs as *E. histolytica* detection methods, no comparison of the commercially available ELISAs exists. In this study we sought to compare the Techlab *E. histolytica* II test and the R-Biopharm Ridascreen *Entamoeba* test.

We screened three cultured trophozoite strains and 110 fecal specimens by both tests. *E. histolytica* HM1:1MSS and Ax 259100 (a patient isolate) were grown axenically in TYI-S-33 (4a), while *E. dispers* SAW760 was grown axenically in LYI medium (2a). Trophozoites were harvested on ice, and twofold dilution curves ranging from 5.0 × 107 trophozoites per ml to 15 trophozoites per ml were prepared in each kit diluent. The ELISAs were run as recommended by the manufacturers, and each test well received 100 μl of the sample. The trophozoite dilution curves were prepared in duplicate, and within a kit, there was never a discrepancy between replicate samples.

We found the Techlab *E. histolytica* II test to be more sensitive with respect to the number of trophozoites detected per well. The Techlab test was able to detect as few as 98 HM1:1MSS and 24 Ax 259100 trophozoites per well, while the lowest numbers of trophozoites that the Ridascreen *Entamoeba* test was able to detect were 195 and 98 per well, respectively. As expected, the Techlab *E. histolytica* II test was specific for *E. histolytica* and did not detect any *E. dispers* trophozoites. The Ridascreen *Entamoeba* test did detect *E. dispers* trophozoites. However, the limits of detection for *E. dispers* were quite high: 25,000 amoebae per well were required to give a positive reaction.

A panel of fecal samples collected from patients with diarrhea at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), was assembled. Upon collection, microscopic examination of a 0.9% saline smear was used to screen samples for ova and parasites, and the samples were then frozen until further use. Samples that tested positive for *Entamoeba* by microscopy were chosen for the ELISA screen, as were samples positive for other common intestinal organisms, including *Ascaris lumbricoides*, *Giardia* spp., *Blastocystis hominis*, *Trichuris trichiura*, *Cryptosporidium* spp., and *Entamoeba coli*. Microscopy-negative samples were also included.
in the panel, since microscopy is known to be an insensitive indicator of *Entamoeba* infection (5, 10).

All samples were diluted in the appropriate kit diluent, and the tests were run and interpreted according to the manufacturers’ instructions. Discrepant samples were retested by both ELISAs and analyzed by PCR. The QIAamp DNA stool mini-kit (Qiagen, Hilden, Germany) was used to extract DNA from the fecal specimens, and PCR for *E. histolytica* and *E. dispar* was carried out as previously described (7, 14).

A total of 110 fecal specimens were tested with the R-Biopharm Ridascreen *Entamoeba* and Techlab *E. histolytica II* ELISA kits. The *E. histolytica II* ELISA kit detected 50 *E. histolytica*-positive samples, while the Ridascreen *Entamoeba* test identified only 34 positive samples. Twenty-eight samples were positive, and 54 were negative, by both tests. All 22 of the Techlab test-positive, R-Biopharm test-negative samples tested positive for *E. histolytica* by PCR. Of the six samples that tested positive by the R-Biopharm test but negative by the Techlab test, two contained *Entamoeba dispar*, two contained *E. histolytica*, one contained both *E. dispar* and *E. histolytica*, and one was negative for both organisms, as determined by PCR. While the PCR-negative sample may represent a false-negative result, it is also possible that the sample contained *E. moshkovskii*, since the Ridascreen *Entamoeba* test is not specific for *E. histolytica*.

The Techlab *E. histolytica II* ELISA correctly identified approximately 35% (19 of 53) more *E. histolytica*-positive samples than the Ridascreen *Entamoeba* test. Additionally, the *E. histolytica* II test identified exclusively *E. histolytica*-positive samples, while samples containing *E. dispar* and possibly *E. moshkovskii* were detected by the Ridascreen *Entamoeba* test. Our results indicate that the Techlab *E. histolytica II* ELISA is not only more specific than the R-Biopharm Ridascreen *Entamoeba* test but also more sensitive.

Sensitive and specific detection of *Entamoeba histolytica* infection is required in order to ensure that patients receive the proper treatment. Because extraction of DNA from fecal specimens and PCR remain expensive and require skilled technicians, commercially available ELISAs may currently represent the most practical method for the identification of *E. histolytica* in fecal samples. While both ELISAs used in this study were relatively quick and easy to perform, the Techlab *E. histolytica II* ELISA outperformed the R-Biopharm Ridascreen *Entamoeba* test in our hands.

This work was conducted at the ICDDR,B with the support of NIH grant 5RO1 AI043596-09 through W. A. Petri, Jr., and the University of Virginia. W. A. Petri, Jr., has a patent licensing agreement with Techlab for a diagnostic test for amoebiasis; the royalties from this test accrue to the American Society of Tropical Medicine and Hygiene without benefit to the licensor.

REFERENCES


