Immunochromatographic Strip-Based Detection of *Entamoeba histolytica*-*E. dispar* and *Giardia lamblia* Coproantigen

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BIOSITE Triage was 68.3% sensitive and 100% specific for the detection of *Entamoeba histolytica*-*E. dispar* \((n = 71)\) compared to Alexon-Trend's ProSpecT test (reference standard) using fresh-fresh stool. Neither test is able to distinguish *E. histolytica* from *E. dispar*. Triage was 83.3% sensitive and 100% specific compared to microscopy (formalin-ether concentrates and permanent stains) for the detection of *Giardia lamblia*.

*Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium parvum* are three of the major causes of protozoan-induced diarrheal disease (2, 3, 13). *E. histolytica* is responsible for approximately 100,000 deaths worldwide each year, making it second only to malaria as a cause of mortality due to a protozoan parasite (13). *G. lamblia* is among the most commonly reported parasitic infections in the United States, and on a global scale, giardiasis is responsible for approximately 100 million infections annually (7). Infection with the coccidian parasite *C. parvum* is usually self-limiting in immunocompetent individuals, but can be chronic and potentially life-threatening in the immunocompromised host (3). Contamination of municipal drinking water with *C. parvum* resulted in over 400,000 infections in Milwaukee during 1993 (8). Traditionally, the laboratory diagnosis of *E. histolytica*, *G. lamblia*, or *C. parvum* infection has relied upon the microscopic examination of fresh or fixed stool. However, microscopic diagnosis has several limitations, and recent studies have reported that stool antigen immunoassays equal (*G. lamblia*) or surpass (*E. histolytica*-*E. dispar*) microscopic detection of these pathogens (1, 6, 9–11).

Since multiple protozoal infections may coexist, a stool enzyme immunoassay capable of simultaneously detecting *E. histolytica*-*E. dispar* complex, *G. lamblia*, and *C. parvum* has recently been developed (Triage Parasite Panel, Biosite Diagnostics, San Diego, Calif.). Triage is a single immunochromatographic (IC) strip coated with monoclonal antibodies specific for 29-kDa surface antigen (*E. histolytica*-*E. dispar*), alpha-1-giardin (*G. lamblia*), and protein disulfide isomerase (*C. parvum*) (12). In a previous study which assessed all available stool antigen enzyme-linked immunosorbent assay (ELISA) kits (Techlab’s *Entamoeba* test and *E. histolytica* test, Merlin Diagnostika’s Optimun S, and Alexon-Trend’s ProSpecT), we showed that ProSpecT is the most sensitive and specific stool antigen ELISA currently available for the detection of the *E. histolytica*-*E. dispar* complex (11). In addition, ProSpecT outperformed microscopy (formalin-ether concentration and permanently stained smears) carried out in either community laboratories or referral centers (11). In this study, we evaluated the performance of Triage compared blindly to those of (i) ProSpecT for the detection of *E. histolytica*-*E. dispar* antigen in patient specimens as well as in mock reconstitution experiments and (ii) microscopy (formalin-ether concentration and permanently stained smears) performed at a referral center for the detection of *G. lamblia* in patient specimens.

Patients presenting between 1993 and 1998 to the Tropical Disease Unit of The Toronto Hospital with gastrointestinal symptoms (with diarrhea defined as $\geq 3$ loose bowel movements/day which conform to the container, abdominal pain, nausea, weight loss, or bloody stool) or risk factors for *E. histolytica*-*E. dispar*, *G. lamblia*, or *C. parvum* infection (travel to the tropics within 6 months, men who have sex with men, or immigrants from the tropics or sub tropics within the previous 2 years) were eligible for inclusion in this study. Verbal informed consent was obtained from each patient, and the study was approved by the Ethical Review Committee of The Toronto Hospital. Subjects were requested to provide fresh stool samples (within 1 h of passage) for microscopy and ELISA analysis. Stool specimens (minimum of two) were transported to the parasitology laboratory for routine evaluation of ova and parasite by microscopy (formalin-ether concentration and permanent stains [iron hematoxylin and modified acid fast]). Aliquots of fresh unpreserved stool were frozen (−20°C) for subsequent ELISA analysis.

Once thawed, samples were resuspended in the specimen dilution buffer (buffered protein solution–0.1% NaNO$_3$) provided in each kit. The assay procedure for both Triage and ProSpecT was carried out according to the manufacturer’s recommendations. A positive reaction in the Triage kit is identified by a qualitative colorimetric reaction when the antibody conjugate (alkaline phosphatase) reacts with the substrate (ionic phosphate), resulting in a dark blue-purple line on the IC strip. The ProSpecT ELISA was read at $\lambda_{\text{abs}}$ with a microplate reader (Thermomax; Molecular Devices Corp., Sunnyvale, Calif.). The ELISA plate format of the ProSpecT allows for multiple tests (up to 96 per ELISA plate), which reduces the assay time per sample. The Triage kit had the advantage of being completely self-contained and can be stored at room temperature. Triage also contains internal positive and negative controls for each test strip.

The results of stool antigen detection by Triage and ProSpecT are summarized in Table 1. Triage was 100% specific and 68.3% sensitive compared to ProSpecT (used as the reference standard) for the detection of the *E. histolytica*-*E. dispar*.
complex. ProSpecT was used as the comparative test in this study, since this assay had the highest specificity (98%) and sensitivity (100%) in a previous study (n = 112) that compared all available ELISA kits for *E. histolytica-E. dispar* detection with microscopy (11). Based on these performance characteristics, ProSpecT was chosen as the reference standard. The lower sensitivity of the Triage IC strip was explained in part by a fourfold difference in the limit of detection for *E. histolytica-E. dispar* trophozoites. HM1:IMSS trophozoites grown axenically in Y1-S medium were serially diluted in specimen dilution buffer and subjected to both the Triage and ProSpecT assays (5). The results of these reconstitution experiments are indicated in Table 2. ProSpecT was able to detect *E. histolytica-E. dispar* antigen at 250 trophozoites per ml, whereas Triage required >1,000 trophozoites per ml for an unequivocal positive signal. Although Triage is a qualitative colorimetric test, we found that band intensity correlated well with trophozoite number independent of the strip reader (Table 2). At 500 (before filtration) or 1,000 (after filtration) trophozoites per ml, band intensity was positive but weak and therefore prone to subjective reader error. No association has been established between parasite burden (number of cysts or trophozoites shed in stool) and clinical severity. Molecular epidemiological data indicate that *E. histolytica* is associated with invasive disease, while the genetically distinct species *E. dispar* results in asymptomatic infection (4, 11).

Comparison of Triage with microscopy for the detection of *G. lamblia* is summarized in Table 3. A minimum of two stools per patient were examined by microscopy (formalin-ether concentration and permanent stains). Aliquots of frozen stools were subsequently tested by IC strip. Triage was 83.3% sensitive and 100% specific compared to microscopy performed blindly at our referral center (n = 71). Triage was able to detect two mixed infections containing both *E. histolytica-E. dispar* and *G. lamblia*. No *C. parvum* infections were detected in these samples by either microscopy or Triage IC strip.

In summary, the Triage IC strip is highly specific for the detection of *E. histolytica-E. dispar* complex. However, Triage is less sensitive (68.3%) than an alternative ELISA diagnostic kit (ProSpecT). The lower sensitivity of Triage may be due to its inability to detect *E. histolytica-E. dispar* antigen at or below 1,000 trophozoites per ml, especially following the required filtration of the sample. Triage has the advantage of being able to detect multiple protozoal pathogens in a single test. Compared to reference microscopy, Triage was 83.3% sensitive and 100% specific for the detection of *G. lamblia*. These results indicate that Triage may be a useful alternative system for the detection of multiple pathogens in stools. However, both of these tests are limited by an inability to distinguish pathogenic *E. histolytica* from nonpathogenic *E. dispar* and by the requirement for fresh or fresh-frozen stool.

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