Detection of *Giardia lamblia*, *Entamoeba histolytica*/Entamoeba dispers, and *Cryptosporidium parvum* Antigens in Human Fecal Specimens Using the Triage Parasite Panel Enzyme Immunoassay

LYNNE S. GARCIA, ROBYN Y. SHIMIZU, AND CAROLINE N. BERNARD

LSG & Associates, Diagnostic Medical Parasitology Consulting/Training Services, Santa Monica, California, and Department of Pathology and Laboratory Medicine, University of California, Los Angeles, California

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The Triage parasite panel (BIOSITE Diagnostics, San Diego, Calif.) is a new qualitative enzyme immunoassay (EIA) panel for the detection of *Giardia lamblia*, Entamoeba histolytica*/dispers*, and *Cryptosporidium parvum* in fresh or fresh, frozen, unfixed human fecal specimens. By using specific antibodies, antigens specific for these organisms are captured and immobilized on a membrane. Panel performance was evaluated with known negative and positive stool specimens (a total of 444 specimens) that were tested by the standard ova and parasite (O&P) examination as the “gold standard,” including staining with both trichrome and modified acid-fast stains. Specimens with discrepant results between the reference and Triage methods were retested by a different method, either EIA or immunofluorescence. A number of samples with discrepant results with the Triage device were confirmed to be true positives. After resolution of discrepant results, the number of positive specimens and the sensitivity and specificity results were as follows: for *G. lamblia*, 170, 95.9%, and 97.4%, respectively; for *E. histolytica*/dispers*, 99, 96.0%, and 99.1%, respectively; and for *C. parvum*, 60, 98.3%, and 99.7%, respectively. There was no cross-reactivity with other parasites found in stool specimens, including eight different protozoa (128 challenges) and three different helminths (83 challenges). The ability to perform the complete O&P examination should remain an option for those patients with negative parasite panel results but who are still symptomatic.

With the increasing interest in rapid diagnostic testing, potential waterborne outbreak situations, fewer well-trained microscopists, and confirmation that *Giardia lamblia*, *Entamoeba histolytica*/dispers*, and *Cryptosporidium parvum* can cause severe symptoms in humans, laboratories are reviewing their options with regard to immunoassay kits that can be incorporated into their routine testing protocols (2, 4-6, 15, 17-21, 24-27, 32). Not only must these methods be acceptable in terms of sensitivity and specificity but they must provide clinically relevant, cost-effective, rapid results, particularly in a potential waterborne outbreak situation (1, 3, 11, 23). It is well known that protozoan cysts, in particular, *Giardia* cysts, are not shed in the stool on a consistent basis and that their numbers vary from day to day; this is also true of coccidian oocysts. Examination of stool specimens collected on consecutive days or even within the recommended 10-day time frame may not confirm infection with *Giardia*, *E. histolytica*/dispers*, or *C. parvum* (13). In patients who are infected with one or more of these parasites, the use of routine diagnostic methods such as concentration and trichrome and modified acid-fast staining may be insufficient to demonstrate the presence of these organisms (16, 33). Renewed awareness of potential waterborne transmission of these parasites is based on the number of well-documented outbreaks during the past few years and the publicity surrounding water regulations and testing.

Among patients with cryptosporidiosis, the majority of immunocompetent patients have initially been symptomatic, with large numbers of oocysts present in their stools. In this situation, a number of diagnostic procedures would be acceptable (8, 12, 13). However, as the acute infection resolves and the patient becomes asymptomatic, the number of oocysts dramatically decreases. Also, the number of oocysts passed by patients, including those with AIDS, varies from day to day and week to week. It has also been established that the infective dose of *Cryptosporidium* oocysts in humans can be relatively low (7, 10).

Antigen detection assays for *G. lamblia*, *E. histolytica*/dispers*, and *C. parvum* have proved to be very useful in the diagnosis of these infections (4-6, 9, 14-22, 28-31). The advantages of these assays include labor, time, and batching efficiencies that may lead to cost reductions. Certainly, these reagents offer alternative methods to the routine ova and parasite (O&P) examination method and provide the added sensitivity required to confirm infections in patients with low parasite numbers.

On the basis of the need for improved diagnostic procedures, a rapid immunoassay device for the detection of *Giardia*, *E. histolytica*/dispers*, and *Cryptosporidium* antigens has been developed (Fig. 1). This BIOSITE Diagnostics (San Diego, Calif.) Triage rapid qualitative enzyme immunoassay (EIA) can be performed in approximately 15 min with fresh or fresh, frozen, unfixed human fecal specimens. This device was tested against known positive and negative fecal specimens on the basis of the results of the O&P examination for the detection of *G. lamblia* and *E. histolytica*/dispers* and the basis of the results of modified acid-fast staining for the detection of *C. parvum*. Specimens with discrepant results were retested by EIA or fluorescent-antibody methods.

* Corresponding author. Mailing address: LSG & Associates, Consulting/Training Services, 512-12th St., Santa Monica, CA 90402. Phone: (310) 393-5059, Fax: (310) 899-9722. E-mail: lgarcia1@gateway .net.
FIG. 1. BIOSITE EIA Triage parasite panel demonstrating positive results. (A) Positive and negative controls and positive test zone for G. lamblia (GIARD); (B) positive and negative controls and positive test zone for E. histolytica/E. dispar (E. HIST); (C) right, positive and negative controls and positive test zone for C. parvum (CRYPT).
and commercial EIA and immunofluorescence kits for testing
G. lamblia and C. parvum infections with trichrome stain and the modified acid-fast stain
Cryptosporidium screen in their test menus; both are separate,
to client in-service information distribution.
In infections (12). Some laboratories
have included both the O&P examination and a
positive specimens (n = 58) and negative samples (n = 386) were tested with the Triage parasite panel. Additional positive specimens (n = 2) were identified with the Triage parasite panel. All specimens with discrepant results with the Triage parasite panel were retested by immunofluorescence (Tables 1 and 2). If positive by any two methods, the specimen was considered truly positive. After resolution, the number of positive specimens was 60, the sensitivity was 98.3%, the specificity was 99.7%, and the NPV was 99.7%.

**TABLE 1. Comparison of results prior to and after testing of specimens with discrepant results**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Result (no. of specimens)</th>
<th>O&amp;P examination, permanent stains (reference methods)</th>
<th>Triage parasite panel</th>
<th>After EIA or FA to resolve discrepancies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pos</td>
<td>Neg</td>
<td>Pos</td>
</tr>
<tr>
<td>G. lamblia</td>
<td>Pos (170)</td>
<td>142</td>
<td>302</td>
<td>170</td>
</tr>
<tr>
<td></td>
<td>Neg (274)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total (444)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. histolytica/E. dispers</td>
<td>Pos (99)</td>
<td>42</td>
<td>401</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>Neg (344)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total (443)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. parvum</td>
<td>Pos (60)</td>
<td>58</td>
<td>386</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Neg (384)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total (444)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Abbreviations: FA, fluorescent antibody; Pos, positive; Neg, negative.

**DISCUSSION**

The selection of a particular diagnostic kit and approach for incorporation into the work flow should be the responsibility of each laboratory. These decisions are based on a number of factors, including clinical relevance, cost-containment, anticipated workload, ease of kit performance, number of trained staff, single-sample versus batched-sample testing, physician clients, physician ordering patterns, size and configuration of client base, laboratory size, availability of equipment, ease with which a new procedure fits into the routine laboratory work flow, turnaround time for achieving a result, reporting limitations (computer system), and the necessity for staff training and client in-service information distribution.

The rapid immunoassays do not replace routine O&P examinations, but they are very useful when trying to confirm Giardia and Cryptosporidium infections (12). Some laboratories have included both the O&P examination and a Giardia or Cryptosporidium screen in their test menus; both are separate, orderable tests. On the basis of the results of the O&P examination with trichrome stain and the modified acid-fast stain and commercial EIA and immunofluorescence kits for testing of specimens with discrepant results, it is clear that the routine microscopy methods used in this study do not reveal as many positive specimens as the more rapid, newer immunoassay reagents. With the need for strict requirements for specimen collection and fixation, plus the availability of fewer well-trained microscopists who can recognize the subtle differences between organisms for organism differentiation, additional more rapid tests will serve as excellent adjunct methods to the O&P examination, provided that the pros and cons of each approach are clearly recognized. Fecal specimen panels and potential modifications in laboratory test menus should be reviewed in light of these and other published results (2, 4–6, 15, 17–21, 24–33).

It has been reported that the Giardia EIA can detect Giardia in at least 30% more specimens than the microscopic examination (31), and it has been reported to have a sensitivity and specificity of 98 and 100%, respectively (4). In another study, the sensitivity and specificity of ColorPAC (Becton Dickinson) for Giardia detection were 100 and 100%, respectively (15), while an earlier study reported an EIA sensitivity of 97% and a specificity of 96% (30). Other studies reported a range in sensitivity from 91.4 to 100% and a range in specificity from 97.8 to 100% (6). Sensitivities and specificities in studies for the detection of C. parvum have ranged from 66.3 to 100% and 93 to 100%, respectively, with the sensitivities and specificities in the majority of studies ranging from 93 to 100% and 98 to 100%, respectively (2, 15, 16). Various studies looking at antigen detection in stool specimens for the detection of E. histolytica/E. dispar have reported sensitivities and specificities that range from 68.3 to 95% and 97 to 99%, respectively (18, 19, 25, 27). Stool antigen studies for pathogenic E. histolytica provide sensitivities and specificities that range from 87 to 97.6% and 92.6 to 98%, respectively (5, 17, 20, 21).

Although the sensitivities and specificities reported for all of the available immunoassay kits are similar, some formats are more time-consuming and labor-intensive. The ability to con-

**TABLE 2. Sensitivity, specificity, and NPV data compared with data for true-positive and true-negative specimens**

<table>
<thead>
<tr>
<th>Method</th>
<th>G. lamblia</th>
<th>E. histolytica/E. dispar</th>
<th>C. parvum</th>
</tr>
</thead>
<tbody>
<tr>
<td>O&amp;P examination, permanent stains (reference methods)</td>
<td>79.4</td>
<td>97.4</td>
<td>88.4</td>
</tr>
<tr>
<td>Triage parasite panel</td>
<td>95.9</td>
<td>97.4</td>
<td>97.4</td>
</tr>
</tbody>
</table>

Sensitivity (%) Specificity (%) NPV (%) Sensitivity (%) Specificity (%) NPV (%) Sensitivity (%) Specificity (%) NPV (%)
currently detect and distinguish between *G. lamblia*, *E. histolytica/E. dispar*, and *C. parvum* antigens in fresh or frozen, frozen fecal specimens with a 15-min qualitative EIA panel provides the laboratorian with another very useful diagnostic tool, and this can be accomplished with the BIOSITE Triage parasite panel. The Triage parasite panel procedure is simple to perform, requires minimal training, and can be used for single-specimen or batch-testing approaches. The Triage parasite panel will provide diagnostic laboratories with a simple, convenient, alternative method for performing simultaneous, discrete detection of *Giardia*, *Cryptosporidium*, and *E. histolytica/E. dispar*-specific antigens in patient fecal specimens.

**REFERENCES**


