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Comparison of Nine Commercially Available Enzyme-Linked Immunosorbent Assays for Detection of *Giardia lamblia* in Fecal Specimens

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Overall performance, including ease of use, total hands-on time, incubation and processing times, sensitivity, and specificity, of each of nine enzyme-linked immunosorbent assays (ELISAs) were compared by using 222 individual fecal samples submitted for the detection of *Giardia lamblia*. The assays evaluated were manufactured by Alexon, Inc., Cambridge Biotech Corp., Meridian, Inc., and Trend Scientific, Inc. All assays used polyclonal antibodies except the "new and improved" Microplate (direct and diluted methods) by Alexon, which is a monoclonal antibody assay. Seventy specimens were positive for *G. lamblia* by ELISA, ova and parasite test, and/or direct fluorescent-antibody assay. One hundred fifty two were negative by all three methods. Sensitivities and specificities ranged from 88.6 to 100% and 99.3 to 100%, respectively. The total hands-on time needed to run one specimen ranged from 1 min to 2 min 17 s per specimen. All except one commercially available ELISA were found to be rapid, sensitive, and specific for the detection of *G. lamblia* in fecal specimens.

Several recent studies have found enzyme-linked immunosorbent assays (ELISAs) to be a sensitive, cost-effective, and rapid method for the detection of *Giardia lamblia* in stool specimens (1, 3, 5, 7–9). This study compared the performance of nine commercial ELISAs for the detection of *G. lamblia* in fecal specimens. Four of the assays evaluated utilize monoclonal antibodies: the ProSpecT *Giardia* EZ Microplate assay uses diluted stool specimens simultaneously incubated in each well with the antibodies, the ProSpecT *Giardia* Rapid assay uses diluted stool specimens applied to a reaction device membrane on which anti-GSA 65 antibodies are immobilized, and the "new and improved" ProSpecT *Giardia* Microplate assay (direct and diluted methods) uses stool specimens that can be diluted before being added to each well (diluted method) or can be added to each well along with specimen dilution buffer (direct method). The other five assays evaluated utilize polyclonal antibodies; they are the ProSpecT *Giardia* Microplate assay (Alexon, Inc., Sunnyvale, Calif.) in which stool specimens are added directly to each microplate well, the *Giardia lamblia* Antigen Detection Microwell ELISA (Cambridge Biotech Corp., Worcester, Mass.), the Premier (Meridian Diagnostics, Inc., Cincinnati, Ohio), and the *Giardia lamblia* Direct Detection System and *Giardia* Direct Detection RS Detection Test System (Trend Scientific, Inc., St. Paul, Minn.). The kits were compared for their overall performance, including ease of use, total hands-on time, incubation and processing times, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

Only the assays manufactured by Alexon, Inc., and Trend, Inc., are currently available. Meridian, Inc., and Cambridge, Inc., are no longer manufacturing their assays.

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MATERIALS AND METHODS

The study was performed on 222 clinical fecal specimens submitted to the Associated Regional and University Pathologists, Inc., Microbiology Laboratory, Salt Lake City, Utah, for *Giardia* ELISA testing. All specimens were obtained from patients symptomatic with gastrointestinal distress where *Giardia* infection was suspected. Fecal specimens were preserved in 10% buffered formalin and examined by an ova and parasite test (O&P), ELISA, and direct fluorescent-antibody assay (DFA). Each specimen was coded and processed separately to eliminate the possibility of observer bias. The first technologist processed, coded, and ran all samples for the ELISA to prevent the second examining technologist, who performed the O&P examination and DFA, from recognizing the samples.

All O&P examinations were performed with a formalin–Hemo-De (Scientific Safety Solvents, Keller, Tex.; distributed by Fisher Scientific [catalog no. 15182507A]) concentration procedure (2). To confirm the presence or absence of *Giardia*, six slides were made from each specimen (stained with Dobell's iodine and covered by a 22- by 40-mm coverslip), and they were examined in their entirety by using overlapping fields under 10 and 40× objectives.

In preparing the DFA stain (Meridian Diagnostics), approximately 10 μl of the concentrated specimen was smeared onto a DFA well slide and allowed to air dry. Staining was performed according to the manufacturer's instructions. Each specimen had a total of six wells examined in their entirety by fluorescence light microscopy under a 50× oil objective.

Samples were prepared and each ELISA was performed according to the manufacturer's instructions (Table 1). All of the kits accept specimens preserved in formalin or sodium acetate-acetic acid-formalin or frozen unpreserved stools. ProSpecT Rapid, EZ, and Trend assays cannot use Merthiolate-iodine-formalin, and Cambridge, Meridian, and Trend cannot accept Cary-Blair or swab samples. Dilutions of the preserved specimens ranged from none to 1:20, and dilutions of frozen unpreserved specimens ranged from 1:5 to 1:20.

Each step listed by the manufacturer was timed by the processing technologist and tabulated for the total hands-on time needed to perform each assay. Results were interpreted by the following two methods: (i) visually inspecting the specimens for a color change in each well before and after the detection reagent was added and (ii) obtaining the optical density at 450-nm wavelength with a spectrophotometer. The spectrophotometer was set to interpret positive results according to each manufacturer's instructions. The spectrophotometer reading was used to calculate the sensitivity, specificity, PPV, and NPV of each assay. A Denley (Durham, N.C.) microplate washer was used to ensure that no assay would be subject to any difference in washing technique. The ProSpecT *Giardia* Rapid assay by Alexon, Inc., was the only membrane assay evaluated in the study. Unlike the other assays evaluated, the ProSpecT *Giardia* Rapid assay can only be read and washed manually, thus not requiring the use of a spectrophotometer or microplate washer.

Specimens that met the following criteria were counted as true positives: (i) ELISA positive and O&P and/or DFA positive and (ii) O&P and/or DFA positive.

TABLE 1. Test procedures for ELISAs

Step no.	Procedure	Alexon ProSpecT *Giardia* Microplate	Alexon ProSpecT *Giardia* new and improved Microplate (direct and diluted methods)	Alexon ProSpecT *Giardia* Rapid	Alexon ProSpecT *Giardia* EZ Microplate	Cambridge Microwell ELISA	Meridian Premier	Trend *G. lamblia* Direct Detection System	Trend *Giardia* Detection RS System
1	Add wells to holder	Yes	Yes	No[a]	Yes	Yes	Yes	Yes	Yes
2	Add conjugate	No	No	No	Yes	No	No	No	No
3	Add dilution buffer to each well	Yes	Yes	No	No	No	No	Yes	Yes
4	Add positive and negative controls	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
5	Add specimen	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	Incubation no. 1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	Wash no. 1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	Add reagent 1	No	No	No	No	Yes	No	Yes	Yes
8a	Add enzyme conjugate	Yes	Yes	Yes	No	No	No	No	No
8a	Add detection antibody	No	No	No	No	No	Yes	No	No
9	Incubation no. 2	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
10	Wash no. 2	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
11	Add reagent 2	No	No	No	No	Yes	No	Yes	Yes
11a	Add conjugate	No	No	No	No	No	Yes	No	No
12	Incubation no. 3	No	No	No	No	Yes	Yes	Yes	Yes
13	Wash no. 3	No	No	No	No	Yes	Yes	Yes	Yes
13a	Wash with distilled water	No	No	No	No	No	Yes	No	No
14	Add solution A or B	No	No	No	No	Yes	Yes	No	No
14a	Add reagent 3	No	No	No	No	No	No	Yes	No
14a	Add substrate	No	No	No	No	No	No	No	Yes
14a	Add color substrate	Yes	Yes	Yes	Yes	No	No	No	No
15	Incubation no. 4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	Wash no. 4	No	No	No	No	No	No	Yes	No
17	Add substrate	No	No	No	No	No	No	Yes	No
18	Incubation no. 5	No	No	No	No	No	No	Yes	No
19	Add stop solution	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	Read results	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Total steps		13	13	10	10	15	16	19	16

[a] Uses one reaction membrane per patient.

Any specimen that was negative by O&P, DFA, and ELISA was counted as a true negative.

RESULTS

Of the 222 specimens, 70 were confirmed as true positives for *G. lamblia* and 152 were confirmed as true negatives. Sensitivity and specificity (Table 2) of each assay ranged from 88.6 to 100% and 99.3 to 100%, respectively. The only assay to produce a false-positive result was the Trend *G. lamblia* Direct Detection System. The PPVs and NPVs of all the assays ranged from 98.6 to 100% and 95.0 to 100%, respectively (Table 2). Tables 1 and 3 are summaries of the number of steps required to perform each assay and the total hands-on time for each parameter measured: processing time, specimen application time, wash time, reagent application time, and incubation time. Table 2 compares the cost per test of each kit from each manufacturer. Only listed, nondiscounted prices were compared.

There were differences with respect to the number of steps required to perform each assay. Alexon's ProSpecT *Giardia* Rapid and the *Giardia* EZ had the fewest steps ($n = 10$), whereas the Trend *G. lamblia* Direct Detection System required 19 steps (Table 1).

Our findings revealed that color change to blue should be considered only a presumptive positive result. After application of the detection reagent, any initial blue that was lighter than the positive control but darker than the negative control could disappear and give a negative result when observed visually. In all of these specimens the negative result was confirmed by a negative spectrophotometer result.

TABLE 2. Sensitivity, specificity, PPV, NPV, and cost per test of ELISAs[a]

Assay	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Cost/test
Alexon ProSpecT *Giardia* Microplate	100	100	100	100	$ 6.20
Alexon ProSpecT *Giardia* new and improved Microplate (direct and diluted methods)[b]	100	100	100	100	$ 6.20
ProSpecT *Giardia* Rapid	90	100	100	95.6	$14.75
ProSpecT *Giardia* EZ Microplate	95.7	100	100	98.1	$ 6.20
Cambridge Microwell ELISA	88.6	100	100	95.0	$ 5.50
Meridian Premier	92.9	100	100	96.8	$ 6.00
Trend *G. lamblia* Direct Detection System	98.6	99.3	98.6	99.3	$ 5.16
Trend *Giardia* Detection RS Test System	97.1	100	100	98.7	$ 5.16

[a] $n = 222$.
[b] Each assay had a sensitivity and specificity of 100%.

TABLE 3. Total hands-on time per individual specimen for ELISAs

Procedure	Alexon EZ	Alexon Rapid	Alexon Microplate (direct)	Alexon new and improved (dilute)	Alexon new and improved (direct)	Cambridge	Meridian	Trend	Trend RS
Processing	48 s	51 s	0	1 min 17 s	0	39 s	49 s	43 s	6 s
Application of specimen	23 s	11 s	49 s	24 s	40 s	39 s	38 s	41 s	36 s
Washes	13 s	11 s	27 s	20 s	10 s	28 s	21 s	38 s	14 s
Application of reagents	14 s	16 s	13 s	12 s	10 s	14 s	15 s	15 s	10 s
Incubation	1 h 40 min	7 min	1 h 40 min	1 h 40 min	1 h 40 min	40 min	40 min	1 h 5 min	1 h 5 min
Total hands-on time	1 min 38 s	1 min 29 s	1 min 29 s	2 min 13 s	1 min	2 min	2 min 3 s	2 min 17 s	1 min 6 s

DISCUSSION

In any given patient population infected with *Giardia*, different levels and patterns of organism excretion can be observed (4). It has been shown that, especially when small numbers of organisms are excreted, two to three O&P examinations may be required before the organism is detected (10). Given the cost and labor considerations described above, an O&P examination is neither a cost-effective nor a rapid method of detecting the presence of *Giardia* in stool specimens.

Because of different levels and patterns of excretion of *Giardia*, we found that performing just one O&P examination would have missed 10 of the 70 confirmed positive samples. Detecting the presence of *Giardia* in these 10 samples required examining up to six additional individual slides. This occurred because we extended our examination for *Giardia* cysts or trophozoites to a maximum of seven individual slide preparations on initial-negative O&P examinations. This result contrasted with that of our previous study (3), where we found 34% more positive stool samples by ELISA than with the O&P test and/or DFA. Because of the high associated cost, no routine O&P test includes examining six slide preparations before a negative result is given. The above findings illustrate the increased sensitivity that one ELISA provides in detecting *G. lamblia*. The ELISA also has other advantages. It may be read visually or with a spectrophotometer. The ELISA required less hands-on processing time (1 to 2 min per specimen) (Table 3) than the O&P test and/or DFA stain (30 and 20 min, respectively).

Each assay's specificity was above 99%, and sensitivities varied from 88.6 to 100% (Table 2). Our results are similar to those reported in two recent papers (5, 6). Nine assays were likewise evaluated by Garcia and Shimizu (5), with sensitivities ranging from 94 to 100% and specificities of 100% for all kits. Both papers demonstrate high sensitivity and specificity for the enzyme immunoassay (EIA) kit. One additional kit not evaluated in our study was the CELISA from Tech Lab. We have no firsthand experience with it, but it is a commercially available kit in the United States and should be considered when making a decision to purchase a commercial product. The kits with the highest sensitivity, greater than 90%, would be more suitable for larger laboratories which are required to process a large number of samples daily, while the Alexon ProSpecT *Giardia* Rapid would be more cost-effective for the small laboratory or a physician's office. In contrast to the two previous papers, our evaluation extended beyond an assessment of performance. We have included data on sample requirements, cost, processing, number of washes, and total hands-on time, factors that are important to turnaround time and that reflect hidden costs (Tables 2 and 3).

Regardless of the product used, the ELISA should not substitute for an O&P examination if the epidemiology and other historical data are suggestive of disease other than giardiasis. Additional parasites were detected from positive and negative ELISA specimens, including *Ascaris lumbricoides* eggs, *Cryptosporidium* oocysts, *Endolimax nana* cysts, *Blastocystis hominis*, *Entamoeba hartmanni* cysts, *Iodamoeba buetschlii* cysts, and *Chilomastix mesnili* cysts.

We suggest replacement of three O&P examinations with EIA on a single stool sample when giardiasis is the most likely clinical diagnosis. Mank et al. (6) demonstrated a NPV for both evaluated EIAs on a single stool sample to be only slightly less than that of microscopy performed on two consecutive samples. We agree that in the event of a negative test and persistent symptoms, microscopic examination of multiple stool samples would be indicated.

In summary, five of the nine evaluated kits had excellent sensitivity and three of the remaining four had acceptable sensitivities. The decision as to which kit to use depends upon the laboratory or physician's office setting. While the Alexon Microplate assays were slightly more expensive, they had the best overall sensitivity and specificity and the highest throughput of all the kits. The Alexon ProSpecT Rapid assay, despite its higher cost ($14.75/test), might be useful as a screening test in the physician's office setting, because it had the shortest time to a positive result (approximately 10 min), so that only negative results would be sent for an O&P examination. While the Trend products were the most cost-effective, the Trend *G. lamblia* Direct Detection System was the only assay to have a specificity less than 100%.

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