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Evaluation of Nine Immunoassay Kits (Enzyme Immunoassay and Direct Fluorescence) for Detection of *Giardia lamblia* and *Cryptosporidium parvum* in Human Fecal Specimens

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It is well known that *Giardia lamblia* and *Cryptosporidium parvum* can cause severe symptoms in humans, particularly those who are immunologically compromised. Immunoassay procedures offer both increased sensitivity and specificity compared to conventional staining methods. These reagents are also helpful when screening large numbers of patients, particularly in an outbreak situation or when screening patients with minimal symptoms. The data obtained by using 9 diagnostic kits were compared: direct fluorescent-antibody assay (DFA) kits (TechLab *Giardia*/*Crypto* IF kit, TechLab *Crypto* IF kit, and Meridian Merifluor *Cryptosporidium*/*Giardia*) and enzyme immunoassay (EIA) kits (Alexon ProSpecT *Giardia* EZ Microplate Assay, Alexon ProSpecT *Cryptosporidium* Microplate Assay, Cambridge *Giardia lamblia* Antigen Microwell ELISA, Meridian Premier *Giardia lamblia*, Meridian Premier *Cryptosporidium*, TechLab *Giardia* CELISA, Trend *Giardia lamblia* EIA). The test with the Meridian Merifluor *Cryptosporidium*/*Giardia* kit was used as the reference method. In various combinations, 60 specimens positive for *Giardia*, 60 specimens positive for *Cryptosporidium*, 40 specimens positive for a *Giardia*-*Cryptosporidium* mix, and 50 negative fecal specimens were tested. Different species (nine protozoa, three coccidia, one microsporidium, five nematodes, three cestodes, and one trematode) were included in the negative specimens. The sensitivity of EIA for *Giardia* ranged from 94% (Alexon) to 99% (Trend and Cambridge); the specificity was 100% with all EIA kits tested. The sensitivity of EIA for *Cryptosporidium* ranged from 98% (Alexon) to 99% (Meridian Premier); specificities were 100%. All DFA results were in agreement, with 100% sensitivity and specificity; however, the TechLab reagents resulted in fluorescence intensity that was generally one level below that seen with the reagents used in the reference method. In addition to sensitivity and specificity, factors such as cost, simplicity, ease of interpretation of results (color, intensity of fluorescence), equipment, available personnel, and number of tests ordered are also important considerations prior to kit selection.

With the increasing interest in potential waterborne outbreak situations and confirmation that both *Giardia lamblia* and *Cryptosporidium parvum* can cause severe symptoms in humans, laboratories are reviewing their options with regard to diagnostic kits that can be incorporated into their routine testing protocols (1–7, 9–16, 18, 19, 22, 24, 27–33, 35, 37–41). Not only must these methods be acceptable in terms of sensitivity and specificity but they must also be clinically relevant and cost-effective and they must provide rapid results, particularly in a potential waterborne outbreak situation.

It is well known that *Giardia* cysts are shed sporadically and that their numbers may vary from day to day. Routine examinations of stool specimens collected on consecutive days or even within the recommended 10-day time frame may not confirm infection with this organism (11, 23). In patients with either giardiasis or cryptosporidiosis, the use of routine diagnostic methods such as concentration and trichrome or modified acid-fast staining may be insufficient to demonstrate the presence of these organisms (11, 23, 41). Renewed awareness of potential waterborne transmission of both organisms 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 procedures would be acceptable for use in order to confirm the diagnosis (7, 10, 26). However, as the acute infection resolves and the patient becomes asymptomatic, the number of oocysts dramatically decreases. 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 (8).

As laboratories continue to review their testing approach and their options with regard to diagnostic reagents, more interest is being shown in the new immunoassay reagent kits. Certainly, these reagents offer alternative methods to the routine "ova and parasite examination" (O&P) method and provide the added sensitivity required to confirm infections in patients with low parasite numbers. The data obtained by these more sensitive methods will also provide for a more accurate assessment of the prevalence of these organisms not only in humans but also in environmental sources. In the current environment of managed care and cost-containment, with cross-trained individuals, fewer well-trained microbiologists, and increased concern about potential waterborne outbreak situations, these reagents may support diagnostic testing that is more accurate, rapid, and cost-effective.

MATERIALS AND METHODS

Specimens. Human fecal specimens ($n = 210$) were collected in 10% formalin and submitted to the laboratory. By the Merifluor *Cryptosporidium*/*Giardia* direct

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TABLE 1. Discrepancies among the results of the tests with the five diagnostic kits (EIAs for *G. lamblia*) compared with the results of the reference method[a]

Result with the reference kit	Result with the following diagnostic kit:				
	TechLab	Cambridge	Premier Meridian	Alexon	Trend
Many Gl	+	+	[−]	[−]	+
Few Gl	+	+	+	[−]	+
Rare Gl	[−]	+	+	[−]	+
Few Gl	+	+	+	[−]	+
Few Gl	+	+	+	[−]	+
Rare Gl (one organism/ slide)	[−]	[−]	[−]	[−]	[−]
Few Gl	[−]	+	+	+	+
Rare Gl	[−]	+	+	+	+

[a] Gl, *G. lamblia* isolates; +, positive; [−], negative.

fluorescent-antibody assay (DFA) (the reference method), 60 specimens were positive for *Giardia*, 60 specimens were positive for *Cryptosporidium*, 40 specimens contained both *Giardia* and *Cryptosporidium*, and 50 specimens were negative for both organisms. Different parasites (nine protozoa, three coccidia, one microsporidium, five nematodes, three cestodes, and one trematode) were included in the negative specimens.

EIA diagnostic kits. The following enzyme immunoassay (EIA) diagnostic kits were used according to the manufacturer's directions: (i) Alexon ProSpecT *Giardia* EZ Microplate Assay (Alexon, Inc., Sunnyvale, Calif.), (ii) Alexon ProSpecT *Cryptosporidium* Microplate Assay, (iii) Cambridge *Giardia lamblia* Antigen Microwell ELISA (Cambridge Biotech Corporation, Worcester, Mass.), (iv) Meridian Premier *Giardia lamblia* (Meridian Diagnostics, Inc., Cincinnati, Ohio), (v) Meridian Premier *Cryptosporidium*, (vi) TechLab *Giardia* CELISA (TechLab, Blacksburg, Va.), and (vii) Trend *Giardia lamblia* EIA (Trend Scientific, Inc., St. Paul, Minn.).

Specimen preparation for EIA methods. All EIA kits required unconcentrated, formalinized stool specimens.

Test performance. Unless indicated otherwise, EIA diagnostic kit procedures were followed and the results were examined and interpreted according to the manufacturers' directions.

DFA diagnostic kits. The following DFA diagnostic kits were used according to the manufacturer's directions, unless indicated below: (i) TechLab *Giardia/Crypto* IF Kit, (ii) TechLab *Crypto* IF Kit, and (iii) Meridian Merifluor *Cryptosporidium/Giardia* (reference method).

Specimen preparation for DFA methods. Stool sediment was obtained by using the routine formalin-ethyl acetate sedimentation concentration method with centrifugation at 500 × *g* for 10 min (11, 23). One drop (10 μl) of the sediment was spread thinly onto the wells, air dried, and methanol fixed before staining (11).

Preparation of eight-well, Teflon-coated slides for DFA methods. The eight-well, Teflon-coated slides were coated with a 1% white glue adhesive as reported previously (12). For purposes of batch testing, we used a 7-mm-well slide rather than the 12.5-mm-well slide included in the kits.

Slide examination method. Each well on the fluorescence slide was scanned at a magnification of ×100, and organism confirmation was made at a magnification of ×250. The *Giardia* cysts were oval, measuring approximately 11 to 15 μm, and the *Cryptosporidium* oocysts were round, measuring approximately 4 to 6 μm. Both organisms showed apple green fluorescence against a dark background free of nonspecific fluorescence. All slides were stored in the dark before being read and were read blinded within 1 h of test completion with a Zeiss (Carl Zeiss, Inc., New York, N.Y.) fluorescence microscope with a 465- to 505-nm exciter filter, a 515-nm dichromatic beam splitter, and a 520- to 560-nm barrier filter. A positive smear was determined on the basis of the presence of one or more *Giardia* cysts (fluorescence, 2+ to 4+) or *Cryptosporidium* oocysts (fluorescence, 2+ to 4+).

RESULTS

EIA for *Giardia*. Specimens positive for *G. lamblia* ($n = 100$) and negative samples ($n = 50$) were tested by using four different kits. A total of 92 specimens were positive in tests with all test kits. Data for the eight specimens with discrepant results are presented in Table 1. The terms "rare," "few," and "many" are used in Table 1 to provide the reader with additional information regarding those specimens containing parasites that were missed by one or more of the kits; no specific quantitation is used or reported with the results. All systems performed in accordance with the expected values regarding sensitivity and specificity, as stated by the manufacturers and presented in Table 2.

DFA for *Giardia*. Specimens positive for *G. lamblia* ($n = 100$) and negative samples ($n = 50$) were tested by using the TechLab *Giardia/Crypto* IF kit. A total of 100 specimens were positive compared with the results of the reference method. This kit performed well, with 100% sensitivity and 100% specificity, although sensitivity and specificity values for this test have not yet been established by the manufacturer. The TechLab reagents resulted in fluorescence intensity that was generally one level below that seen by the reference method.

EIA for *Cryptosporidium*. Specimens positive for *Cryptosporidium* ($n = 100$) and negative samples ($n = 50$) were tested by using the Meridian Premier *Cryptosporidium* kit and the Alexon ProSpecT *Cryptosporidium* Microplate Assay kit. A total of 98 specimens were positive in tests with both test kits. The two positive specimens that were missed contained rare oocysts; one positive specimen was missed by both kits, and a second positive specimen was missed by the Alexon kit. Both systems performed in accordance with expected values regarding sensitivity and specificity, as stated by the manufacturers (Meridian, 91 and 99%, respectively; Alexon, 97 and 100%, respectively).

DFA for *Cryptosporidium*. Specimens positive for *Cryptosporidium* ($n = 100$) and negative samples ($n = 50$) were tested by using two diagnostic kits. There was 100% agreement among the results obtained by the TechLab *Giardia/Crypto* IF kit, the TechLab *Crypto* IF kit, and the reference method, although sensitivity and specificity values for this test have not yet been established by the manufacturer. The TechLab fluorescence intensity results were generally one level of fluorescence below that seen by the reference method.

DISCUSSION

All kits used in the evaluation performed well, with sensitivities and specificities of 94% or greater. Tests with any one of these kits would provide an acceptable method for any laboratory. The selection of a particular 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 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 proce-

TABLE 2. Sensitivity and specificity data for five diagnostic kits (EIAs for *G. lamblia*) compared with those for the reference method[a]

Diagnostic kit	Sensitivity (%)	Expected sensitivity (%)[b]	Specificity (%)	Expected specificity (%)[b]
Merifluor *Cryptosporidium/ Giardia*	100	100	100	100
TechLab	96	NE[c]	100	NE
Cambridge	99	100	100	99
Premier Meridian	98	97	100	99
Alexon	94	97	100	98
Trend	99	96	100	97

[a] A total of 100 positive specimens and 50 negative specimens were tested.
[b] Expected performance as stated in kit documentation (from the manufacturer).
[c] NE, not yet established.

dure 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. Depending on the priorities and requirements of the individual laboratory, one or more of these considerations may take precedent.

These diagnostic kits do not take the place of routine O&Ps but they are very useful when trying to confirm *Giardia* and *Cryptosporidium* infections. A screening approach may be helpful when handling a potential outbreak situation, particularly if a waterborne outbreak is suspected. Clinically relevant approaches with non-outbreak-related clinical specimens in which fewer routine O&Ps are coupled with an immunoassay screening test also depend on whether the patient continues to be symptomatic after the first stool examinations and screenings are reported as negative. If the patient continues to be symptomatic and additional routine O&Ps are performed, the yield of protozoa can be increased considerably (*Entamoeba histolytica*, 22.7%; *Dientamoeba fragilis*, 31.1%) (14).

With renewed interest in the cross-training of personnel and continued reductions in staffing, diagnostic procedures that do not require extensive examinations at the microscope are also advantageous. Training in diagnostic parasitology requires extended periods of time, and the expertise required is based on experience in examining organism morphology with a light microscope. With approximately 60% of the laboratory's expense budget being allocated to personnel, it would be appropriate to review for possible implementation an approach that could decrease this percentage.

Renewed awareness of potential waterborne transmission of both organisms is based on the number of well-documented outbreaks during the past few years and the publicity surrounding water regulations and testing. The importance of an early diagnosis and appropriate therapy cannot be overemphasized, particularly for immunocompromised patients (17, 20, 21, 25, 34, 36).

REFERENCES

1. **Addis, D. G., J. P. Davis, J. M. Roberts, and E. E. Mast.** 1992. Epidemiology of giardiasis in Wisconsin: increasing incidence of reported cases and unexplained seasonal trends. Am. J. Trop. Med. Hyg. **47:**13–19.
2. **Addis, D. G., H. M. Mathews, J. M. Stewart, S. P. Walquist, R. M. Williams, R. J. Finton, H. C. Spencer, and D. D. Juranek.** 1991. Evaluation of a commercially available enzyme-linked immunosorbent assay for *Giardia lamblia* antigen in stool. J. Clin. Microbiol. **29:**1137–1142.
3. **Alles, A. J., M. A. Waldron, L. S. Sierra, and A. R. Mattia.** 1995. Prospective comparison of direct immunofluorescence and conventional staining methods for detection of *Giardia* and *Cryptosporidium* spp. in human fecal specimens. J. Clin. Microbiol. **33:**1632–1634.
4. **Atherton, F., C. P. Newman, and D. P. Casemore.** 1995. An outbreak of waterborne cryptosporidiosis associated with a public water supply in the UK. Epidemiol. Infect. **115:**123–131.
5. **Berkelman, R. L.** 1994. Emerging infectious diseases in the United States, 1993. J. Infect. Dis. **70:**272–277.
6. **Centers for Disease Control and Prevention.** 1994. *Cryptosporidium* infections associated with swimming pools—Dane County, Wisconsin, 1993. Morbid. Mortal. Weekly Rep. **43:**561–563.
7. **Current, W. L., and L. S. Garcia.** 1991. Cryptosporidiosis. Clin. Microbiol. Rev. **3:**325–358.
8. **DuPont, H. L., C. L. Chappell, C. R. Sterling, P. C. Okhuysen, J. B. Rose, and W. Jakubowski.** 1995. The infectivity of *Cryptosporidium parvum* in healthy volunteers. N. Engl. J. Med. **332:**855–859.
9. **Elshewy, K., R. T. Kilani, M. M. Hegazi, L. M. Makhlouf, and W. M. Wenmann.** 1994. Identification of low-molecular-mass coproantigens of *Cryptosporidium parvum*. J. Infect. Dis. **169:**460–463.
10. **Flanigan, T. P., and R. Soave.** 1993. Cryptosporidiosis, p. 1–20. *In* T. Sun (ed.), Progress in clinical parasitology, vol III. Springer-Verlag, New York, N.Y.
11. **Garcia, L. S., and D. A. Bruckner.** 1997. Diagnostic medical parasitology, 3rd ed. ASM Press, Washington, D.C.
12. **Garcia, L. S., A. C. Shum, and D. A. Bruckner.** 1992. Evaluation of a new monoclonal antibody combination reagent for direct fluorescence detection of *Giardia* cysts and *Cryptosporidium* oocysts in human fecal specimens. J. Clin. Microbiol. **30:**3255–3257.
13. **Hayes, E. B., T. D. Matte, T. R. O'Brien, T. W. McKinley, G. S. Logsdon, J. B. Rose, B. L. P. Ungar, D. M. Word, P. F. Pinsky, M. S. Cummings, M. A. Wilson, E. G. Long, E. S. Hurwitgs, and D. D. Juranek.** 1989. Large community outbreak of cryptosporidiosis due to contamination of a filtered public water supply. N. Engl. J. Med. **320:**1372–1376.
14. **Hiatt, R. A., E. K. Markell, and E. Ng.** 1995. How many stool examinations are necessary to detect pathogenic intestinal protozoa? Am. J. Trop. Med. Hyg. **53:**36–39.
15. **Johnson, D. W., N. J. Pieniazek, D. W. Griffin, L. Misener, and J. B. Rose.** 1995. Development of a PCR protocol for sensitive detection of *Cryptosporidium* oocysts in water samples. Appl. Environ. Microbiol. **61:**3849–3855.
16. **Juranek, D. D.** 1995. Cryptosporidiosis: sources of infection and guidelines for prevention. Clin. Infect. Dis. **21:**S57–S61.
17. **Loeb, M., C. Walach, J. Philips, I. Fong, I. Salit, A. Rachlis, and S. Walmsley.** 1995. Treatment with letrazuril of refractory cryptosporidial diarrhea complicating AIDS. J. Acquired Immune Defic. Syndr. Hum. Retrovirol. **10:**48–53.
18. **Mackenzie, W. R., N. J. Hoxie, and M. E. Proctor.** 1994. A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. N. Engl. J. Med. **331:**161–167.
19. **Mackenzie, W. R., W. L. Schell, K. A. Blair, D. G. Addiss, D. E. Peterson, N. J. Hoxie, J. J. Kazmierczak, and J. P. Davis.** 1995. Massive outbreak of waterborne cryptosporidium infection in Milwaukee, Wisconsin: recurrence of illness and risk of secondary transmission. Clin. Infect. Dis. **21:**57–62.
20. **Marshall, R. J., and T. P. Flanigan.** 1992. Paromomycin inhibits *Cryptosporidium* infection of a human enterocyte cell line. J. Infect. Dis. **165:**772–774.
21. **Moore, J. A., and J. K. Frenkel.** 1991. Respiratory and enteric cryptosporidiosis in humans. Arch. Pathol. Lab. Med. **115:**1160–1162.
22. **Nash, T. E., D. A. Herrington, and M. M. Levine.** 1987. Usefulness of an enzyme-linked immunosorbent assay for detection of *Giardia* antigen in feces. J. Clin. Microbiol. **25:**1169–1171.
23. **National Committee for Clinical Laboratory Standards.** 1993. Procedures for the recovery and identification of parasites from the intestinal tract. Proposed guideline M28-P. National Committee for Clinical Laboratory Standards, Villanova, Pa.
24. **Newman, R. D., K. L. Jaeger, T. Wuhib, A. A. M. Lima, R. L. Guerrant, and C. L. Sears.** 1993. Evaluation of an antigen capture enzyme-linked immunosorbent assay for detection of *Cryptosporidium* oocysts. J. Clin. Microbiol. **31:**2080–2084.
25. **Pilla, A. M., M. J. Rybak, and P. H. Chandrasekar.** 1987. Spiramycin in the treatment of cryptosporidiosis. Pharmacotherapy **7:**188–190.
26. **Public Health Laboratory Service Study Group.** 1990. Cryptosporidiosis in England and Wales: prevalence and clinical and epidemiological features. Br. Med. J. **301:**774–777.
27. **Riggs, J. L., K. W. Dupuis, K. Nakamura, and D. P. Spath.** 1983. Detection of *Giardia lamblia* by immunofluorescence. Appl. Environ. Microbiol. **45:**698–700.
28. **Rosenblatt, J. E., and L. M. Sloan.** 1993. Evaluation of an enzyme-linked immunosorbent assay for detection of *Cryptosporidium* spp. in stool specimens. J. Clin. Microbiol. **31:**1468–1471.
29. **Shaw, P. K., R. E. Brodsky, D. D. Lyman, B. T. Wood, C. P. Hibler, G. R. Healy, K. I. E. MacLeod, W. Stahl, and M. G. Schultz.** 1977. A community wide outbreak of giardiasis with evidence of transmission by a municipal water supply. Ann. Intern. Med. **87:**426–432.
30. **Siddons, C. A., P. A. Chapman, and B. A. Rush.** 1992. Evaluation of an enzyme immunoassay kit for detecting cryptosporidium in faeces and environmental samples. J. Clin. Pathol. **45:**479–482.
31. **Skeels, M. R., R. Sokolow, C. V. Hubbard, J. K. Andrus, and J. Baisch.** 1990. *Cryptosporidium* infection in Oregon public health clinic patients, 1985–1988: the value of statewide laboratory surveillance. Am. J. Public Health **80:**305–308.
32. **Smith, P. D., F. D. Gillin, W. R. Brown, and T. E. Nash.** 1981. IgG antibody to *Giardia lamblia* detected by enzyme-linked immunosorbent assay. Gastroenterology **80:**1476–1480.
33. **Sorvillo, F. J., K. Fujioka, B. Nahlen, et al.** 1992. Swimming-associated cryptosporidiosis. Am. J. Public Health **82:**742–744.
34. **Tanyuksel, M., H. Gun, and L. Doganci.** 1995. Prevalence of *Cryptosporidium* sp. in patients with neoplasia and diarrhea. Scand. J. Infect. Dis. **21:**69–70.
35. **Unger, B. L. P., R. H. Yolken, T. E. Nash, and T. C. Quinn.** 1984. Enzyme-linked immunosorbent assay for the detection of *Giardia lamblia* in fecal specimens. J. Infect. Dis. **149:**90–97.
36. **White, A. C., C. L. Chappell, C. S. Hayat, K. T. Kimball, T. P. Flanigan, and R. W. Goodgame.** 1994. Paromomycin for cryptosporidiosis in AIDS: a prospective, double-blind trial. J. Infect. Dis. **170:**419–424.
37. **Wilson, M., P. Schantz, and N. Pieniazek.** 1995. Diagnosis of parasitic infections: immunologic and molecular methods, p. 1159–1170. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

38. **Wittner, M., S. Maayan, W. Farrer, and H. B. Tanowitz.** 1983. Diagnosis of giardiasis by two methods, immunofluorescence and enzyme-linked immunosorbent assay. Arch. Pathol. Lab. Med. **107:**524–527.
39. **Wolfe, M. S.** 1992. Giardiasis. Clin. Microbiol. Rev. **5:**93–100.
40. **Woods, K. M., M. V. Nesterenko, and S. J. Upton.** 1995. Development of a microtitre ELISA to quantify development of *Cryptosporidium parvum* in vitro. FEMS Microbiol. Lett. **1:**89–93.
41. **Zimmerman, S. K., and C. A. Needham.** 1995. Comparison of conventional stool concentration and preserved-smear methods with Merifluor Cryptosporidium/Giardia direct immunofluorescence assay and ProSpecT Giardia EZ microplate assay for detection of *Giardia lamblia*. J. Clin. Microbiol. **33:**1942–1943.