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# Evaluation of Three Commercial Assays for Detection of *Giardia* and *Cryptosporidium* Organisms in Fecal Specimens

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**There is an increasing demand for diagnostic testing for *Giardia intestinalis (G. lamblia)* and *Cryptosporidium parvum*, with a priority being placed on obtaining diagnostic results in an efficient and timely manner. Several commercial companies have developed rapid diagnostic tests that are simple to perform and can be completed in less time than traditional methods for detecting *Giardia* and *Cryptosporidium*. We compared one of these rapid tests, the ImmunoCard STAT! (Meridian Bioscience, Inc.) lateral-flow immunoassay, with the MERIFLUOR direct fluorescent-antibody (DFA) test, the ProSpecT EZ microplate assay for *Giardia* and the ProSpecT microplate assay for *Cryptosporidium*, and modified Kinyoun's acid-fast stained smears for the detection of *Cryptosporidium* using 246 specimens. The MERIFLUOR DFA (Meridian Bioscience, Inc.) test detected the largest number of cases (32 *Giardia* and 37 *Cryptosporidium*) infections and was used to calculate the sensitivity and specificity of the other tests. For *Giardia*, the sensitivities of the ImmunoCard STAT! and the ProSpecT *Giardia* EZ microplate assay (Alexon-Trend, Inc.) were 81 and 91%, respectively. For detection of *Cryptosporidium*, the sensitivities of the ImmunoCard STAT!, the ProSpecT *Cryptosporidium* microplate assay (Alexon-Trend, Inc.), and modified Kinyoun's acid-fast stained smears were 68, 70, and 78%, respectively. Test specificities were equal to or greater than 99%. Specimens with very small numbers of organisms were not detected by the ImmunoCard STAT!, the ProSpecT microplate assay or modified Kinyoun's acid-fast stained smears.**

*Giardia intestinalis* (*G. lamblia*) and *Cryptosporidium parvum* are recognized as two of the most common intestinal protozoan parasites infecting humans in the United States (7, 9). Outbreaks of giardiasis and cryptosporidiosis, which occur via fecal-oral transmission, are associated with consumption of contaminated food (21, 22) and drinking water (2) and use of day care centers (26) and recreational water venues (2, 3, 16). Definitive diagnosis requires the microscopic identification of *G. intestinalis* cysts or trophozoites or *C. parvum* oocysts in stool samples (11, 19). Giardiasis is often hard to diagnose because of intermittent shedding of organisms (6), requiring examination of stool specimens collected over several days. *C. parvum* may be challenging to detect on modified Kinyoun's acid-fast stained smears due to its small size (4 to 6 μm) (20) and variable staining of the oocysts. Furthermore, microscopic identification requires trained microscopists and involves time and labor for preparing, staining, and examining smears (17, 18, 23, 25, 27). As a result, immunoassays for the detection of *Giardia* and *Cryptosporidium* stool antigens have replaced microscopy as the routine diagnostic procedure of choice in many hospitals and public health laboratories (12). These immunoassays are reported to be as sensitive and specific as traditional microscopic methods and increase laboratory efficiency by reducing labor, time, and costs (12).

The most widely used antigen detection immunoassays for *Giardia* and *Cryptosporidium* are the direct fluorescent-antibody (DFA) tests (13), which detect intact organisms, and enzyme immunoassays (EIAs), which detect soluble stool antigens (10, 12). DFA tests utilize fluorescein-labeled antibodies directed against cell wall antigens of *Giardia* cysts and *Cryptosporidium* oocysts and allow visualization of the intact parasites, providing a definitive diagnosis. The sensitivity and specificity of the most commonly used commercial DFA test, the MERIFLUOR DFA test, have been reported to be 96 to 100% and 99.8 to 100%, respectively, for both *Giardia* and *Cryptosporidium* (12, 13, 15, 25, 27). This test has a greater sensitivity than traditional examination of permanent smears for *Giardia* (17) and a sensitivity equal to or greater than that of traditional examination of permanent smears prepared from concentrated stool specimens for *Cryptosporidium* (15). Commercially available EIAs use antibodies for the qualitative detection of *Giardia*- and *Cryptosporidium*-specific antigens in preserved stool specimens (5, 24). The reported sensitivities of EIAs range from 94 to 97% and specificities range from 99 to 100% (12, 15, 27). Advantages of the EIA are as follows: (i) numerous samples can be screened at one time (11), and (ii) tests can be read objectively on a spectrophotometer instead of subjectively on a fluorescence microscope. However, problems with false-positive (8) and false-negative (14) test results have been reported.

Immunochromatographic lateral-flow immunoassays (rapid assays) for both *Giardia* and *Cryptosporidium* have become popular diagnostic tools (10) because they eliminate the need for trained microscopists and costly equipment and can be completed in 10 min rather than the 1 to 2 h required to perform DFA tests or EIAs. These tests are simple, 10-min

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TABLE 1. Comparison of diagnostic procedures for the detection of *Giardia* and *Cryptosporidium* in stool specimens

| MERIFLUOR DFA results | No. of specimens | No. of positive specimens by: | | |
|---|---|---|---|---|
| | | ProSpecT EIA | ImmunoCard STAT! | Modified Acid-Fast Stained Smears |
| *Giardia* positive | 32 | 29 | 26 | NA[a] |
| *Giardia* negative | 214 | 1 | 1 | NA |
| *Cryptosporidium* positive | 37 | 26 | 25 | 29[b] |
| *Cryptosporidium* negative | 209 | 1 | 2 | 40[b] |

[a] NA, not applicable.
[b] A total of 69 specimens were examined for the presence of *Cryptosporidium* using modified Kinyoun's acid-fast stained smears.

card assays that have a reported sensitivity of greater than 97% and a specificity of 100% (4, 10).

We compared the ImmunoCard STAT! (a rapid assay) (Meridian Bioscience, Inc., Cincinnati, Ohio) to the MERIFLUOR *Cryptosporidium*/*Giardia* DFA test (Meridian Bioscience, Inc.), ProSpecT *Giardia* EZ microplate assay (EIA) (Alexon-Trend, Inc., Ramsey, Minn.), and ProSpecT *Cryptosporidium* microplate assay (EIA) (Alexon-Trend, Inc.) for the detection of *Giardia* and *Cryptosporidium*, respectively, as well as modified Kinyoun's acid-fast stained smears for the detection of *Cryptosporidium*, to determine the usefulness of the ImmunoCard STAT! test for the diagnosis of giardiasis and cryptosporidiosis.

## MATERIALS AND METHODS

**Stool specimens.** A total of 246 human fecal specimens from two different sites were collected and preserved in 10% formalin; 177 of these specimens were collected from adults and children in Leogane, Haiti, and 69 were collected in the United States in conjunction with a *C. parvum* recreational-water outbreak. All specimens were collected and used for this study under guidelines established in Institutional Review Board-approved protocols.

**Assays.** A formalin-ethyl acetate concentration procedure was performed on all specimens before performing the DFA test and preparation of modified acid-fast stained smears. Specimens were centrifuged twice at 500 × *g* for 10 min to concentrate (19). The MERIFLUOR *Cryptosporidium*/*Giardia* DFA test was performed on each specimen as specified by the manufacturer (Meridian Bioscience Inc.). One drop, approximately 10 μl, of the concentrated sediment was thinly spread onto each well of the treated slide. The entire well was examined by fluorescence light microscopy with a 20× objective, and the results were recorded. Organisms detected by the DFA test were counted and classified as rare (≤25), few (26 to 175), moderate (176 to 275), and many (≥275). Smears of formalin–ethyl-acetate-concentrated material from the 69 U.S. specimens were stained with modified Kinyoun's acid-fast stain (1). Smears approximately 20 by 20 mm were examined with a 100× oil immersion objective, and the presence or absence of *Cryptosporidium* organisms was recorded. The ProSpecT *Giardia* EZ and ProSpecT *Cryptosporidium* microplate assays were performed on unconcentrated formalin-fixed specimens as specified by the manufacturer (Alexon-Trend, Inc.). Specimens were considered positive if the optical density at 450 nm was 0.050 or greater.

The ImmunoCard STAT! *Cryptosporidium*/*Giardia* rapid assay was performed on unconcentrated formalin-fixed stool specimens as specified by the manufacturer (Meridian Bioscience, Inc.). Results were visualized after 10 min. A positive control line was visible on the device each time the test was completed successfully. A positive reaction appeared as a grey-black band visible at the *Giardia* or *Cryptosporidium* area in the test window. Any reaction in the test window, regardless of color intensity, was interpreted as a positive result. No reaction in the test window and a positive control line was interpreted as a negative result. Tests on samples with weak or faint reactions using the ImmunoCard STAT! were repeated, and the results were read by two readers. Any of the assays above that gave discrepant results were tested twice for accuracy, and the original results were confirmed in all instances.

**Calculations.** For this study, the MERIFLUOR DFA test was considered the "gold standard" for detecting both *Giardia* and *Cryptosporidium* and was assigned sensitivity and specificity rates of 100%. Sensitivity was calculated as the number of positive test results divided by the sum of the DFA-positive results and multiplied by 100. Specificity was defined as the number of negative test results divided by the sum of the DFA-negative results and multiplied by 100.

## RESULTS

A total of 246 formalin-fixed stool specimens were examined for the presence of *Giardia* and/or *Cryptosporidium*. Of these specimens, 32 were positive for *Giardia* and 37 were positive for *Cryptosporidium* using the MERIFLUOR DFA test. The results of all tests are shown in Table 1, and sensitivity and specificity rates for all methods are shown in Table 2.

Results from 26 of 32 DFA-positive *Giardia* specimens were concordant. The ImmunoCard STAT! rapid assay failed to detect six specimens and the ProSpecT EZ microplate assay failed to detect three specimens which were positive for *Giardia* using the DFA test. One specimen was positive for *Giardia* using the ProSpecT EZ microplate assay and the ImmunoCard STAT! rapid assay. Test results were concordant for 25 of the specimens that were positive for *Cryptosporidium*. A total of 14 specimens produced discordant results (Table 3). Of these, 12 specimens were negative by ImmunoSTAT! rapid assay, 11 were negative by the ProSpecT microplate assay, and 8 were negative by modified Kinyoun's acid-fast stained smears. Two specimens were negative using the DFA test and modified Kinyoun's acid-fast stained smears but generated positive results using the ImmunoCard STAT! rapid assay and/or the ProSpecT microplate assay. We also examined the results based on the relative number of parasites present in the specimen (Table 4). Most of the discrepant negative results were seen in specimens with rare or few parasites.

Interpretation of the ImmunoCard STAT! results from the specimens was sometimes problematic because of the low intensity of the bands produced in the test. Also, the intensity of

TABLE 2. Sensitivity and specificity of assays for the detection of *Giardia* and *Cryptosporidium* in stool specimens[a]

| Assay | Sensitivity (%) | Specificity (%) |
|---|---|---|
| *Giardia* | | |
| ProSpecT microplate EZ | 90.6 | 99.5 |
| ImmunoCard STAT! | 81.3 | 99.5 |
| *Cryptosporidium* | | |
| ProSpecT microplate | 70.3 | 99.5 |
| ImmunoCard STAT! | 67.6 | 99.0 |
| Acid-fast stained smears | 78.4 | 100.0 |

[a] The MERIFLUOR DFA test was used as the gold standard.

TABLE 3. Discrepancies between *Giardia* and *Cryptosporidium* test methods

| Genus | No. of specimens | Result obtained with[a]: | | | |
|---|---|---|---|---|---|
| | | MERIFLUOR DFA test | Pro-SpecT EIA | Immuno-Card STAT! | Acid-fast stained smears |
| *Giardia* | 3 | + | − | − | NA |
| | 3 | + | + | − | NA |
| | 1 | − | + | + | NA |
| *Cryptosporidium* | 8 | + | − | − | − |
| | 3 | + | − | − | + |
| | 1 | + | + | − | + |
| | 1 | − | − | + | − |
| | 1 | − | + | + | − |

[a] +, positive by that test; −, negative by that test; NA, not applicable.

the positive control band was always much stronger than that of any of the specimens tested. Furthermore, band intensity was not proportional to the numbers of organisms in the specimen, as determined by the DFA test and modified Kinyoun's acid-fast stained smears, or to the EIA optical density readings.

## DISCUSSION

We compared the ImmunoCard STAT! rapid assay to other currently available techniques for the detection of *Giardia* and *Cryptosporidium*. The specificity of all tests was ≥99%. The ImmunoCard STAT! was the least sensitive test evaluated for both *Giardia* and *Cryptosporidium*.

The results of this study indicate that the MERIFLUOR DFA is the most sensitive detection method tested in this study for these organisms in stool specimens. This test was accepted as the gold standard in this study; however, no testing was performed to determine if there were any false-negative results. Our results also show that although the ImmunoCard STAT! and the ProSpecT microplate assays generally detect positive samples with >175 organisms/10 μl, they often fail to detect samples with small numbers of parasites. This problem is most apparent with *Cryptosporidium* infections: the ImmunoCard STAT! missed 12 of 12 and the ProSpecT EIA missed 11 of 12 samples with parasite densities of <175/10 μl. Although the *Giardia* assays appear to be less strongly affected by parasite density rates, the six samples not detected by the ImmunoCard STAT! assay contained sufficiently few organisms to be classified as containing rare organisms.

In all cases when positive results were obtained using the DFA test but not the other tests, the patients were symptomatic with diarrhea. Our data suggest that a large percentage of ill persons would have not have been properly diagnosed if the ProSpecT EIA or ImmunoCard STAT! had been the sole method for detecting *Giardia* or *Cryptosporidium*.

One *Giardia* and two *Cryptosporidium* specimens were positive by the ProSpecT EIA and/or the ImmunoCard STAT! but were negative by the MERIFLUOR DFA test and were therefore classified as false-positive results. The DFA test detects only intact *Giardia* cysts or *Cryptosporidium* oocysts, and the EIA and the rapid assay detect antigen, which may persist after the patient stops shedding intact organisms. Therefore, the results we obtained may not be false-positives but may represent recently cured cases.

Over the past several years, EIA kits for the detection of *Cryptosporidium* antigens in stool have been reported to have problems with specificity and sensitivity, resulting in significant numbers of false-positive (8) and false-negative (14) results. In this study, the ImmunoCard STAT! and ProSpecT EIA were less sensitive than the MERIFLUOR DFA test for the detection of *Giardia* and *Cryptosporidium*. In low-prevalence populations, tests such as the EIA or the rapid assay, with a low sensitivity as described, should not be used as screening tests or as the sole method of diagnosing giardiasis and cryptosporidiosis.

TABLE 4. Immunoassay results classified by the relative number of parasites present

| Relative parasite no.[a] | No. of positive results obtained by: | | | |
|---|---|---|---|---|
| | MERIFLUOR DFA test | Pro-SpecT EIA | Immuno-Card STAT! | Modified acid-fast stained smear |
| *Giardia* | | | | |
| Rare | 10 | 8 | 4 | NA |
| Few | 6 | 5 | 6 | NA |
| Moderate | 4 | 4 | 4 | NA |
| Many | 12 | 12 | 12 | NA |
| Total | 32 | 29 | 26 | |
| *Cryptosporidium* | | | | |
| Rare | 10 | 0 | 0 | 6 |
| Few | 2 | 1 | 0 | 4 |
| Moderate | 3 | 3 | 3 | 4 |
| Many | 22 | 22 | 22 | 15 |
| Total | 37 | 26 | 25 | 29 |

[a] Organisms detected by the DFA test were counted and classified as rare (≤ 25), few (26 to 175), moderate (176 to 275), and many (≥ 275).

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Use of trade names and commercial sources is for identification only and does not imply endorsement by CDC or the U.S. Department of Health and Human Services.

## REFERENCES

1. **Ash, L. R., and T. C. Orihel.** 1987. Parasites: a guide to laboratory procedures and identification. American Society of Clinical Pathologists Press, Chicago, Ill.
2. **Barwick, R. S., D. A. Levy, G. F. Craun, M. J. Beach, and R. L. Calderon.** 2000. Surveillance for waterborne-disease outbreaks-United States, 1997–1998. Morb. Mortal. Wkly. Rep. **49:**1–21.
3. **Centers for Disease Control and Prevention.** 2001. Protracted outbreaks of cryptosporidiosis associated with swimming pool use—Ohio and Nebraska, 2000. Morb. Mortal. Wkly. Rep. **50:**406–410.
4. **Chan, R., J. Chen, M. K. York, N. Setijono, R. L. Kaplan, F. Graham, and H. B. Tanowitz.** 2000. Evaluation of a combination rapid immunoassay for detection of *Giardia* and *Cryptosporidium* antigens. J. Clin. Microbiol. **38:** 393–394.
5. **Chapman P. A., B. A. Rush, and J. McLauchlin.** 1990. An enzyme immunoassay for detecting *Cryptosporidium* in faecal and environmental samples. J. Med. Microbiol. **32:**233–237.
6. **Danciger, M., and M. Lopez.** 1975. Numbers of *Giardia* in the feces of infected children. Am. J. Trop. Med. Hyg. **24:**237–242.
7. **Dietz, V., and J. M. Roberts.** 2000. National surveillance for infection with *Cryptosporidium parvum* 1995–1998: what have we learned?. 2000. Public Health Rep. **115:**358–363.
8. **Doing, K. M., J. L. Hamm, J. A. Jellison, J. A. Marquis, and C. Kingsbury.**

1999. False-positive results obtained with the Alexon ProSpecT *Cryptosporidium* enzyme immunoassay. J. Clin. Microbiol. **37:**1582–1583.
9. **Furness B. W., M. J. Beach, and J. M. Roberts.** 2000. Giardiasis surveillance—United States, 1992–1997. Morb. Mortal. Wkly. Rep. **49:**1–13.
10. **Garcia, L. S., and R. Y. Shimizu.** 2000. Detection of *Giardia lamblia* and *Cryptosporidium parvum* antigens in human fecal specimens using the ColorPAC combination rapid solid-phase qualitative immunochromatographic assay. J. Clin. Microbiol. **38:**1267–1268.
11. **Garcia, L. S.** 1999. Practical guide to diagnostic parasitology. ASM Press, Washington, D.C.
12. **Garcia, L. S., and R. Y. Shimizu.** 1997. Evaluation of nine immunoassay kits (enzyme immunoassay and direct fluorescence) for detection of *Giardia lamblia* and *Cryptosporidium parvum* in human fecal specimens. J. Clin. Microbiol. **35:**1526–1529.
13. **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.
14. **Hanson, K. L., and C. P. Cartwright.** 2001. Use of an enzyme immunoassay does not eliminate the need to analyze multiple stool specimens for sensitive detection of *Giardia lamblia*. J. Clin. Microbiol. **39:**474–477.
15. **Kehl, K. S. C., H. Cicirello, and P. L. Havens.** 1995. Comparison of four different methods for the detection of *Cryptosporidium* species. J. Clin. Microbiol. **33:**416–418.
16. **Levy, D. A., M. S. Bens, G. F. Craun, R. L. Calderon, and B. L. Herwaldt.** 2000. Surveillance for waterborne-disease outbreaks—United States, 1995–1996. Morb. Mortal. Wkly. Rep. **49:**1–62.
17. **Mank, T. G., J. O. M. Zaat, A. M. Deelder, J. T. M. van Eijk, and A. M. Polderman.** 1997. Sensitivity of microscopy versus enzyme immunoassay in the laboratory diagnosis of giardiasis. Eur. J. Clin. Microbiol. Infect. Dis. **16:**615–619.
18. **Marshall, M. M., D. Naumovitz, Y. Ortega, and C. R. Sterling.** 1997. Waterborne protozoan pathogens. Clin. Microbiol. Rev. **10:**67–85.
19. **National Laboratory 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.
20. **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.
21. **Olsen, S. J., L. C. MacKinnon, J. S. Goulding, N. H. Bean, and L. Slutsker.** 2000. Surveillance for foodborne-disease outbreaks—United States, 1993–1997. Morb. Mortal. Wkly. Rep. **49:**1–62.
22. **Quiroz, E. S., C. Bern, J. R. MacArthur, L. Xiao, M. Fletcher, M. J. Arrowood, D. K. Shay, M. E. Levy, R. I. Glass, and A. Lal.** 2000. An outbreak of cryptosporidiosis linked to a foodhandler. J. Infect. Dis. **181:**695–700.
23. **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.
24. **Rosoff, J. D., C. A. Sanders, S. S. Sonnad, P. R. De Lay, W. K. Hadley, F. F. Vincenzi, D. M. Yajko, and P. D. O'Hanley.** 1989. Stool diagnosis of giardiasis using a commercially available enzyme immunoassay to detect *Giardia*-specific antigen 65 (GSA 65). J. Clin. Microbiol. **9:**1997–2002.
25. **Scheffler, E. H., and L. L. Van Etta.** 1994. Evaluation of rapid commercial enzyme immunoassay for detection of *Giardia lamblia* in formalin-preserved stool specimens. J. Clin. Microbiol. **32:**1807–1808.
26. **Thacker, S. B., D. G. Addiss, R. A. Goodman, B. R. Hooloway, and H. C. Spencer.** 1992. Infectious diseases and injuries in child day care: opportunities for healthier children. JAMA **268:**1720–1726.
27. **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.