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Evaluation of a New Monoclonal Antibody Combination Reagent for Direct Fluorescence Detection of *Giardia* Cysts and *Cryptosporidium* Oocysts in Human Fecal Specimens

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***Giardia lamblia* and *Cryptosporidium parvum* can cause severe symptoms in humans, particularly in the immunologically compromised. Monoclonal antibody reagents offer increased sensitivity and an excellent alternative to conventional staining methods. These reagents are helpful when screening large numbers of patients or those with minimal symptoms. Problems of false-positive and false-negative results with routine staining methods for stool parasites can be eliminated with monoclonal antibody reagents. Known positive formalinized specimens [*Giardia* sp. (n = 60), *Cryptosporidium* sp. (n = 55), and mixed *Giardia-Cryptosporidium* spp. (n = 10)] and negative formalinized specimens (n = 105), of which 46 contained other yeast or human cells or protozoa), were tested by the MERIFLUOR *Cryptosporidium-Giardia* direct immunofluorescence detection procedure. The MERIFLUOR reagent exhibited ± to 4+ (majority, 2+ to 3+) on all *Giardia* cysts and 2+ to 4+ (majority, 3+ to 4+) on all *Cryptosporidium* oocysts. The cysts were generally oval (11 to 15 μm), while the oocysts were round (4 to 6 μm); both showed apple-green fluorescence against a background free of nonspecific fluorescence. All specimens positive for *Giardia* sp. and/or *Cryptosporidium* sp. showed fluorescence, and all specimens negative for the two organisms showed no fluorescence. There were eight specimens previously negative by the ova and parasite examination which were positive by the direct fluorescence method; four contained *Giardia* sp., and four contained *Cryptosporidium* sp. These positive results were confirmed after the examination of additional trichrome and modified acid-fast smears. The MERIFLUOR reagent was very easy to use, and even with a lower fluorescence intensity for *Giardia* sp. cysts, no false-negative or false-positive results among the specimens tested for either organism were found.**

With the increased awareness that both *Cryptosporidium parvum* and *Giardia lamblia* can cause severe symptoms in humans and with renewed interest in water testing, the development and implementation of many new diagnostic techniques have been reported, including various concentration and staining methods (1–9, 11–15). The majority of patients with cryptosporidiosis have been symptomatic, with various degrees of diarrhea. In symptomatic patients, the large number of oocysts present ensures that the diagnosis can be made by a number of techniques. However, some patients may exhibit fewer symptoms or be asymptomatic when the number of oocysts present is very few. In these situations, the use of routine methods such as concentrations and modified acid-fast stains may be insufficient to demonstrate the presence of the parasites. It is well known that there is a correlation between the number of oocysts present in the stool and the clinical presentation of the patient; the more severe the diarrhea, the more oocysts are found in the stool. The number of oocysts passed by patients, including those with AIDS, varies from day to day and week to week (6).

Giardia sp. cysts are shed sporadically and may vary in numbers. Examination of several sequential fecal specimens may not reveal infection with this parasite, particularly if the numbers are low. There has also been renewed interest in waterborne transmission of both parasites, with numerous well-documented outbreaks occurring in the last few years (3, 4, 8). Examples of coinfection with both organisms have also been documented elsewhere (10).

Monoclonal antibody reagents with increased sensitivity offer an alternative method to the routine concentrations and stains, both of which may not reveal infections when few parasites are present. Incidence data obtained from these more sensitive methods would also provide a more accurate reflection of the prevalence of such parasites not only in humans but in environmental water sources. The use of monoclonal antibody methods has also been shown to eliminate the potential for false-positive and false-negative results that may be obtained by routine methods for testing stool specimens (6). Reagents that use the direct method also have the advantage of saving time for the clinical laboratory, a valuable benefit during this era of cost containment.

MATERIALS AND METHODS

Specimens. Human fecal specimens (n = 230) were collected in 10% formalin and submitted to the laboratory. There were 60 *Giardia* sp.-positive specimens (by routine ova and parasite examination), 55 *Cryptosporidium* sp.-positive specimens (by modified acid-fast stains), 10 specimens containing both *Giardia* sp. and *Cryptosporidium* sp. (confirmed by routine ova and parasite examination and modified acid-fast stains, respectively), and 105 specimens negative for both parasites (by routine ova and parasite examination), of which 46 contained yeastlike fungi, parasites, other organisms, or combinations thereof (Table 1).

Monoclonal direct immunofluorescence detection kit. The MERIFLUOR *Cryptosporidium-Giardia* direct immunofluo-

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TABLE 1. Organisms used to test monoclonal antibody reagent for specificity

Organism	No. of samples
Protozoa	
Entamoeba histolytica trophozoites and/or cysts	3
Entamoeba coli trophozoites and/or cysts	7
Entamoeba hartmanni trophozoites and/or cysts	4
Endolimax nana trophozoites and/or cysts	8
Iodamoeba bütschlii trophozoites and/or cysts	4
Chilomastix mesnili trophozoites and/or cysts	1
Dientamoeba fragilis trophozoites	1
Trichomonas hominis trophozoites	1
Balantidium coli trophozoites and/or cysts	1
Blastocystis hominis central body forms	15
Isospora belli oocysts	1
Helminth eggs and larvae	
Ascaris lumbricoides	3
Trichuris trichiura	2
Hookworm	1
Strongyloides stercoralis	3
Taenia spp.	1
Hymenolepis nana	1
Hymenolepis diminuta	1
Diphyllobothrium latum	1
Clonorchis sinensis	1
Paragonimus westermani	1
Fasciola or *Fasciolopsis* sp.	1
Schistosoma mansoni	1
Bacteria	
Shigella flexneri	1
Salmonella group B	1
Salmonella group D	1
Campylobacter jejuni	1
Mycobacterium avium-M. intracellulare	1
Yeastlike fungi	
Candida albicans	1
Candida guilliermondii	1
Candida tropicalis	1
Candida krusei	1
Candida pseudotropicalis	1
Candida parapsilosis	1
Candida (Torulopsis) glabrata	1
Cryptococcus neoformans	1
Cryptococcus laurentii	1
Saccharomyces cerevisiae	1
Geotrichum sp.	1
Trichosporon cutaneum	1
Rhodotorula rubra	1

rescence detection kit procedure was used (Meridian Diagnostics, Inc., Cincinnati, Ohio) according to the manufacturer's directions unless indicated below.

Specimen preparation for fluorescence. Stool sediment was washed in 10% formalin and centrifuged at 500 × *g* for 10 min. One drop (10 μl) of the sediment was spread thinly onto the wells and methanol fixed before staining (6). We prefer to use the concentrated specimen at 500 × *g* for 10 min rather than the unspun specimen.

Preparation of 8-well, Teflon-coated slides. The 8-well, Teflon-coated slides were coated with a glycerol-agar adhesive as previously reported (6). For batching purposes, we used a 7-mm-well slide rather than the 12.5-mm-well slide included in the kit.

Method of slide examination. Each well on the fluorescence slide was scanned at a magnification of ×100, and the organism confirmation was made at a magnification of ×250. The *Giardia* sp. cysts were oval, measuring approximately 11 to 14 μm, and the *Cryptosporidium* sp. 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 prior to 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 one or more *Giardia* sp. cysts (fluorescence, 2+ to 3+) or *Cryptosporidium* sp. oocysts (fluorescence, 2+ to 4+).

TABLE 2. Summary of results obtained with the MERIFLUOR *Cryptosporidium-Giardia* direct immunofluorescence detection kit

Fluorescence reaction	No. of positive results with the following tests:			No. of negative results with the following tests:		
	Trichrome[a]	Mod AFB[b]	Stain[c]	Trichrome[a]	Mod AFB[b]	Stain[c]
Positive	56	51	10	4[d]	4[e]	0
Negative	0	0	0	105	105	105

[a] The trichrome stain was used to detect *Giardia* species only.
[b] The modified acid-fast (Mod AFB) stain was used to detect *Cryptosporidium* species only.
[c] The trichrome and modified acid-fast stains were used to detect *Giardia* species and *Cryptosporidium* species, respectively, in the same specimen.
[d] Confirmed positive after examination of additional trichrome-stained fecal smears.
[e] Confirmed positive after examination of additional modified-acid-fast-stained fecal smears.

RESULTS

Sensitivity was 100% with a fluorescence of 2+ to 4+ (majority, 3+ to 4+) for all 55 *Cryptosporidium* sp. oocysts, in both light and heavy infections. (Light infection was defined as 1 to 5 oocysts per field at a magnification of ×100; heavy infection was defined as >50 oocysts per field at a magnification of ×100.) Specificity was also 100% with all 55 positive specimens exhibiting fluorescence and 105 negative specimens showing no fluorescence.

Sensitivity was 100% with a fluorescence of ± to 4+ (majority, 2+ to 3+) for all 60 *Giardia* sp. cysts, in both heavy and light infections. Light and heavy infections were defined as they were for *Cryptosporidium* sp. oocysts. Specificity was also 100% with all 60 positive specimens exhibiting fluorescence and 105 negative specimens showing no fluorescence. Specimens previously negative by the ova and parasite examination (four were found to be positive for *Cryptosporidium* sp. and four were found to be positive for *Giardia* sp. by the direct fluorescence method). These findings were confirmed by repeated sampling and examination of additional trichrome- and modified-acid-fast-stained fecal smears.

Both sensitivity and specificity were 100% for all 10 specimens containing both *Cryptosporidium* sp. oocysts and *Giardia* sp. cysts. The organisms which were used to test the combination reagent for specificity are listed in Table 1. None of the 46 specimens containing these organisms were positive at any level of fluorescence, and there was no background fluorescence. These results are summarized in Table 2.

DISCUSSION

Since there were no false-positive results with either *Cryptosporidium* sp. or *Giardia* sp., the ability to screen the fluorescent-antibody wells with low power (magnification of ×100) was confirmed. A positive diagnosis for either organism can be determined by finding one *Cryptosporidium* sp. oocyst and/or one *Giardia* sp. cyst per well; the size and shape differences are very obvious. The increase in sensitivity was shown by the fact that eight specimens found to be negative by the routine ova and parasite examination were positive with the MERIFLUOR reagent: four with *Cryptosporidium* sp. oocysts and four with *Giardia* sp. cysts. Since routine screening for *Cryptosporidium* sp. is normally performed on request only, these four positive-patient results were unexpected. It was not until additional permanent-stained trichrome and modified-acid-fast smears were prepared and examined that these positive results were confirmed. This ability to detect very low numbers of organisms provides a great improvement in terms of patient care, particularly in patients who are periodically symptomatic but who are passing very few oocysts and/or cysts. The lower limits of permanent-stained smears are hard to define, particularly when the numbers of specimens submitted for examination, the expertise of the examiner, the quality of the smears, and the time spent on smear examination are considered. However, on the basis of our experience with permanent stains and these newer techniques, we continue to believe that use of this combination reagent is at least 10 times more sensitive than that of the permanent-stained smears.

As reported in a previous study, the preparation time for specimens is comparable to processing for the routine ova and parasite examination; however, the examination time for the fluorescent-antibody preparation is approximately 20 to 30 s (6). The examination of permanent-stained smears can take approximately 15 min or longer, depending on the quality of the smear and the expertise of the examiner. With approximately 60% of any laboratory budget being allocated to personnel, the paid-personnel time required for performing and reading these procedures could be significant. However, in addition to the patient populations serviced, the potential positive rate, and issues related to equipment costs, the overall clinical relevance of such procedures needs to be assessed by each laboratory. Certainly, when adequate therapy for cryptosporidiosis is available, the importance of an early diagnosis cannot be overemphasized, particularly for the immunocompromised patient.

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