Detection of *Cryptosporidium* Oocysts in Human Fecal Specimens by an Indirect Immunofluorescence Assay with Monoclonal Antibodies

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An indirect fluorescence assay (IFA) with monoclonal antibodies developed for the detection of *Cryptosporidium* oocysts in fecal smears (Meridian Diagnostics, Inc., Cincinnati, Ohio) was compared with the Zeihl-Neelsen-modified acid-fast stain (MAFS) in 119 human fecal specimens collected between 1984 and 1987. The sensitivity of the IFA was 100%; all 56 specimens positive by MAFS exhibited fluorescence. There were 63 specimens negative for *Cryptosporidium* sp. by MAFS; of these, 61 were negative by IFA (97% specificity). This discrepancy may reflect an increased sensitivity of the IFA to detect oocysts that were not visualized by MAFS because of faint staining or a paucity of organisms. On average, the IFA required less time than the MAFS (1 versus 5 min, respectively) when only rare or few oocysts were present. Cost comparison of reagents showed the IFA to be three times more expensive. The IFA offers a reasonable alternative to the MAFS because of its high sensitivity and specificity, the simplicity of performing it and interpreting results, and its capability of providing a definitive diagnosis of *Cryptosporidium* oocysts.

Cryptosporidium sp. is a coccidial protozoan parasite known to cause enterocolitis and diarrhea in numerous vertebrate species (3, 9, 12). Common techniques currently used by clinical laboratories to identify the oocyst in fecal specimens include modifications of the acid-fast stain of fecal smears or concentrates (3, 7, 12; L. A. Garcia, T. C. Brewer, D. A. Bruckner, and R. Y. Shimizu, Clin. Microbiol. Newsl. 5:60–63, 1983), the Giemsa stain (10), the safranin stain (1), the auramine-phenol fluorescent stain (11), and oocyst flotation with Sheather sucrose solution followed by phase-contrast microscopy (6). In a recent comparative study, the Zeihl-Neelsen-modified acid-fast stain (MAFS) was recommended for concentrated stool specimens. It is a rapid, inexpensive, and sensitive test, particularly when lower numbers of oocysts are present (6). Recently, an indirect fluorescent-antibody assay (IFA) developed by Sterling and Arrowood and utilizing an immunoglobulin M monoclonal antibody was demonstrated to be both 100% sensitive and 100% specific compared with the MAFS (4, 13). This method has subsequently been marketed and released for commercial use by Meridian Diagnostics (Cincinnati, Ohio) as the Merifluor *Cryptosporidium* kit. We examined 119 fecal specimens submitted for fecal parasite identification for *Cryptosporidium* oocysts, utilizing both the Merifluor IFA kit and the MAFS, and compared the two procedures in terms of sensitivity, specificity, time requirement, and cost to determine the potential usefulness of the Merifluor kit in a clinical laboratory setting. Human fecal specimens were collected from patients at Wilford Hall USAF Medical Center (64 specimens from children and 55 specimens from adults). The majority of the specimens positive for *Cryptosporidium* sp. were preselected specimens collected during a waterborne outbreak of *Cryptosporidium* sp. in San Antonio, Texas (1984 to 1987), which were preserved in 10% Formalin prior to the availability of the IFA. The remaining specimens were randomly selected from

fecal specimens submitted throughout 1987. All specimens were concentrated by using the Formalin ethyl acetate method (5). Specimens were pretreated with 10% KOH (Garcia, et al., Clin. Microbiol. Newsl.) during the 10% Formalin wash prior to centrifugation and preserved in 10% Formalin after concentration.

The MAFS was performed by spreading one drop of fecal sediment onto a glass slide (air dried for 60 min), fixing the slide in absolute methanol (1 min), and air drying the slide. Modified Ziehl-Neelsen stain (8) was added (20 min), followed by a tap water rinse, decolorization with acid alcohol (8) until no color was running from the smear, a repeat rinse, counterstaining with methylene blue (1 min), rinsing, and draining.

The IFAs were performed according to the instructions of the manufacturer and were run in four batches of 30 specimens each. Briefly, 10 μl of fecal material in 10% Formalin, the positive control, and the negative control were placed into the respective wells of one slide. Patient specimens were placed in all wells of the remaining slides. Monoclonal antibody (50 μl) was added to each well, and the slides were incubated in a humidity chamber for 30 min at room temperature. Slides were rinsed with phosphate-buffered saline (pH 7.5) provided in the kit and placed in a Coplin jar containing phosphate-buffered saline (5 min). Fluorescein-conjugated goat anti-murine antibody (50 μl) was added to each well, and the slides were incubated in a humidity chamber (30 min) and given a final rinse with phosphate-buffered saline.

Reading of the slides was performed in a blind fashion. Slides stained by MAFS were scanned completely by light microscopy at ×400 magnification, with confirmation of identification done at ×1,000 magnification. Wells of IFA slides were scanned by fluorescence microscopy at ×400 magnification. Oocysts were identified as round, 4- to 6-μm-diameter organisms exhibiting an apple-green fluorescence. Stains were considered positive if one or more oocysts were visualized.

Results showed that the sensitivity of the IFA was 100%.

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TABLE 1. Results for fecal smears tested for *Cryptosporidium* oocysts by MAFS and IFA

No. of oocysts	No. of specimens negative or positive by:	
	MAFS	IFA
Negative (0)	63	61[a]
Rare (<5)	9	11[a]
Few (6–20)	8	8
Moderate (21–200)	7	7
Many (>200)	32	32

[a] Two fecal specimens from an acquired immunodeficiency patient with *G. lamblia* cysts showed rare oocysts by repeated IFAs but were repeatedly negative by MAFS.

All 56 specimens positive by MAFS exhibited fluorescence. There were 63 specimens negative for *Cryptosporidium* sp. by MAFS. Of these, 61 were negative by IFA (97% specificity). The two specimens negative for oocysts by MAFS on repeat examinations but positive by IFA on three separate examinations occurred in two separate stool specimens from an acquired immunodeficiency syndrome patient with *Giardia lamblia* cysts and demonstrated only rare oocysts (fewer than five). These oocysts exhibited the typical roundness and apple-green fluorescence present in the control. With the exception of these two specimens, both stains gave comparable results (Table 1). Fecal specimens containing other protozoa were negative for oocysts by both MAFS and IFA. Organisms used to test the IFA for specificity were *G. lamblia* (9 specimens), *Blastocystis hominis* (11 specimens), *Endolimax nana* (3 specimens), *Dientamoeba fragilis* (3 specimens), *Entamoeba coli* (6 specimens), *Iodoamoeba butschlii* (1 specimen), *Entamoeba histolytica* (4 specimens), and *Entamoeba hartmani* (5 specimens).

The 97% specificity of the IFA relative to the MAFS as the standard most likely reflects the increased sensitivity of the fluorescent-antibody stains (4, 13) to detect oocysts that were not visualized by MAFS because of faint staining or a paucity of organisms (1, 2). Because no false positives were observed in the other seven stool specimens containing *G. lamblia* cysts, this discrepancy was not believed to represent cross-reactivity. We consider these two specimens positive by IFA alone to be true positives and believe that the IFA may be a preferable test to use as the standard in the detection of *Cryptosporidium* oocysts. These results are similar to those of Garcia et al., who reported the IFA to be more sensitive than the MAFS and found no cross-reactivity with other enteric organisms, including *G. lamblia* (4).

The time required to read the negative slides was comparable in both groups (17 min by IFA and 14 min by MAFS). Oocysts were readily identifiable by both methods within seconds when moderate to many oocysts were present. When only rare or a few oocysts were present, however, the IFA generally required less than 1 min for a positive reading, compared with 5 min by MAFS. Garcia et al. (4) were able to scan wells at ×100 magnification in 20 to 30 s with confirmation at ×200 without a loss of sensitivity. We chose to scan at ×400 to ensure thoroughness in this study.

The reagent cost of the Merifluor kit was substantially higher than that of the MAFS ($1.30 per specimen versus

$0.50 by MAFS when run in batches of 30 specimens). Although there is a potential for organism carryover when specimens are run in quantities, this was not observed. Slides were placed horizontally in Coplin jars during rinses and incubations as precautionary measures to avoid this problem. Cost reduction due to decreased technician time, particularly that resulting if one scans the wells at ×100, was not addressed in this study.

In summary, the IFA is a highly sensitive and specific assay which requires less technician time than the MAFS when the oocysts are present in low numbers. The IFA offers the advantage of a very readily identifiable apple-green fluorescence which provides a definitive diagnosis and can be used for confirmation testing when the technologist is unfamiliar with the organism. Although the reagent cost is significantly higher and the stain requires the availability of a fluorescence microscope, the reduction in cost from the decrease in technician reading time must also be considered. The IFA may be preferable to the MAFS for the detection of *Cryptosporidium* oocysts in fecal specimens in laboratories which frequently encounter this pathogen.

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