

NOTES

Prospective Comparison of Direct Immunofluorescence and Conventional Staining Methods for Detection of *Giardia* and *Cryptosporidium* spp. in Human Fecal Specimens

AJIT J. ALLES,¹ MARY ANN WALDRON,² LUZ STELLA SIERRA,²
AND ANTHONY R. MATTIA^{1*}

*Department of Pathology¹ and the Clinical Microbiology Laboratory,²
Massachusetts General Hospital, Boston, Massachusetts 02114*

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In a prospective comparative study, 2,696 consecutive fresh stool specimens over the course of 1 year were examined for *Giardia lamblia* and *Cryptosporidium parvum* by using a direct immunofluorescent-monoclonal antibody stain (for unspun specimens) and conventional staining methods (chlorazol black E for *Giardia* cysts and modified Kinyoun acid-fast for *Cryptosporidium* oocysts). The direct immunofluorescent-monoclonal antibody method resulted in a significantly increased detection rate for both giardia (118 versus 79 specimens, 49.4%; $P = 0.006$) and cryptosporidia (39 versus 23 specimens, 69.6%; $P = 0.055$).

Intestinal giardiasis and cryptosporidiosis are common causes of diarrheal disease worldwide, often affecting children and the immunosuppressed (2, 3). Water-borne transmission of infective *Giardia lamblia* cysts and *Cryptosporidium parvum* oocysts is most important, and acquisition by person-to-person contact is also possible. The recent massive outbreak of intestinal cryptosporidiosis linked to the Milwaukee public water supply emphasizes the epidemic potential of this parasite (9) and the need for rapid and sensitive diagnostic techniques. Diagnosis of these infections generally requires the morphologic identification of *Giardia* trophozoites or cysts or *Cryptosporidium* oocysts in stool specimens, intestinal aspirates, or intestinal biopsy specimens. The diagnostic sensitivity of routinely stained smears for giardia and that of modified acid-fast staining for cryptosporidia are often limited by the shedding of organisms intermittently or in low numbers. Sensitivity of these conventional staining methods also depends on the skill of the microscopist, and it is often highest when full-time parasitology technologists are examining specimens (12). Garcia and colleagues reported their initial experience with a recently developed, highly specific direct fluorescent-combination monoclonal antibody (DFA) kit for detecting both *Giardia* cysts and *Cryptosporidium* oocysts which showed greatly increased sensitivity compared with that for routinely stained smears from selected specimens and the ability to detect low numbers of organisms (4). This DFA method allows morphological observation of the parasites but requires fluorescence microscopy. Thus far reported clinical experience with DFA diagnosis of these infections is limited, although subsequent reports generally support the improved sensitivity of DFA staining (6, 14, 15).

The purpose of the present study was to prospectively evaluate detection of giardia and cryptosporidia by DFA compared with that by conventional staining methods in a large number

of stool specimens examined in the routine practice of the parasitology section of a large general hospital laboratory. To this end, 2,696 consecutive fresh stool specimens from 2,100 patients (1,405 outpatients and 695 inpatients) that were submitted for ovum- and parasite-examination were studied over the course of 1 year. Our laboratory protocol requirement for fresh stool specimens dictates that specimens must be delivered to the laboratory within 30 min after collection for loose stools and within 1 h after collection for formed stools; the specimens are processed upon receipt. The routine staining methods included initial direct saline and iodine-stained wet preparations and two permanent smears, one of which was stained with chlorazol black E (11) and the other of which was stained with a modified Kinyoun acid-fast stain (8). The chlorazol black E stain, although not widely used, typically provides excellent staining of protozoa in fresh stool specimens, comparable to that by trichrome or iron hematoxylin (1, 5, 7). The remainder of the specimen was concentrated with formalin-ethyl acetate, and saline and iodine-stained wet preparations were made from the concentrate. For the purposes of this study, a third direct smear was prepared directly from the stool specimen, prior to concentration, and stained with the DFA Merifluor Cryptosporidia/Giardia kit (Meridian Diagnostics Inc., Cincinnati, Ohio) according to the manufacturer's instructions; positive and negative controls were stained simultaneously. All slides were read by two full-time parasitology technologists. The routinely prepared specimens were examined with a Leitz (Ernst Leitz, Rockleigh, N.J.) laboratory microscope using the 10, 40, and 100× oil immersion objectives as necessary. The DFA-stained slides were examined with a Zeiss epifluorescence microscope using the 10 and 40× objectives (excitation filter, 450 to 490 nm; mirror, 510 nm; barrier filter, 520 nm; Carl Zeiss, Inc., New York, N.Y.), and the results were recorded independently. Each of the six routinely stained slides prepared from each specimen required 3 to 5 min of reading time (total of 18 min or longer), whereas the single DFA-stained slide could be scanned for fluorescence in approximately 2 min.

* Corresponding author. Present address: Department of Pathology, University of Vermont, Medical Alumni Building, Burlington, VT 05405-0068. Phone: (802) 656-2210. Fax: (802) 656-3509.

TABLE 1. Results of DFA and routine stains for detection of giardia in 2,696 stool specimens

Method	No. of specimens		Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
	Positive	Negative				
DFA	118	2,578	99.2	100	100	99.96
Routine ^a	79	2,617	66.4	100	100	98.5

^a Saline and iodine-stained direct and concentrated wet preps; chlorazol black E-stained permanent smears.

Of the 2,696 specimens examined, 119 were positive for *Giardia* cysts by at least one method. Of these, 78 were positive by both DFA and routine methods, 40 were positive only by DFA, and 1 was positive only by the routine method (Table 1). Thus the overall detection rate of *Giardia* cysts was increased by 49.4% ($P = 0.006$). The single false-negative DFA result in the giardia group was subsequently reexamined and found to be positive by DFA in a smear made from the concentrated specimen. Thus it is possible that sampling error caused the initial negative result.

In the case of *Cryptosporidium* oocysts, 42 specimens were positive by at least one method. Of these, 20 were positive by both the DFA and the modified acid-fast stains, 19 were positive by DFA alone, and 3 were positive only by the modified acid-fast stain (Table 2). Overall, DFA staining increased the detection rate of cryptosporidia by 69.6% ($P = 0.055$). Of the three false-negative DFA results in the cryptosporidium group, one specimen showed faint positivity (two organisms identified) by DFA when restained from the concentrate; the original modified acid-fast-stained slide demonstrated only few organisms. The other two specimens showed no fluorescence at all. The reason for the faint labeling is unclear; however, the lack of staining in the other two specimens could be due to sampling error.

We observed that fluorescent intensity with the DFA stain was somewhat weaker for organisms isolated by our concentration technique. The reason for this is unclear, but it may be due to interfering substances in the concentrating reagents. However, the organisms remained readily identifiable.

Our results show that the DFA method, in addition to having excellent specificity, exhibits vastly improved sensitivity over those of the routine methods used. These findings support and supplement the initial experiences of others with this technique (4, 6, 14, 15). Technologists can become rapidly proficient in the performance of this simple DFA method, and the fluorescent label allows rapid scanning of stained slides, saving valuable observer time. Additionally, the high quality of the reagents results in minimal background autofluorescence or nonspecific staining and enhances the identification of any organisms present. Stool smears stained by conventional methods require considerable experience and extensive screening time. Thus the conventional techniques used here, even in the hands of experienced microscopists, fall short of the require-

TABLE 2. Results of DFA and modified acid-fast stains for detection of cryptosporidium oocysts in 2,696 stool specimens

Method	No. of specimens		Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
	Positive	Negative				
DFA	39	2,657	93	100	100	99.9
MAF ^a	23	2,673	54.8	100	100	99.3

^a Modified Kinyoun acid-fast stain.

ments for reliable screening of large numbers of specimens. That the few false-negative DFA results were obtained from direct smears of fresh specimens may indicate a sampling problem. Garcia et al. utilized centrifugation of formalin-fixed specimens prior to slide preparation (4), which may be a more appropriate processing method.

A limitation to the use of the DFA method is the requirement for the laboratory to possess a microscope with epifluorescence capabilities, which may not be available in many hospital laboratories. A potential cost-effective alternative to conventional fluorescence microscopy is the UV ParaLens microscope adapter (Becton Dickinson, Sparks, Md.), which effectively adds epifluorescence capability to a standard light microscope (10). In this study, 23 selected DFA-stained slides (eight *Giardia*, eight *Cryptosporidium*, and seven negative slides) were examined by using a ParaLens adapter equipped with a 40 \times objective, which showed excellent visualization of organisms comparable to that with the conventional epifluorescence microscope. With such an instrument, a rapid and sensitive DFA technique becomes accessible to even the small laboratory.

Enzyme immunoassays (EIAs) are also commonly used for the diagnosis of giardiasis. Scheffler and Evans reported that a commercial EIA improved detection of giardia by 26% compared with that by conventional microscopy (13). Reports of the systematic evaluation of the performance of EIA versus that of DFA are not yet available, although our results suggest that the DFA method is at least as sensitive as EIA for giardia detection. However, EIAs are not currently available for detection of cryptosporidia, and microscopy remains the "gold standard" of diagnosis of this infection for the immediate future. The increasing awareness of potential large water-borne outbreaks of cryptosporidiosis dictates the need for use of the most sensitive diagnostic techniques available.

More widespread use of the DFA technique would greatly improve the laboratory diagnosis of both giardiasis and cryptosporidiosis from fecal specimens. However, at present, the DFA method is considerably more expensive than conventional staining methods, and this probably precludes its use in the routine stool ovum- and -parasite examination. A more appropriate use may be as a single test in selected cases in which either or both parasites are suspected clinically. An additional key role for DFA methods in epidemiologic and control studies is also likely (6, 15).

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