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Use of an Enzyme Immunoassay Does Not Eliminate the Need To Analyze Multiple Stool Specimens for Sensitive Detection of *Giardia lamblia*

KEVAN L. HANSON[1] AND CHARLES P. CARTWRIGHT[1,2]*

Department of Laboratory Medicine and Pathology, Hennepin County Medical Center, Minneapolis, Minnesota 55415,[1] and Department of Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, Minnesota 55455[2]

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The relative sensitivities of a commercially available enzyme immunoassay (EIA) (ProSpecT *Giardia*; Alexon-Trend Inc., Ramsey, Minn.) and conventional ovum-and-parasite (O&P) examination for the detection of *Giardia lamblia* in preserved stool specimens were determined. Paired stool samples collected independently within a 7-day period from 103 patients were analyzed by both methods. A total of 54 specimens from 30 patients (18 asymptomatically infected with *G. lamblia* and 12 with symptoms consistent with intestinal giardiasis) were determined to be positive for *G. lamblia*, of which 48 (88.9%) were positive by microscopy and 52 (96.3%) were positive by EIA. Both specimens submitted were positive for *G. lamblia* by O&P examination for 66.7% (20 of 30) of the positive patients; for 26.7% (8 of 30) a single specimen was positive by O&P examination, and for 6.7% (2 of 30) of those determined to be infected with *G. lamblia*, both samples were negative by microscopy. The sensitivity of conventional O&P examination was somewhat higher in symptomatically infected individuals, with 75% (9 of 12) of patients in this category having *G. lamblia* detected in both samples, compared with 61% (11 of 18) of asymptomatic patients. A total of 24 positive patients (80%) had *G. lamblia* antigen detected by EIA in both submitted samples, 4 positive patients (13.3%) had one specimen positive by EIA, and the EIA was negative in both specimens from 2 infected individuals (6.5%), the sensitivity of EIA was substantially equivalent in asymptomatic and symptomatic individuals (77 versus 83% of patients with positive results on both specimens). Although the sensitivity of EIA for the detection of *G. lamblia* on a single stool specimen was somewhat higher than that of conventional O&P examination in symptomatic patients (83 versus 75%), in asymptomatic patients (77 versus 61%), and overall (80 versus 67%), examination of two specimens by either EIA or microscopy was necessary to achieve a diagnostic sensitivity of greater than 90%.

Giardiasis is by far the most common enteric parasitic infection in the United States, with an estimated 500 thousand to 1 million cases occurring annually (5). Until relatively recently, definitive diagnosis of this infection was dependent upon microscopic examination of stool specimens for the characteristic cyst and trophozoite forms of *Giardia lamblia*. Unfortunately, the sensitivity of conventional ovum-and-parasite (O&P) examination on a single stool specimen for *G. lamblia* has been shown in several studies to be less than optimal (1, 4, 9, 11) and in a recent study conducted in our laboratory was determined to be only 74% (3). The poor sensitivity of a single O&P examination for diagnosing giardiasis is primarily due to intermittent or low-level shedding of parasites by infected individuals (4) and is one of the principal reasons that parasitology textbooks and laboratory manuals recommend that multiple, independently collected stool specimens be obtained for O&P examination (6, 7).

The commercial availability of enzyme immunosorbent assays (EIAs) for detecting *Giardia*-specific antigens in stool specimens has provided a potentially attractive alternative to conventional O&P examination for diagnosing giardiasis. A number of clinical evaluations of *Giardia* EIAs have found them to be rapid and, perhaps more importantly, cost-effective tools for diagnosing infection with *G. lamblia* (1, 2, 8, 9, 11, 12, 14). The sensitivity of EIA in these studies has been at worst comparable (8, 14) and in most cases somewhat superior (1, 2, 9, 11, 12) to O&P examination. Indeed, for those laboratories that serve patient populations in whom the prevalence of pathogenic enteric parasites other than *G. lamblia* approaches zero, routine use of *Giardia* EIA rather than O&P examination has been suggested (2).

G. lamblia is the most commonly identified pathogenic enteric parasite in specimens submitted to the Hennepin County Medical Center (HCMC) laboratory for O&P examination, with an annual per-specimen prevalence rate of approximately 10% (3). This high prevalence of *G. lamblia* infection is in part attributable to the institution's role in mandated health screening of newly domiciled refugees, many of whom are asymptomatically infected with intestinal parasites. The issue of how to most cost-effectively utilize *Giardia* EIA testing in a high-prevalence setting was, therefore, the primary motivator for this study, since most of the studies published to date comparing *Giardia* EIA with O&P examination have not assessed whether EIA testing of a single specimen results in a diagnostic yield comparable to that obtained if multiple stool samples are examined microscopically. We sought to answer this question by prospectively comparing the sensitivity of a commercial

* Corresponding author. Mailing address: Clinical Laboratories, MC #812, Hennepin County Medical Center, 701 Park Ave., Minneapolis, MN 55415. Phone: (612) 347-3026. Fax: (612) 904-4229. E-mail: charles.cartwright@co.hennepin.mn.us.

TABLE 1. Detection of *G. lamblia* in paired, independently collected, preserved stool samples using either conventional microscopic O&P examination or ProSpecT *Giardia* EIA

Detection method	No. of positive patients					
	Asymptomatic (n = 18)		Symptomatic (n = 12)		All (n = 30)	
	One specimen positive	Two specimens positive	One specimen positive	Two specimens positive	One specimen positive	Two specimens positive
Microscopy	5	11	3	9	8	20
ProSpecT *Giardia* EIA	2	14	2	10	4	24

Giardia EIA with routine O&P examination in the high-prevalence population served by HCMC.

MATERIALS AND METHODS

Between February and June 1999, 206 stool samples collected from 103 patients attending HCMC or its outlying clinics were evaluated for the presence of *G. lamblia* by both conventional O&P examination and the ProSpecT *Giardia* Microplate assay (Alexon-Trend Inc., Ramsey, Minn.). To be included in the evaluation, patients had to have two stool samples submitted to the laboratory for O&P examination that had been independently collected within a 7-day time period. All specimens were received in the ParaPak ULTRA Stool System (Meridian Diagnostics, Cincinnati, Ohio), consisting of stool preserved in 10% formalin in one vial and stool fixed in polyvinyl alcohol in the other. Formalin-preserved material was concentrated prior to examination by use of a formalin-ethyl acetate sedimentation technique as recommended by the manufacturer of the specimen collection kit. Concentrates were read as wet mounts by examining the entire area under a 22- by 50-mm coverslip, using a 10× objective for screening and 40× objective for parasite identification. Permanently stained preparations of stool specimens were made with the material preserved in polyvinyl alcohol by use of Wheatley's (13) modified trichrome stain (Meridian Diagnostics). Trichrome-stained smears were read by examining at least 300 fields using a 100× oil immersion objective.

Giardia EIA testing was performed on unconcentrated formalin-preserved specimens using the manufacturer's recommended procedure. The EIA testing was performed in a blinded manner; specimens were assigned unique identifiers and unlinked from conventional O&P results. Upon completion of assay procedures, absorbance values were measured using a spectrophometer and interpreted using criteria provided by the manufacturer.

In the absence of a true "gold standard" for determining the diagnostic sensitivities of the two test procedures, specimens meeting the following criteria were considered to be positive for *G. lamblia*: (i) positive by O&P examination and positive or negative by *Giardia* EIA and (ii) negative by O&P examination and positive by *Giardia* EIA if the specimen was positive by *Giardia* EIA upon repeat and (a) the other specimen in the pair was positive by O&P examination or (b) the other specimen in the pair was negative by O&P examination but repeatedly positive by *Giardia* EIA or (c) the other specimen in the pair was negative by *Giardia* EIA but the patient was clinically symptomatic and was treated for giardiasis.

RESULTS

Of the 206 stool samples tested, 54 specimens (26.2%) collected from 30 patients (29.1%) were determined to be positive for *G. lamblia*. Conventional O&P examination detected 88.9% (48 of 54) of the positive specimens, while the *Giardia* EIA exhibited a sensitivity of 96.3% (52 of 54). Two O&P-negative specimens that were positive on initial testing with the *Giardia* EIA were negative upon repeat testing, and since the other sample in each pair was negative by both *Giardia* EIA and conventional O&P examination and neither patient was symptomatic, these were considered false-positive results. The specificity of the *Giardia* EIA was, therefore, determined to be 98.7% (150 of 152).

Of the 103 patients evaluated in the study, only 41 (39.8%) had clinical symptoms of giardiasis at the time of specimen collection. The remaining 62 individuals (60.2%) were asymptomatic but had recently entered the United States as refugees and were being evaluated for enteric parasite infection as part of routine health screening. Of those patients determined to be infected with *G. lamblia*, 40% (12 of 30) were symptomatic and 60% (18 of 30) were asymptomatic. The number of specimens positive for *G. lamblia* by O&P examination and *Giardia* EIA on a per-patient basis is shown in Table 1. The O&P examination of two stool samples rather than one increased the diagnostic sensitivity of this procedure by 26.6% (93.3 versus 66.7%) and improved its negative predictive value by 9.3% (97.3 versus 88%). Testing of two stool samples by *Giardia* EIA resulted in a more modest 13.3% (93.3 versus 80%) increase in diagnostic sensitivity and 4.9% (97.3 versus 92.4%) improvement in negative predictive value compared with analysis of a single specimen. The difference in sensitivity between O&P examination and *Giardia* EIA was somewhat more prominent in asymptomatically infected individuals. Of the 18 individuals asymptomatically infected with *G. lamblia*, 11 (61.1%) had the parasite detected by O&P examination in both specimens examined, whereas both specimens were positive in the *Giardia* EIA for 14 asymptomatic patients (77.8%) (Table 1). In contrast, both methods detected *G. lamblia* in each member of a sample pair with near-identical frequency in symptomatic individuals, on 75% (9 of 12) and 83.3% (10 of 12) of occasions for O&P examination and *Giardia* EIA, respectively (Table 1).

Two patients had positive O&P examinations but were negative by *Giardia* EIA testing. In both instances the patients were asymptomatic, only a single specimen was positive, and rare cyst forms of *G. lamblia* were observed only in fecal concentrates; all of these are findings suggestive of a low parasite burden. Two additional patients were diagnosed as positive for *G. lamblia* by *Giardia* EIA but were negative by conventional O&P examination. One of these patients was asymptomatic and consequently was not treated; however, both stool samples collected from this individual were repeatedly positive by *Giardia* EIA, and thus this was considered a true positive. The second patient had a single stool specimen repeatedly positive by *Giardia* EIA, was symptomatic, had a sibling diagnosed concurrently with giardiasis, and was treated with metronidazole with resolution of symptoms.

DISCUSSION

A number of evaluations of the ProSpecT *Giardia* EIA have been published in the peer-reviewed literature (1, 2, 8, 9, 11), with reported sensitivities varying from 91% (1) to 100% (2) and specificities of 98% (1) to 100% (2). In most of these studies, *Giardia* EIA was compared on a direct per-specimen

basis with microscopic examination of stool specimens, by conventional O&P examination either alone (1, 2, 11) or in combination with direct fluorescent antibody staining (8). In our evaluation, *Giardia* EIA detected 52 of 54 specimens determined to be positive for *G. lamblia* using O&P examination plus clinical criteria as the gold standard, for a diagnostic sensitivity of 96.3% (conventional O&P examination had a sensitivity of 88.9%). Two specimens were deemed to have given false-positive *Giardia* EIA results, and thus the specificity of the test in our hands was 98.7%. Our data substantially confirm, therefore, those of previous studies in demonstrating that the ProSpecT *Giardia* EIA is a sensitive and specific alternative to conventional O&P examination for diagnosing giardiasis.

By examining paired, independently collected stool samples, we determined the diagnostic sensitivity of a single O&P examination to be 66.7% (20 of 30 cases detected). This is similar to the sensitivity of 74% reported for a previous study conducted in our laboratory (3) and comparable to sensitivities reported by other investigators (1, 11, 12). This relative lack of sensitivity of O&P examination, which presumably is reflective of low parasite numbers or intermittent shedding of organisms, means that at least two independently collected stool specimens need to be submitted for O&P examination to obtain a diagnostic sensitivity of greater than 90%. A sensitivity of 93.3% was achieved in the present study by microscopically examining two stool samples for parasites. Given that the sensitivity of the *Giardia* EIA, at least on a per-specimen basis, is higher than that of O&P examination, it seemed conceivable that the sensitivity of a single *Giardia* EIA might approach the sensitivity of paired-stool-sample O&P examination. In the present study, however, the sensitivity of *Giardia* EIA on a single sample was 80% (24 of 30 cases detected), which is a considerable improvement over conventional O&P examination of a single specimen (14%; four additional cases), but not equivalent in sensitivity to O&P examination of two stool specimens. The improvement in sensitivity effected by using *Giardia* EIA rather than conventional O&P examination on a single specimen was somewhat greater in asymptomatically infected individuals undergoing routine health screening (17%; three additional cases) than in individuals with symptoms of intestinal giardiasis (8%; one additional case); however, in neither population was a single EIA comparable in sensitivity to O&P examination of paired specimens.

Only one of the previously published evaluations of *Giardia* EIAs used analysis of paired, independently collected stool samples to evaluate the comparative sensitivity of conventional O&P examination and EIA (9). Mank et al. (9) determined the sensitivity of a single O&P examination to be 80%, with microscopic examination of two stool samples increasing the diagnostic yield to 96.4%. Use of a *Giardia* EIA improved the sensitivity of a single-stool-sample analysis to 92.7%, with a negative predictive value substantially equivalent to that achieved by microscopically examining two specimens (98.7 versus 99.4%). Based on these findings, those authors concluded that a single *Giardia* EIA could replace multiple O&P examinations for patients at minimal risk for infection with parasites other than *G. lamblia*. Interestingly, the 12.7% increased sensitivity (92.7 versus 80%) of single-sample *Giardia* EIA testing over single-sample microscopic examination reported by Mank and colleagues (9) was essentially matched by the 13.3% increase (80 versus 66.7%) observed in our study. The primary difference in findings between the two studies was in the sensitivity of a single O&P examination. In our study, in only 66.7% of documented cases were both stool samples positive for *G. lamblia* by O&P examination, whereas in the study by Mank et al. (9), *G. lamblia* was detected microscopically in both samples on 80% of occasions. A possible explanation for this considerable difference in sensitivity of *G. lamblia* detection by O&P examination is that while all the specimens examined in the previously published study were obtained from patients with symptoms of intestinal giardiasis, in the present study only 40% (41 of 103) of patients from whom specimens were obtained were symptomatic. Indeed, in our study, the sensitivity of a single O&P examination for patients exhibiting symptoms of giardiasis was 75%, compared with only 61% in individuals asymptomatically infected with *G. lamblia* (Table 1). Nevertheless, unlike in the previous study (9), even in symptomatically infected patients the sensitivity of a single *Giardia* EIA was not comparable to that of O&P examination of two stool samples (83.3 versus 100%).

In conclusion, the results of our study confirm previous findings, demonstrating the superior sensitivity of *Giardia* EIA testing to conventional O&P examination in both symptomatic and asymptomatic individuals. The improvement in sensitivity achieved by detecting *G. lamblia* antigen was, however, insufficient to achieve parity between single-specimen testing with *Giardia* EIA and multiple-specimen evaluation by conventional O&P examination. In settings where the prevalence of enteric parasitic infection is low and where the primary pathogen identified is *G. lamblia*, routine testing of a single stool specimen by conventional O&P examination has been recommended (10). Based on our findings and on those of Mank and colleagues (9), utilization of *Giardia* EIA as the routine means of enteric parasite detection appears to be a preferable approach in such environments, since it would improve diagnostic yield, shorten turnaround time, and decrease labor cost. In higher-prevalence settings, however, or for patients who are at increased risk for giardiasis, the negative predictive value of the ProSpecT *Giardia* EIA on a single stool sample is not sufficiently high to exclude the possibility of *G. lamblia* infection. To evaluate patients for whom the level of clinical suspicion for *G. lamblia* infection is moderate or high and infection with other intestinal parasites is low, therefore, we recommend that two stool samples be submitted to the laboratory for *Giardia* EIA testing, with analysis of the second sample being performed if the first assay yields a negative result.

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