RE-EVALUATION OF COMMERCIALLY AVAILABLE ENZYME-LINKED IMMUNOSORBENT ASSAY FOR THE DETECTION OF *GIARDIA LAMBLIA* AND *CRYPTOSPORIDIUM* SPP FROM STOOL SPECIMENS

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Abstract. This study aimed to detect *Giardia lamblia* and *Cryptosporidium* spp infection from stool specimens. A total of 345 stool specimens were examined by microscopy (both direct smear and formalin concentration) and EIA techniques (ProSpecT Microplate Assay) for *G. lamblia* and *Cryptosporidium* spp. Of 73 tests positive for *G. lamblia*, 41(56.2%) were positive by microscopy, and 71(97.3%) were positive by EIA. Of 16 tests positive for *Cryptosporidium* spp, 5 (31.3%) were positive by microscopy, and 16(100%) were positive by EIA technique. The results demonstrate that this EIA method is quick, simple, and more sensitive than the microscopy method and should be used for the detection of *G. lamblia* and *Cryptosporidium* spp where the prevalence of these protozoan parasites is a public health problem.

INTRODUCTION

Diarrheal disease is a major cause of childhood illness in rural areas world-wide. *Giardia lamblia* and *Cryptosporidium* are enteric protozoan parasites that take up residence in the small intestine, causing giardiasis and cryptosporidiosis, respectively. Giardiasis occurs world-wide, but is more prevalent in warmer climates. It is also more common in children than in adults. After ingestion, the cysts excyst in the duodenum thereby releasing trophozoites that rapidly multiply and colonize the microvillous surface of the jejunum. This process can lead to endothelial dysfunction, malabsorbtion of essential nutrients, nausea, diarrhea, abdominal distension, cramps, weight loss, flatulence, anemia and general weakness lasting from a few weeks to several months. In the young this can lead to morbidity and even death. Chronic infection may also be asymptomatic (Black *et al*, 1977; Wolfe, 1979; Garcia, 2001). Cryptosporidiosis is also recognized as a significant human disease in world-wide distribution. Transmission of *Cryptosporidium* is direct, by either the fecal-oral route or ingestion of water contaminated with infective oocysts. Illness is characterized by acute, severe diarrheal illness, and often leads to persistent infection or death in immunocompromized patients. Asymtomatic infection may also occur (Dubey *et al*, 1990; McDonald, 1996).

The examination of stool specimens for detection of intestinal parasites in our laboratory involves six different techniques of microscopic analysis: (1) direct saline and (2) iodine wet mounts of fresh stool, a (3) DMSO (Dimethylsulfoxide)-mAFB stained smear of fresh stool, an (4) iodine wet mount and (5) DMSO-mAFB stained smear of a sample concentrated by the formalin-ethylacetate sedimentation concentration technique from a 10% buffered formalin preserved stool, and a (6) trichrome stained smear from a Zinc sulfate polyvinyl alcohol (Zinc PVA) preserved stool (Bronsdon *et al*, 1984; NCCLS, 1997; Kellogg and Elder, 1999; Garcia, 2001). In patients with either giardiasis or cryptosporidiosis, the use of such routine diagnostic methods may be insufficient to detect these organisms. Additionally, diagnosis of these infections by microscopic examination can be time-consuming and expensive. Recent studies have found enzyme-linked immunosorbent assays (ELISA) to be sensitive, cost-effective, simple and a rapid method for the detection of *G. lamblia* and *Cryptosporidium* in stool specimens (Nash *et al*, 1987; Addiss *et al*, 1991; Garcia and Shimizu, 1997; Aldeen *et al*, 1998). The assays employed in this evaluation are manufactured by Alexon-Trend (now Remel) and are currently available (Remel, Inc, 12076 Santa Fe Drive, Lenexa, KS 66215). ProSpecT *Giardia* and *Cryptosporidium* Microplate assays are solid phase immunoassays for the detection of *Giardia* (GSA 65) and *Cryptosporidium* (CSA) Specific Antigens in stool specimens.

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These antigens are produced by the organisms within the host intestinal tract and can be detected in stool specimens with or without the visible presence of cysts, trophozoites or oocysts. In this study the above assays were used and re-evaluated to determine the true prevalence of *G. lamblia* and *Cryptosporidium* spp in the study area.

MATERIALS AND METHODS

After informed consents were obtained, stool specimens were collected from children 3 months to 5 years of age from the Sangkhla Buri district in the west of Thailand along the Thai-Myanmar border. All stool specimens were processed by preparing a 1:3 suspension of stool and preservative, namely 10% buffered formalin and Zinc PVA (Kellogg and Elder, 1999). Saline and iodine wet mounts of fresh stools were also prepared. The slides of fresh stool smears were fixed in absolute methanol for 10 minutes and air dried before performing DMSO-mAFB staining (Bronsdon *et al*, 1984) for detecting *Cryptosporidium* and *Cyclospora* oocysts. An iodine wet mount and DMSO-mAFB smear were prepared from the formalin preserved specimens after performing a formalin-ethylacetate sedimentation concentration. Trichrome staining was performed on the stool specimens preserved in Zinc PVA (Bronsdon *et al*, 1984; NCCLS, 1997; Kellogg and Elder, 1999; Garcia, 2001).

Part of each specimen was kept in a cryogenic vial at -20˚C for weekly shipment to AFRIMS in a box on dry ice. There it was tested for *G. lamblia* and *Cryptosporidium* spp antigens by enzyme immunoassay (EIA: ProSpecT Microplate Assay, Alexon-Trend 14000 Unity St. NW, Ramsey, MN 55303). Positive ProSpecT *Giardia/Cryptosporidium* Microplate Assay results were followed-up by performing individual assays of ProSpecT *Giardia* Microplate Assay and ProSpecT *Cryptosporidium* Microplate Assay. EIA results were read both visually, according to the manufacturer's guidelines (0 to 4+), and spectrophotometrically via optical density (OD) readings.

RESULTS

From October 2001 through October 2002, a total of 345 stool specimens were examined by microscopy as described above and ProSpecT *Giardia/ Cryptosporidium* Microplate Assay. Of 84 positive results by ProSpecT *Giardia/Cryptosporidium* Microplate Assay, subsequently, 71 tested positive by ProSpecT *Giardia* Microplate Assay and 16 were positive by ProSpecT *Cryptosporidium* Microplate Assay. Upon further analysis of the original 84 positives, 9 stool samples were positive by both ProSpecT *Giardia* and *Cryptosporidium* Microplate Assays and 6 were negative when tested by the individual assays (Table 1).

Of 73 tests positive for *G. lamblia*, 41 (56.2%) were microscopy positive, and 71 (97.3%) were positive by EIA. Of 16 tests positive for *Cryptosporidium* spp, 5 (31.3%) were microscopy positive, and 16 (100%) were positive by EIA technique. The sensitivity of EIA ProSpecT *Giardia/ Cryptosporidium*, *Giardia*, and *Cryptosporidium* Microplate Assays were 95.5, 94.9, and 100%, respectively (Table 2).

Visual readings and OD results showed good correlation when comparing positive results. They yielded the same results, except one specimen, in which the EIA *Giardia* Assay was negative by visual reading (0), but the OD yielded a positive result (OD = 0.081). All negative specimens had OD readings equal to or lower than 0.05 (Table 3). Microscopic examination also revealed a number of co-infections: 13 (3.8%) *Ascaris lumbricoides*, 1 (0.3%) *Trichuris trichiura*, 2 (0.6%) *Blastocystis hominis*, 4 (1.2%) *Trichomonas*

Table 1
Results of EIA ProSpecT *Giardia/Cryptosporidium, Giardia,* and *Cryptosporidium* Microplate Assays.

EIA Microplate Assays	No. of stool specimens
Giardia/Cryptosporidium positive results	84
Giardia positive results alone	62
Cryptosporidium positive results alone	7
Both *Giardia* and *Cryptosporidium* positive results	9
Both *Giardia* and *Cryptosporidium* negative results	6

Table 2
Results and sensitivity of EIA and microscopic examination for *G.lamblia* and *Cryptosporidium* in stool specimens.

EIA Microplate Assays Specimens (n= 345)	Result (no.) of microscopic examination and EIA				Sensitivity[a] of EIA *vs* microscopy
	M+ E+	M+ E-	M- E+	M- E-	
Giardia/Cryptosporidium	42	2	42	259	42/44 (95.5)
Giardia	37	2	34	272	37/39 (94.9)
Cryptosporidium	5	0	11	329	5/5 (100)

M: microscopic examination, E: EIA Microplate Assays
[a] Values are number of EIA positive /number of microscopic positive tests (%)

Table 3
Visual reading and OD of positive results by EIA.

	Giardia/Cryptosporidium		*Giardia*		*Cryptosporidium*	
	Visual	OD	Visual	OD	Visual	OD
No.of positive	84	84	70	71	16	16
Mean	3	1.306	3	1.372	2.5	1.738
SD	1.168	0.96	0.617	0.539	1.366	1.772
Min	1	0.063	1	0.067	1	0.057
Max	4	3.926	4	2.153	4	3.947

hominis, and 6 (1.7%) *Cyclospora* spp. These organisms showed no evidence of cross reactivity with *Giardia lamblia* or *Cryptosporidium* spp by EIA.

DISCUSSION

By virtue of its rural location, Sangkhla Buri is characterized by a high incidence of both *G. lamblia* and *Cryptosporidium* spp, 18.4 and 3.4%, respectively (Boonchai *et al*, 2002, unpublished data). The endemic nature of these intestinal parasites and their ability to be transmitted person-to-person (Black *et al*, 1977; Garcia, 2001) calls for reliable diagnostic methods. Accurate and rapid diagnosis can lead to implementation of preventative measures and - in cases of giardiasis - treatment to limit the spread of disease.

Choices for diagnostic testing are ever increasing. Historically, a diagnosis of giardiasis relied on detection of cysts and/or trophozoites in the stool by a highly trained microscopist, while a diagnosis of cryptosporidiosis relied on detection of small oocysts, again, by microscopic examination. These examinations are labor-intensive, subjective, require a well-trained microscopist who sees repeated positives, require consistency in staining, and rely on an infected person shedding the cysts, trophozoites, or oocysts, an event which is known to be intermittent.

For *G. lamblia* detection, advances in laboratory techniques include DFA, CIE, and ELISA. At least one study comparing two of these methods against the "gold standard"-microscopy-found DFA to be no more sensitive than microscopy (Aldeen *et al*, 1998). Detecting *Cryptosporidium* is being performed by an array of stains: auramine, DMSO-mAFB (Bronsdon, 1984), DFA, and ELISA. The drawback of DFA is its high cost. In contrast, EIA techniques to detect these two pathogens are recognized as rapid, easy to use, highly sensitive and specific, less labor-intensive and subjective than microscopic methods, and less dependent on expensive equipment (Dagan *et al*, 1995; Parisi and Tierno, 1995).

Our experience with ProSpecT Microtiter Assays confirmed earlier reports citing their ease of use and increased sensitivity when compared to "gold standard" methods (Dagan *et al*, 1995; Parisi and Tierno, 1995). In contrast to microscopy, ELISA

detected 30 additional *G.lamblia* cases and 11 additional *Cryptosporidium* cases in an infant and child population. The lack of cross-reactivity with five other parasites highlighted the test's specificity. Freezing of specimens did not adversely affect test results. Additionally, high correlation between visual and OD readings made this group of three ELISA tests independent of equipment expenditures. Thus, these ELISA tests served as reliable and sensitive adjuvants to microscopic ova and parasite examinations.

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