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Evaluation of a New Direct ELISA, the *Giardia* II Test, for the Detection of *Giardia Lamblia* Antigen in Fecal Specimens

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Introduction

Giardiasis, caused by the protozoan parasite *Giardia lamblia,* is the most frequently diagnosed parasitic infection in the United States[1]. Microscopy, enzyme-linked immunosorbent assay (ELISA), and immunofluorescence (IFA) are used most commonly in conjunction with clinical history to diagnose giardiasis. Microscopy requires experienced technicians and the costs associated with IFA are often prohibitive. Both microscopy and IFA can also be time consuming. Additionally, *Giardia* cysts may be shed intermittently or at low levels during the course of infection[2] and *Giardia* antigens sometimes are present in fecal samples even if cysts are not. Therefore, ELISAs that detect antigen may be more effective in detecting infections and ELISA may represent the easiest and most cost-effective diagnostic aid for giardiasis. In this study we evaluated a new direct ELISA for detection of *Giardia* cyst wall protein 1 (CWP1) in fecal specimens. The new test, *Giardia* II Test (G2) represents a second generation ELISA developed by TechLab®, Inc. of Blacksburg, VA. G2 is simpler to perform and more sensitive than our first generation *Giardia* Test. G2 utilizes a direct format and offers at least a two-fold increase in sensitivity. G2 consists of a 60 minute incubation with the fecal specimen followed by a 30 minute incubation with conjugate and a 10 minute substrate incubation. The test uses a monoclonal capture antibody and a polyclonal conjugate antibody both specific for *Giardia* CWP1 antigen.

Target Antigen

Like our first generation test, the *Giardia* II test detects the presence of cyst wall protein 1 (CWP1), a heat-stable protein that is produced and shed in the external environment (intestinal lumen)by encysting trophozoites. CWP1 is a useful diagnostic marker for *Giardia* because it is stable and readily detected by specific antibodies.

Aims

The purpose of this study was to evaluate the performance characteristics of the new *Giardia* II Test (G2) by TechLab®, Inc. (Blacksburg, VA). We compared G2 to the ProSpecT® *Giardia* Microplate Assay (PA) by Alexon-Trend (Minneapolis, MN). Both G2 and PA detect CWP1 in fecal specimens[3].

Methods

•124 fecal samples were evaluated. The samples were preserved in SAF (n=56), 10% formalin (n=32) or fresh (n=36). The *Giardia* negative samples were from healthy controls (n=23) and from patients with parasitic infections other than *Giardia lamblia* (n=14). The *Giardia* positive samples were confirmed by microscopy with trichrome staining.

•Discrepant samples were resolved using Merifluor *Cryptosporidium / Giardia* (IFA) (Meridian Bioscience, Inc., Cincinnati, Ohio). IFA was run according to the protocol of the manufacturer.

•G2 and PA were run according to instructions of the manufacturer. The cut-off value for positive samples is $OD_{450} \geq 0.05$ (after subtraction of negative control) for G2 and PA.

Statistical Summary of Test Results

Analysis of G2 and PA vs Microscopy

		Microscopy +	Microscopy -
G2	+	86	0
	-	1	37

		Microscopy +	Microscopy -
PA	+	85	0
	-	2	37

Analysis of G2 vs PA

		PA +	PA -
G2	+	85	1
	-	0	38

Positive by Microscopy and IFA

Performance Characteristics

n=127	TechLab® Inc. *Giardia* II Test (G2) vs Microscopy	Alexon-Trend ProSpecT® *Giardia* Microplate Assay (PA) vs Microscopy	G2 vs PA
Sensitivity	98.9%	97.7%	100.0%
Specificity	100.0%	100.0%	97.4%
PPV	100.0%	100.0%	98.8%
NPV	97.4%	94.9%	100.0%
Correlation	99.2%	98.4%	99.2%

References:

1) Garcia LS, and Bruckner D. (1993). Diagnostic medical parasitology, *American Society for Microbiology, Washington, D.C.* p. 31-48.

2) Burke JA. (1977). The clinical laboratory diagnosis of giardiasis, *Crit. Rev. Lab. Sci.* 7:373-391.

3) Boone JH, Wilkins TD, Nash TE, Brandon JE, Macias EA, Jerris RC, Lyerly DM, (1999). TechLab and Alexon *Giardia* Enzyme-Linked Immunosorbent Assay Kits Detect Cyst Wall Protein 1, *J. Clin. Microbiol.* 37(3):611-4.

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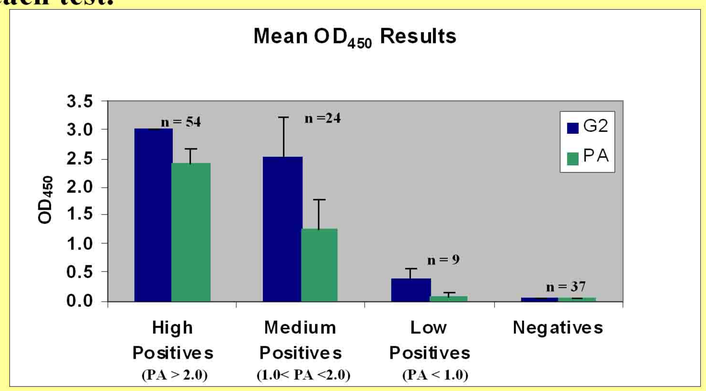
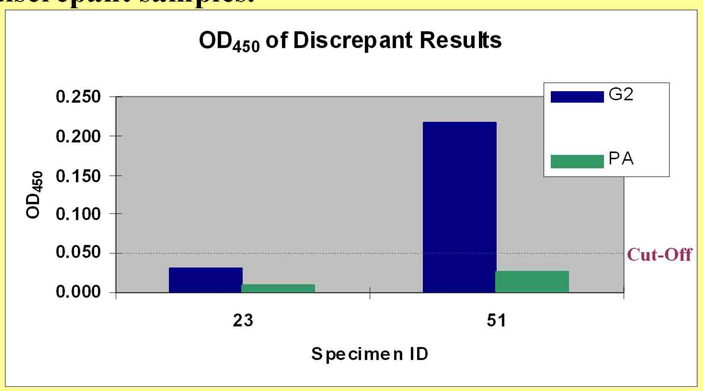
Results

•All samples from healthy persons and from patients with parasitic infections other than *Giardia* (n=37) were negative in both kits.

•Positive samples (n=87) exhibited a higher OD_{450} on G2 than on PA. The graph below shows the average values for samples, with standard deviation bars, in each test.



•Of 87 microscopy positive samples, two samples were negative on PA (23 & 51) and one of the samples was negative on G2 (23). Both discrepant samples contained at least one *Giardia* cyst (per slide) by IFA. The graph below shows the OD values of the discrepant samples.



Conclusion

•TechLab® Inc.'s new *Giardia* II Test (G2) represents a sensitive and specific test for *Giardia* antigen in fecal specimens.

•Fresh fecal samples and samples preserved with SAF or 10% formalin can be used in G2.

•G2 provided higher OD values for positive specimens than PA. G2 may be more effective than PA for detecting low level positive samples.