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Evaluation of *GIARDIA/CRYPTOSPORIDIUM CHEK*™, a New ELISA for the Detection of *Giardia* and *Cryptosporidium* in Human Fecal Specimens

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INTRODUCTION

The current study examined the diagnostic capabilities of two *Giardia* and *Cryptosporidium* combination ELISA tests, the *GIARDIA/CRYPTOSPORIDIUM CHEK*™ (TECHLAB®, Inc.) and the ProspecT® *Giardia/Cryptosporidium* Microplate Assay (Remel, Inc.).

The tests are designed to detect *Giardia* antigen and *Cryptosporidium* antigen in fresh and preserved human fecal specimens. Both require fecal dilution, specimen incubation, conjugate incubation, two washings steps, development, and interpretation of results (visual or spectrophotometric). Total assay time is under 2 hours.

A positive result with both tests indicates the presence of *Giardia* and/or *Cryptosporidium* in the specimen; further testing is required to identify the parasite(s) present within positive specimens. The tests are designed as an efficient and cost-effective method of screening specimens for the presence *Giardia* and *Cryptosporidium* in situations where a large percentage of specimens are routinely found to be negative.

METHODS

Study Specimens: 588 human fecal specimens stored frozen, stored in Cary Blair transport medium, preserved in 10% neutral-buffered formalin, or preserved in sodium acetate formalin (SAF). Of the 588 specimens, 288 were determined to be positive for *Giardia* and/or *Cryptosporidium* and 300 were determined to be negative for both parasites. The *GIARDIA/CRYPTOSPORIDIUM CHEK*™ and the ProspecT® *Giardia/Cryptosporidium* Microplate Assay were tested using the same 588 specimens.

Specimens were tested at LSG & Associates, Santa Monica, CA (163 specimens), the International Center for Diarrheal Disease Research (ICDDR), Dhaka, Bangladesh (153 specimens), and TECHLAB®, Inc., Blacksburg, VA (272 specimens).

Fecal panels at each location were characterized with regard to the presence of *Giardia* and/or *Cryptosporidium* using the *GIARDIA/CRYPTOSPORIDIUM CHEK*™, the ProspecT® *Giardia/Cryptosporidium* Microplate Assay, and additional assays routinely used to identify the presence of *Giardia* and *Cryptosporidium* in human fecal specimens at each testing location. The additional assays included light microscopy, IFA-confirmed microscopy, RT-PCR, and commercially available antigen detection tests specific for *Giardia* or *Cryptosporidium*.

For 582 of the 588 specimens, the *GIARDIA/CRYPTOSPORIDIUM CHEK*™ and the ProspecT® *Giardia/Cryptosporidium* Microplate Assay results agreed as to the positive/negative status of the specimen. Positive results indicated the presence of *Giardia* and/or *Cryptosporidium* in a specimen.

For the 6 discrepant specimens, the final positive/negative status of the specimens was resolved by the individual testing sites using IFA-confirmed microscopy (2 specimens), RT-PCR (1 specimen), or tests ELISA tests specific for *Giardia* or *Cryptosporidium* (3 specimens, tested on the TECHLAB®, Inc. *GIARDIA II and CRYPTOSPORIDIUM II*).

SUMMARY OF TESTING

FECAL SPECIMEN ANALYSIS WITH THE *GIARDIA/CRYPTOSPORIDIUM CHEK*™ ELISA		Characterized Fecal Panel	
ELISA (*n* = 588)		Positive	Negative
TECHLAB®, Inc.'s *GIARDIA/CRYPTOSPORIDIUM CHEK*™	Positive	286	0
	Negative	2	300

Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Correlation
99.3%	100%	100%	99.3%	99.7%

FECAL SPECIMEN ANALYSIS WITH THE ProspecT® *Giardia/Cryptosporidium* Microplate Assay		Characterized Fecal Panel	
ELISA (*n* = 588)		Positive	Negative
Remel, Inc.'s ProspecT® *Giardia/Cryptosporidium* Microplate Assay	Positive	285	1
	Negative	3	299

Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Correlation
99.0%	99.7%	99.7%	99.0%	99.3%

RESULTS

Both tests were sensitive and specific for the detection of *Giardia* and *Cryptosporidium* in human fecal specimens.

Both tests performed well with specimens stored unpreserved, stored in Cary Blair transport medium, preserved in 10% neutral-buffered formalin, or preserved in SAF. Discrepant samples did not correlate to specimen type.

Based on results with the confirming assays, false negative results for both tests were from specimens containing low-levels of *Giardia* or *Cryptosporidium*.

For the 6 out of 588 specimens displaying discrepant results between the TECHLAB®, Inc. *GIARDIA/CRYPTOSPORIDIUM CHEK*™ and the Remel, Inc. ProspecT® *Giardia/Cryptosporidium* Microplate Assay, 4 were resolved in favor of the *GIARDIA/CRYPTOSPORIDIUM CHEK*™ and 2 were resolved in favor of the ProspecT® *Giardia/Cryptosporidium* Microplate Assay according to confirming assays performed at the individual testing sites.

Visual interpretation correlated with spectrophotometric analysis (100%).

Neither tests displayed cross-reactivity with other microorganisms including *Blastocystis hominis*, *Entamoeba* spp., *Dientamoeba fragilis*, *Endolimax nana*, and a variety of intestinal nematodes and cestodes.

CONCLUSIONS

Giardia/Cryptosporidium combination ELISA tests effectively identify the presence of *Giardia* and *Cryptosporidium* in human fecal specimens.

Giardia/Cryptosporidium combination ELISA tests can be used as a time saving and cost-effective primary screen of parasitology specimens to eliminate the often large number of *Giardia*-negative and *Cryptosporidium*-negative specimens.

Specific identification of *Giardia* or *Cryptosporidium* in combination test-positive specimens can be achieved through reflexive testing with ELISA tests specific for the individual parasites.

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