

# Detection of *Giardia* spp. and *Cryptosporidium* spp. Antigen in Human Fecal Specimens using the *GIARDIA/CRYPTOSPORIDIUM CHEK™* ELISA

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## ABSTRACT

**Background:** The *GIARDIA/CRYPTOSPORIDIUM CHEK™* test (TechLab, Inc., Blacksburg, VA) is a 96-well ELISA assay that detects *Giardia* spp. antigens and *Cryptosporidium* spp. antigens in human fecal specimens (fresh, frozen, stored in Cary Blair transport media, fixed in 10% formalin, or fixed in SAF). Positive samples are indicated by a yellow color in the well that can be analyzed spectrophotometrically or interpreted visually. A positive test result indicates the specimen contains *Giardia* spp. antigen and/or *Cryptosporidium* spp. antigen. Assay steps include tube preparation (diluent, patient sample); addition of sample, conjugate and development reagents to the assay wells; two plate washing steps; and, interpretation of results (Total incubation time = 100 minutes). **Methods:** Test performance was evaluated using 218 positive and negative human fecal specimens (75 *Giardia* positive, 47 *Cryptosporidium* positive, 3 positive for both parasites, 93 negative) based on results from trichrome, iron-hematoxylin, or modified acid-fast stains, or the Meridian Biosciences, Inc. *Giardia/Cryptosporidium* Merifluor combination reagent (FA). Specimens showing discrepant results were retested using the Merifluor combination reagent. **Results:** Based on the reference methods, the sensitivity was 97.6% and the specificity was 100% for the *GIARDIA/CRYPTOSPORIDIUM CHEK™* test. False negative *Cryptosporidium* results (3) were obtained from specimens with low parasite numbers; each contained fewer than 5 oocysts per FA well. No false negative results were obtained with samples containing *Giardia* spp. No crossreactivity was seen with 9 different protozoa (113 challenges), 7 different helminths (26 challenges), or human cells (2 challenges) found in fecal samples. Visual interpretation of the test correlated with spectrophotometric results. **Conclusion:** The *GIARDIA/CRYPTOSPORIDIUM CHEK™* test may be very beneficial in the absence of trained microscopists; however, in patients who remain symptomatic after a negative result, the O&P examination and special stains for coccidia and the microsporidia should always remain options.

## METHODS

**Study Samples:** 218 anonymous human fecal specimens previously submitted for routine parasitology screening. Specimens were preserved at a 1:4 dilution (1 part fecal specimen to 3 parts fixative) in polyvinyl alcohol (PVA) for permanent staining and either 10% neutral-buffered formalin or sodium acetate formalin (SAF) for ELISA and FA analysis.

Each specimen was processed using trichrome, iron-hematoxylin, and acid-fast stains and was analyzed using light microscopy for the presence of intestinal parasites. Further testing with the Meridian Biosciences, Inc. *Giardia/Cryptosporidium* Merifluor combination reagent (FA) was used to specifically confirm the presence of *Giardia* and *Cryptosporidium* in the specimens. Any samples showing discrepant results between microscopic analysis and ELISA antigen detection were retested with the FA test and the ELISA test to reconfirm the initial results.

Specimens were tested for the presence of *Giardia* and *Cryptosporidium* antigens using the TECHLAB®, Inc. *GIARDIA/CRYPTOSPORIDIUM CHEK™* ELISA. The test utilizes a single assay well per sample and provides an enzyme-based color reaction for samples containing *Giardia* and/or *Cryptosporidium* antigen. A positive result with the test indicates the presence of either *Giardia* or *Cryptosporidium* and indicates that reflexive testing is required to identify if *Giardia*, *Cryptosporidium* or both parasites are present.

The presence of other organisms identified through light microscopy was recorded to examine antigen assay cross-reactivity.

## Comparison of the *GIARDIA/CRYPTOSPORIDIUM CHEK™* ELISA to IFA-Confirmed Microscopic Analysis for *Giardia* spp. and *Cryptosporidium* spp.

ANALYSIS FOR <i>GIARDIA</i> AND <i>CRYPTOSPORIDIUM</i>		FA-Confirmed Microscopy		
ELISA		Positive	Negative	
TECHLAB®, Inc.'s	Positive	122	0	
<i>GIARDIA/CRYPTOSPORIDIUM CHEK™</i>				
(n = 218)	Negative	3	93	
Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Correlation
97.6%	100%	100%	96.9%	98.6%

ANALYSIS FOR <i>GIARDIA</i> ONLY		FA-Confirmed Microscopy		
ELISA		Positive	Negative	
TECHLAB®, Inc.'s	Positive	75	0	
<i>GIARDIA/CRYPTOSPORIDIUM CHEK™</i>				
(n = 168)	Negative	0	93	
Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Correlation
100%	100%	100%	100%	100%

ANALYSIS FOR <i>CRYPTOSPORIDIUM</i> ONLY		FA-Confirmed Microscopy		
ELISA		Positive	Negative	
TECHLAB®, Inc.'s	Positive	47	0	
<i>GIARDIA/CRYPTOSPORIDIUM CHEK™</i>				
(n = 143)	Negative	3	93	
Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Correlation
94.0%	100%	100%	96.9%	97.9%

## RESULTS

The TECHLAB®, Inc. *GIARDIA/CRYPTOSPORIDIUM CHEK™* ELISA displayed high correlation to FA-confirmed microscopy with a preserved human fecal parasitology panel.

The TECHLAB®, Inc. *GIARDIA/CRYPTOSPORIDIUM CHEK™* ELISA did not display a false-positive reaction. Three false-negative reactions were shown by FA microscopy to contain low levels of *Cryptosporidium* oocysts (<5 oocysts/FA well). There were no false-negative reactions for samples containing *Giardia* cysts.

Visual interpretation correlated well with spectrophotometric analysis.

The TECHLAB®, Inc. *GIARDIA/CRYPTOSPORIDIUM CHEK™* ELISA did not display cross-reactivity with other organisms including *Blastocystis hominis*, *Entamoeba* spp., *Dientamoeba fragilis*, *Endolimax nana*, and a variety of intestinal nematodes and cestodes.



## CONCLUSIONS

The TECHLAB®, Inc. *GIARDIA/CRYPTOSPORIDIUM CHEK™* ELISA is sensitive and specific for the detection of *Giardia* cysts and *Cryptosporidium* oocysts in human fecal samples.

The TECHLAB®, Inc. *GIARDIA/CRYPTOSPORIDIUM CHEK™* ELISA can be utilized as an effective screening assay for parasitology samples in the absence of trained microscopists.

O&P examination and special stains for coccidia and microsporidia should always remain an option for symptomatic patients displaying negative results.

## CONTACTS

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