

Detection of *Cryptosporidium* spp. Antigen in Human Fecal Specimens using the *CRYPTOSPORIDIUM II* ELISA

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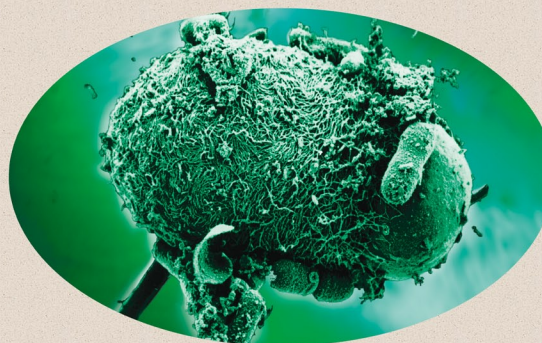
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ABSTRACT

BACKGROUND: The *CRYPTOSPORIDIUM II* test (TECHLAB[®], Inc., Blacksburg, VA) is a 96-well ELISA that detects *Cryptosporidium* spp. antigen in human fecal specimens (fresh, frozen, stored in Cary Blair transport media, preserved in 10% formalin, or preserved 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 *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 185 human fecal specimens, 44 positive for *Cryptosporidium* based on results from the Meridian Biosciences, Inc. Merifluor *Giardia/Cryptosporidium* combination reagent direct immunofluorescence assay (IFA). Specimens showing discrepant results were retested using the Merifluor combination reagent assay. **RESULTS:** Based on the reference methods, the *CRYPTOSPORIDIUM II* test had a sensitivity of 97.7%, a specificity of 100%, a positive predictive value of 100%, a negative predictive value of 99.3%, and a correlation of 99.5%. A single false negative result was obtained from a specimen with low parasite numbers, fewer than 5 oocysts per IFA well. No cross reactivity was seen with 10 different protozoa (174 challenges), 8 different helminths (21 challenges), or human cells (3 challenges) found in fecal samples. Visual interpretation of the test correlated with spectrophotometric results (100%). **CONCLUSION:** The *CRYPTOSPORIDIUM II* ELISA is a new reliable assay for the detection of *Cryptosporidium* spp. in human fecal specimens.

METHODS

- Study Samples: Human fecal specimens submitted for routine parasitological screening preserved in sodium acetate formalin (SAF) or 10% buffered formalin at a 1:4 dilution (1 part feces to 3 parts preservative).
- Samples were analyzed for pathogenic microorganisms using routine staining and microscopic procedures. The presence of *Cryptosporidium* spp. was confirmed using immunofluorescence assay (IFA) microscopy (Merifluor, Meridian Biosciences).
- Samples were further analyzed using the TECHLAB[®], Inc. *CRYPTOSPORIDIUM II* test. The test utilizes a single assay well per sample, and indicates a positive result with a colored reaction for samples containing *Cryptosporidium* spp. antigen.



ANALYSIS FOR *CRYPTOSPORIDIUM*

ELISA		IFA-Confirmed Microscopy		
(n = 185)		Positive	Negative	
TECHLAB [®] , Inc.'s	Positive	43	0	
<i>CRYPTOSPORIDIUM II</i>	Negative	1	141	
Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Correlation
97.7%	100%	100%	99.3%	99.5%

RESULTS

- *CRYPTOSPORIDIUM II* test results correlated well with IFA-confirmed microscopy.
- The test did not display false-positive results.
- The single false-negative result was with a sample containing ≤ 5 *Cryptosporidium* oocysts per well (as identified by IFA).
- The *CRYPTOSPORIDIUM II* test was compatible with positive and negative fecal samples preserved in SAF and 10% buffered formalin (dilution of 1 part feces to 3 parts preservative).
- Visual interpretation of assay results correlated with spectrophotometric analysis for all samples (100%).
- The *CRYPTOSPORIDIUM II* test did not display cross-reactivity with other microorganisms including *Blastocystis hominis*, *Entamoeba* spp., *Dientamoeba fragilis*, and *Endolimax nana*.

CONCLUSIONS

- The TECHLAB[®], Inc. *CRYPTOSPORIDIUM II* test is a sensitive and specific ELISA for the presence of *Cryptosporidium* spp. in human fecal specimens.
- *CRYPTOSPORIDIUM II* test results correlate well with IFA-confirmed microscopy and the test is more sensitive than conventional microscopic techniques (data not shown).
- The *CRYPTOSPORIDIUM II* test requires less sample preparation and assay time compared to complete microscopic analysis:
 1. Specimen concentration is not required.
 2. Specimens maintained in SAF, 10% buffered formalin, or Cary Blair medium may be added directly to the assay well.
 3. Fresh and frozen specimens require a simple one-step dilution - neither filtering nor centrifugation is required.
 4. The test utilizes a two-step direct assay format with a 60-minute sample incubation, 30-minute conjugate incubation, and a 10-minute development step.
- *CRYPTOSPORIDIUM II* test results may be read visually.
- The monoclonal antibody-based format allows the *CRYPTOSPORIDIUM II* test to yield highly specific results with no cross-reactivity with other protozoan parasites.

