#254: Diagnosis of *Giardia* and *Cryptosporidium* Enteric Infections with a New Point-of-Care Rapid Assay

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Background

Giardia spp. and *Cryptosporidium* spp. are pathogenic protozoan parasites able to colonize the human intestine and are among the leading causes of traveler's diarrhea. Infection can result in chronic debilitating diarrhea and nutrient malabsorption. Here, we report the clinical evaluation of the *GIARDIA/CRYPTOSPORIDIUM QUIK CHEK*, a rapid membrane-based assay capable of detecting *Giardia* cyst antigen and *Cryptosporidium* oocyst antigen in human fecal specimens. Specimens tested were obtained from the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) from a cohort of children in an area where *Giardia* and *Cryptosporidium* infection are prevalent. The test utilized immobilized capture antibodies and a soluble peroxidase-conjugated antibody that is combined with a diluted specimen.

Objective

To develop a new Point-of-Care diagnostic test for the simultaneous qualitative detection of *Giardia* and *Cryptosporidium* antigen in human fecal specimens.

Methods

Commercially available *Giardia* and *Cryptosporidium* ELISAs were used in accordance with the manufacturer's instructions (TechLab, Inc., Blacksburg, VA). For fresh specimens, 100 µL of fecal sample was added to 400 µL of diluent in a tube and mixed thoroughly. Preserved specimens were used directly from their Para-Pak vials. One hundred microliters of diluent and 50 µL of the specimen mixture were added to the micro assay plate and were left at room temperature for incubation for 1 hour. The wells were washed five times and a drop (50 µL) of conjugate was added to each well, which was incubated at room temperature for 30 minutes followed by washing. Two drops (100 µL) of substrate were added to each well. After 10 minutes of incubation, 1 drop (50 µL) of stop solution was added to each well. The plate was read using an ELISA reader, with ≥0.150 being the cutoff for the sample to be considered positive at an optical density of 450 nm.

The *GIARDIA/CRYPTOSPORIDIUM QUIK CHEK* (TechLab, Inc., Blacksburg, VA) is a rapid membrane-based assay capable of detecting *Giardia* cyst antigen and *Cryptosporidium* oocyst antigen in fresh, frozen and preserved human fecal specimens. For liquid and formed specimens, 25 µL of specimen (or its solid equivalent) was added to a tube containing a mixture of 500 µL diluent and 1 drop conjugate. For preserved specimens in Para-Paks, 100 µL of specimen was added to a tube containing 400 µL diluent and 1 drop conjugate mixture. Once mixed, 500 µL of the diluted sample-conjugate mixture was transferred into the Sample Port and incubated at RT for 15 minutes. The Reaction Window membrane was washed and 2 drops of substrate was added to the Reaction Window. Results were read and recorded after 10 minutes.

Results

Results from the rapid test were compared to ELISAs specific for *Giardia* and *Cryptosporidium*. The 307 human fecal samples tested at the ICDDR,B included 129 fresh and 178 frozen specimens. The *Giardia* line compared to ELISA had 100% positive agreement, 100% negative agreement, 100% overall agreement. The *Cryptosporidium* line compared to ELISA had 100% positive agreement, 100% negative agreement, 100% overall agreement.

Of the positive specimens there were 81 *Giardia*-positives, 36 *Cryptosporidium*-positives, and 7 dual positive specimens. *Giardia* and *Cryptosporidium* results obtained with the *GIARDIA/CRYPTOSPORIDIUM QUIK CHEK* were confirmed by individual *Giardia* and *Cryptosporidium* ELISAs.

TABLE 1

Number of *Giardia* positive specimens by *GIARDIA/CRYPTOSPORIDIUM QUIK CHEK and GIARDIA* II ELISA .

	G/C QUIK CHEK (+)ve	G/C QUIK CHEK (-)ve	Total
***Giardia* ELISA (+) ve**	**88**	**0**	88
***Giardia* ELISA (-) ve**	**0**	**219**	219
Total	88	219	307

TABLE 2

Number of *Cryptosporidium* positive specimens by *GIARDIA/CRYPTOSPORIDIUM QUIK CHEK and CRYPTOSPORIDIUM* II ELISA .

	G/C QUIK CHEK (+)ve	G/C QUIK CHEK (-)ve	Total
***Cryptosporidium* ELISA (+) ve**	**43**	**0**	43
***Cryptosporidium* ELISA (-) ve**	**0**	**264**	264
Total	43	264	307

TABLE 3

Performance of the *QUIK CHEK* compared to ELISA for the detection of *Giardia* and *Cryptosporidium* for all fresh and formalin preserved specimens.

	G/C QUIK CHEK (+)ve	G/C QUIK CHEK (-)ve	Total
G/C ELISAs (+)ve	**131**	**0**	131
G/C ELISAs (-)ve	**0**	**176**	176
Total	131	176	307

Conclusions

- The major conclusion of this study is that the *GIARDIA/CRYPTOSPORIDIUM QUIK CHEK* allows streamlined testing of multiple specimens and offers a cost-efficient means of screening stool samples for *Giardia* and *Cryptosporidium* infection.
- The *GIARDIA/CRYPTOSPORIDIUM QUIK CHEK* test was examined for compatibility with fresh and formalin preserved specimens during the evaluation. No decrease in sensitivity or specificity was observed with specimens preserved in 10% formalin when compared to fresh and frozen specimens.
- The data from this clinical evaluation indicate that the assay is a reliable method for the identification of *Giardia* and *Cryptosporidium* in fresh and formalin preserved human fecal specimens.

GIARDIA/CRYPTOSPORIDIUM QUIK CHEK

Negative control | **Positive control** | ***Giardia*** | ***Cryptosporidium*** | ***Giardia* & Crypto**