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Evaluation of a Screening Test for Detection of *Giardia* and *Cryptosporidium* Parasites▽

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The *Giardia*/*Cryptosporidium Chek* test (TechLab, Inc.), a screening test for *Giardia* and *Cryptosporidium*, was evaluated with 136 fecal samples. Using the results of the *Giardia II* test and *Cryptosporidium II* test as gold standards, it was 98.4% sensitive and 100% specific and had positive and negative predictive values of 98.7% and 99.3%.

Giardia is a binucleated flagellated protozoan parasite which exists as a noninfectious trophozoite inhabiting the small intestine or as a highly infectious cyst form (7). The noninfectious trophozoite can last only a matter of hours outside the body, while the infectious cyst form can survive for several days in an external environment (7). *Cryptosporidium* is also an enteric protozoan parasite of vertebrates (8). Both parasites are transmitted through fecal-oral routes (9, 10, 26, 32), by consumption of contaminated food or water (9, 18, 30, 32, 33), or by sexual transmission (33). They cause common intestinal waterborne diseases in the United States (6, 20, 21, 31). Contraction of the diseases is more frequent among younger children, and they especially pose a threat to immune system-compromised individuals (1, 3, 19, 31, 33). In developing countries, crowded urban areas and a lack of sanitation enhance the risk of transmission (4, 5, 22, 30, 33). There is a need for rapid diagnostic tests, especially for point-of-care use and in waterborne-outbreak investigations (2, 16, 24, 25, 27–29, 30, 31, 34).

Giardia/*Cryptosporidium Chek* is an immunoassay developed to allow the simultaneous qualitative detection of *Giardia* and *Cryptosporidium* antigens in fecal specimens. It is a microtiter plate enzyme-linked immunosorbent assay (ELISA) that detects both *Giardia* and *Cryptosporidium* cyst antigens from fecal specimens. Positive results of the test signify the presence of either *Giardia* or *Cryptosporidium* or the presence of both pathogens. It should be noted, however, that the test cannot distinguish between the two pathogens.

One hundred thirty-six fecal samples, of both diarrheal and nondiarrheal specimens, were tested at the International Centre for Diarrheal Disease Research, Bangladesh. As a qualitative standard of measurement of the presence/absence of either or both of the antigens, individual ELISAs for *Giardia* and *Cryptosporidium*, *the Giardia II* test and the *Cryptosporidium II* test (TechLab), were used as the gold standard. All specimens with discrepant results were retested by the *Giardia II* test and the *Cryptosporidium II* test and by PCR tests. If the specimen was positive with the *Giardia II* test and/or the *Cryptosporidium II* test and further confirmed by the PCR tests, the specimen was considered a true positive.

The *Giardia*/*Cryptosporidium Chek* assay was used in accordance to the manufacturer's directions. Four hundred microliters of diluent was added to 100 μl of fecal sample in a tube and was mixed thoroughly. One hundred microliters of diluent and 50 μl of the mixture were added to the microassay plate and was left at room temperature for incubation for 1 h. The wells were washed five times using the wash solution provided, and a drop (50 μl) of conjugate was added to each well, which was incubated at room temperature for 30 min, followed by washing. Subsequently, 2 drops (100 μl) of substrate was added to each well. After 10 min of incubation at room temperature, 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.

Using the results of the individual ELISAs as gold standards, the *Giardia*/*Cryptosporidium Chek* assay had a sensitivity of 98.4%, 100% specificity, a positive predictive value of 98.7%, and a negative predictive value of 99.3% (Table 1). One specimen, giving a false-negative result by the *Giardia*/*Cryptosporidium Chek* test, was found to be positive for *Cryptosporidium* by the *Cryptosporidium II* test. The optical density at 450 nm of the false-negative specimen, 0.146, however, was very close to the cutoff value of the *Giardia*/*Cryptosporidium Chek* test, 0.15. The use of *Giardia II* test and *Cryptosporidium II* test ELISAs, instead of the use of PCR, as a measurement of the standard may have overestimated the sensitivity of *Giardia*/*Cryptosporidium Chek*. Nonetheless, the results of the *Giardia*/*Cryptosporidium Chek* test show comparable sensitivity to other diagnostic tests.

Already-existing rapid diagnostic tests for *Cryptosporidium* and *Giardia* include the ImmunoCard Stat! test (Meridian Bioscience, Inc.) (11), the ColorPAC *Giardia*/*Cryptosporidium* test (Becton Dickinson) (12), and the Triage parasite panel (Biosite Diagnostics, San Diego, CA) (13). The other diagnostic tests take comparable amounts of time to perform and with the exception of ImmunoCard Stat! appear to have comparable sensitivities and specificities to those of the *Giardia*/*Cryp-*

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TABLE 1. Performance of *Giardia*/*Cryptosporidium Chek* test

Organism	No. of specimens with result			
	True positive	False positive	True negative	False negative
Giardia	32	0	104	0
Cryptosporidium	32	0	103	1[a]

[a] The one specimen with a false-negative result with the *Giardia*/*Cryptosporidium Chek* test was confirmed to be positive by PCR and *Cryptosporidium* ELISA.

tosporidium Chek test (11, 12, 14, 15, 17, 20, 23). The *Giardia*/*Cryptosporidium Chek* test's ability to test multiple specimens simultaneously, 96 on each plate, offers the promise of a cost- and time-efficient means of screening stool samples for these infections.

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