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Evaluation of Rapid Antigen Point-of-Care Tests for Detection of *Giardia* and *Cryptosporidium* Species in Human Fecal Specimens

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In Bangladesh, a new parasite rapid antigen test was investigated demonstrating accuracy and feasibility. For *Giardia* species, it had a sensitivity and specificity of 94% and 100%, respectively. For *Cryptosporidium* species, it had a sensitivity and specificity of 100% and 100%, respectively. These are higher than or equal to the sensitivities and specificities of other tests on the market.

TABLE 1 Results of the three rapid antigen tests for *Giardia* and *Cryptosporidium* species compared to real-time PCR as the reference standard[a]

Parameter[b]	Result by:					
	Quik Chek		ImmunoCard STAT!		Xpect	
	Giardia	*Cryptosporidium*	*Giardia*	*Cryptosporidium*	*Giardia*	*Cryptosporidium*
Sensitivity (%)	78	100	78	100	56	58
Specificity (%)	100	100	44	50	78	100
Positive predictive value (%)	100	100	58	80	71	100
Negative predictive value (%)	82	100	67	100	64	55
No. of samples TN/TP	9/7	6/12	4/7	3/12	7/5	6/7
No. of samples FN/FP	2/0	0/0	2/5	0/3	4/2	5/0

[a] A total of 18 samples with discrepant results between the rapid antigen tests and ELISA were tested by real-time PCR.
[b] TN/TP, true negative/true positive; FN/FP, false negative/false positive.

The organisms *Giardia* and *Cryptosporidium* are protozoan parasites transmitted by the fecal-oral route, most commonly by the consumption of contaminated food and water. These parasites are common causes of diarrhea (1, 2, 4). Infections are more often seen in young children as well as those who are immunocompromised (4, 10). Enteric infections in children can have devastating consequences affecting intestinal absorption, nutrition, and childhood development (1, 3). While these infections are seen in developed countries, they are especially a threat to those living in developing countries. There is an increased risk of transmission in developing countries due to urban crowding and poor sanitation (1, 4).

A simple yet accurate method of detection is necessary for quick and effective treatment. Rapid antigen cartridge tests are available for the detection of these pathogens. These tests can be used in situations where there is limited time, labor, and resources. Results are available during the course of a clinic visit, thus ensuring that treatment can be provided (5, 7). Currently there are two rapid antigen tests for stool samples available: ImmunoCard STAT! *Cryptosporidium*/*Giardia* (Meridian Bioscience, Inc.) and Xpect *Giardia*/*Cryptosporidium* (Remel, Inc.). Here we report the results of a new rapid antigen cartridge test for the detection of *Giardia* and *Cryptosporidium* species, TechLab, Inc.'s *Giardia*/*Cryptosporidium* Quik Chek. We examined the performance levels of the three tests compared to the results of enzyme-linked immunosorbent assay (ELISA) and PCR testing.

Stool specimens were obtained from a cohort of children in an area of Dhaka, Bangladesh, where *Giardia* and *Cryptosporidium* infections are prevalent (9). The sample panel included 117 diarrheal and nondiarrheal specimens that were tested at the International Centre for Diarrheal Disease Research, Bangladesh. All samples were fresh fecal specimens stored at −20°C and defrosted for use. As a qualitative standard of measurement of the presence/absence of either or both of the antigens, individual ELISAs for *Giardia* and *Cryptosporidium* were performed using the *Giardia* II and *Cryptosporidium* II tests (TechLab, Inc.). The 117 stool samples were tested by all three rapid antigen tests. PCR tests were performed on all specimens with discrepant results, in addition to 10% of the other samples.

The Quik Chek assay was used in accordance with the manufacturer's directions. Fresh fecal specimens were used for testing; however, this test is reportedly compatible with samples preserved in 10% formalin, sodium acetate formalin, and transport media such as Cary Blair or C&S. Five hundred microliters of diluent was added to a tube. One drop of conjugate was then added. Twenty-five microliters of specimen was added to the tube. Five hundred microliters of the mixture was

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TABLE 2 Results of the three rapid antigen tests for *Giardia* and *Cryptosporidium* species using both ELISA as the reference standard and, for ELISA discrepant results, real-time PCR

Parameter[a]	Result by: Quik Chek		ImmunoCard STAT!		Xpect	
	Giardia	*Cryptosporidium*	*Giardia*	*Cryptosporidium*	*Giardia*	*Cryptosporidium*
Sensitivity (%)	94	100	94	100	88	82
Specificity (%)	100	100	94	97	98	100
Positive predictive value (%)	100	100	86	90	94	100
Negative predictive value (%)	98	100	98	100	95	95
No. of samples TN/TP	83/32	89/28	78/32	86/28	81/30	89/23
No. of samples FN/FP	2/0	0/0	2/5	0/3	4/2	5/0

[a] TP/TN, true positive/true negative; FN/FP, false negative/false positive.

added to the sample well of the test device. After the test device was left to incubate for 15 min, 300 μl of wash buffer was added to the reaction window, followed by 2 drops of substrate. Ten minutes later, the results were read (14). The Xpect assays were also used in accordance with the manufacturer's directions. One hundred microliters of unpreserved stool sample diluted 1:4 in deionized water was added to 4 drops of specimen dilution buffer in a tube. Four drops of Conjugate was added to a tube. Two hundred microliters of the sample-Conjugate mixture was transferred to the well of the test device. After 15 min, the assay was read visually (11). The ImmunoCard STAT! assay was performed according to the manufacturer's directions. Sixty microliters of unpreserved stool sample diluted 1:4 in deionized water was added to 2 drops of sample treatment buffer. Two drops of Conjugate reagent A and 2 drops of Conjugate reagent B were also added to the tube. The sample was mixed, and then the entire contents were transferred into the sample well of the test device. The results were read visually after 10 min (8). A control line was present on all three tests if the test was run correctly. Samples were considered positive if a line, regardless of intensity, appeared in the appropriate position in the results window at the specified cutoff time (8, 11, 14).

TechLab, Inc., *Giardia* II, and *Cryptosporidium* II kits were used for ELISA testing of the samples. Both kits allowed for unpreserved, frozen, or preserved samples to be used. The kits were used in accordance with the manufacturer's directions (12, 13). Multiplex PCR was conducted by the method of Haque et al. (6). The primers and TaqMan probes for *Giardia* (accession no. M54878) were designed on the small subunit rRNA gene. The primers and TaqMan probes for *Cryptosporidium* were designed on *Cryptosporidium* oocyst wall protein (COWP; accession no. AF248743). The amplified targets were 62 bp for *Giardia* and 151 bp for *Cryptosporidium* (6).

To determine the sensitivity and specificity of each of the rapid antigen tests, ELISA was used as the reference standard. Using ELISA as the reference standard, the *Giardia* Quik Chek had a sensitivity, specificity, positive predictive value, and negative predictive value of 100%, 98.8%, 96.8%, and 100%, respectively. For *Cryptosporidium*, Quik Chek had a sensitivity, specificity, positive predictive value, and negative predictive value of 100%, 100%, 100%, and 100% respectively. This compared favorably to ImmunoCard STAT! and Xpect tests.

PCR testing was performed on 18 samples that were discrepant. For these 18 samples, ELISA results did not match those of the rapid antigen tests, or there were differences in the results obtained between the three antigen tests. The cause of the discrepancies could not be identified. In each antigen test performed, the control line was visible when results were collected. This indicated that the test was run correctly. For 15 of the 18 samples, the PCR results matched those of the ELISA (Table 1). PCR results matched the results of the Quik Chek in all but two cases.

Using both ELISA and, for the 18 discrepant samples PCR as reference standards for *Giardia*, Quik Chek had a sensitivity, specificity, positive predictive value, and negative predictive value of 94%, 100%, 100%, and 98% respectively. For *Cryptosporidium*, Quik Chek had a sensitivity, specificity, positive predictive value, and negative predictive value of 100%, 100%, 100%, and 100% respectively (Table 2).

The major conclusion of this study is that the *Giardia*/*Cryptosporidium* Quik Chek can be used reliably for routine diagnosis of *Giardia* and *Cryptosporidium* in stool samples. For the detection of *Giardia* and *Cryptosporidium* species, the Quik Chek test has sensitivity and specificity higher than or equal to those of the other two products, ImmunoCard STAT! and Xpect. The Quik Chek is a rapid test that can be run at the bedside of the patient and requires minimal equipment and training, making it a valuable tool for patient care.

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