

Diagnosis of *Giardia* and *Cryptosporidium* Infection in Children with Diarrheal Illness Using Rapid Immunochromato- graphic Assay

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Abstract

Background: *Giardia* and *Cryptosporidium* are both enteric pathogens causing diarrhea with no reliable method for their diagnosis. The aim of our study was to evaluate the diagnostic performance of a rapid immunochromatographic (IC) ImmunoCard STAT! *Crypt/Giardia* assay (Meridian Bioscience, Inc.) in comparison to composite reference standard (CRS) of both wet mount microscopy and ELISA copro-antigen assay in the diagnosis of both pathogens.

Methods and Findings: One hundred twenty two children complaining of chronic diarrhea were checked for infection by *Giardia* and *Cryptosporidium* using wet mount microscopy, ELISA copro-antigen assay and the rapid IC test. The rapid assay had a sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPP) and diagnostic accuracy (DA) of 89, 100, 100, 94% and 96% for *Giardia* and 78, 100, 100, 98 and 98% for *Cryptosporidium* in reference to CRS.

Conclusions: The rapid IC assay proved to be simple, time-saving and objective; it can be used as a rapid screening test for giardiasis and cryptosporidiosis or integrated with other diagnostic modalities to confirm diagnosis.



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Introduction

Giardia lamblia and *Cryptosporidium parvum* are both common enteric parasites in human causing gastrointestinal infections [1-3], manifested with a wide range of clinical spectrum ranging from asymptomatic carrier to long-lasting diarrhea with malabsorption [4-6]. Both parasites are transmitted through fecal-oral routes [7, 8], by consumption of contaminated food [9, 10] or water [3, 11]. In developing countries, crowded urban areas, poor water quality and a lack of sanitation enhance the risk of transmission [3, 12].

Contraction of the diseases is more frequent among younger children especially those in day care centers [13, 14]. These

infections in childhood are of major importance as being the most frequent cause of malabsorptive diarrhea that have a negative impact on growth and development of children [15-17].

Conventional microscopical diagnosis of *Giardia* and *Cryptosporidium* infections is time-consuming, and relies crucially on the microscopist's skills and experience [18]. Rapid antigen detection assays have the advantage of being more precise, rapid, simple and cost-effective modality, offering a relevant alternative method to the routine examination method and provide the added sensitivity required to confirm infections in patients with low parasite numbers [19].

The purpose of this study is to evaluate the diagnostic performance of an immunochromatographic (IC) test, ImmunoCard STAT! *Crypt/Giardia* (Meridian Bioscience, Inc.) for diagnosing giardiasis and cryptosporidiosis by comparing it with composite reference standard (CRS) of wet mount microscopy and ELISA copro-antigen assay.

Material and methods

The study was carried out on a sample of 122 children attending the Outpatient Clinics at Mansoura University Children Hospital. Recruited children were in the age of 6 months to 14 years, suffering from persistent and/or recurrent diarrhea with the exclusion of children who had received anti-parasite treatment in the last three months before specimen collection. Diarrhea was defined as an increase of stool frequency to three or more times a day and/or the change of stool consistency into more liquid form.

Fresh stool specimens were collected in sterile leak proof containers and send immediately to laboratory. Each specimen was divided into three parts. The first part was left fresh for wet mount microscopy using formalin-ethyl acetate concentration method and mounted smears were stained by Trichrome [18] for detecting *Giardia* and Kinyoun modified acid-fast and Auramine-Rhodamine stains for detecting *Cryptosporidium* [20].

The second part was preserved in 10% formalin for performing the rapid IC ImmunoCard STAT! *Crypt/Giardia* assay according to the manufacturer's instructions (Meridian Bioscience, Inc.). The assay procedure involves the addition of 2 drops of sample treatment buffer to a tube with 60µL of stool specimen then adding 2 drops of colloidal carbon-conjugated detection reagent for *Giardia* and *Cryptosporidium*. After mixing, the sample was immediately poured into the test device. Results were visualized after 10 min. positive results were visualized as gray-black lines. A positive control line was visible on the device each time the test was completed successfully.

The last third part of each stool specimen was immediately frozen and stored at -20°C. Subsequently, the frozen part were thawed and mixed thoroughly before testing for *Giardia* and *Cryptosporidium* copro-antigen using Ridascreen *Giardia* and Ridascreen *Cryptosporidium* (R-Biopharm) enzyme immunoassay respectively. Both tests were performed according to the manufacturers' instructions.

Analysis

The validation parameters of the rapid IC assay were determined compared to those of CRS of wet mount microscopy and copro-antigen assay. Specimen was labeled as positive if the results of either wet mount or copro-antigen were positive. On the other hands, Specimen was labeled negative if both mount preparations and copro-antigen were negative. Data entry and analyses were performed using the SPSS statistical package version 16 (SPSS, Inc., Chicago, IL, USA). The description of qualitative data was done in form frequency and proportions. The chi-square (χ^2) test was used to test for the potential association between groups for qualitative data. A p value of <0.05 was considered a significant difference at confidence interval 95%.

Result

Of 122 stool samples, 45 were confirmed as true positives for *Giardia*, and 9 for *Cryptosporidium* reporting 38.5% and 7.4% incidence of *Giardia* and *Cryptosporidium* in children suffering from chronic diarrheal illness, respectively. The results obtained with the different methods are shown in **Table 1**. It was noted that any positive sample by any method was also positive by Ridascreen *Giardia* enzyme immunoassay for diagnosis of *Giardia* and Kinyoun modified acid-fast for diagnosis of *Cryptosporidium*. So, the number of positive samples for Ridascreen *Giardia* enzyme immunoassay and Kinyoun modified acid-fast will represent the total number of positives for *Giardia* and *Cryptosporidium*, respectively.

Table 1. Result of various diagnostic assays for both *Giardia* and *Cryptosporidium*.

Protozoan and Diagnostic Assay	Positive number	Positive rate (%)
Number of <i>Giardia</i> specimen = 122		
Formaline-ethyl acetate conc.	39	31.96
Trichrome stain	37	30.32
Ridascreen <i>Giardia</i>	47	38.52
ImmunoCard STAT! <i>Giardia</i>	42	34.42
Number of <i>Cryptosporidium</i> specimen =122		
Auramine-Rhodamine stain	6	4.91
Kinyoun modified acid-fast	9	7.37
Ridascreen <i>Cryptosporidium</i>	7	5.73
ImmunoCardSTAT! <i>Cryptosporidium</i>	7	5.73

In validating the immunochromatographic ImmunoCard STAT! *Crypt/Giardia* assay against the reference methods, the calculated sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPP) and diagnostic accuracy (DA) were 89, 100, 100, 94% and 96% for *Giardia* and 78, 100, 100, 98 and 98% for *Cryptosporidium*, respectively (Table 2). There were 5 false negative specimen for giardiasis and 2 false negative cryptosporidiosis recorded by ImmunoCard STAT! *Crypt/Giardia* assay.

Discussion

The detection of *Giardia* and *Cryptosporidium* antigens in stool specimens using enzyme immunoassays has become an accepted approach to diagnosis but, it's laborious, time-consuming and can be used in epidemiological survey for screening multiple specimens simultaneously but not as a routine laboratory technique [21]. So, there is need for better diagnostic method that surpasses the limitations of the currently available techniques and not to miss any case of infection. In this context, the immunochromatographic rapid test was tried and its performance was assessed in reference to other conventional methods.

In this study, the overall incidence of both *Giardia* and *Cryptosporidium* in children with diarrheal illness was 38.5% and 7.4% respectively. A similar study done in Cairo [22] reported 31.1% incidence for *Giardia* and 19.8% for *Cryptosporidium*,

this difference may be attributed to the different epidemiological patterns of parasitic infection in Egypt.

The performance of ImmunoCardSTAT! *Cryptosporidium* against the CRS was 78, 100, 100, 98 and 98% for sensitivity, specificity, PPV, NPV and DA respectively. The sensitivity of the ImmunoCardSTAT! was less than that of Kinyoun modified acid-fast, equal to Ridascreen immunoassay and more than Auramine-Rhodamine stain. The ImmunoCard STAT! *Cryptosporidium* assay may eliminate some of the skill needed in performing complicated staining procedures and recognizing the morphology of the small oocyst. However, staining holds importance due to its low cost in addition to having a comparable efficacy with the assay [23].

A recent study [24] conducted in Egypt to evaluate the ImmunoCard STAT! *Cryptosporidium* assay on 315 immunocompromised patients, have reported 96% (95% CI, 87% to 104%) sensitivity and 97% (95% CI, 93-103) as the total accuracy of the assay. Another study [19] reported 98.8% sensitivity, 100% specificity, 100% PPV, and 99.7% for NPV for the same assay using a total of 401 specimens. On the other hand, a similar study [25] carried on 246 specimens reported 67.6% sensitivity, 99% specificity for ImmunoCardSTAT! *Cryptosporidium*. The difference in sensitivities may be due to diversity of genotypes of *Cryptosporidium* among different population, the immune status of patients and also due to the different number of samples used in each study.

Table 2. Performance of different assays in the diagnosis of *Giardia* and *Cryptosporidium*.

Protozoan and Assay	(%)Sensitivity (95% CI) ^a	(%)Specificity (95% CI)	PPV ^b (%)	NPV ^c (%)	DA ^d (%)
For <i>Giardia</i>					
Formaline-ethyl acetate conc.	83 (0.69; 0.92)	100 (0.94; 1.00)	100 (0.89;1.00)	90 (0.81; 0.95)	93 (0.87;0.97)
Trichrome stain	79 (0.64; 0.89)	100 (0.94; 1.00)	100 (0.88;1.00)	88 (0.79; 0.94)	92 (0.85;0.96]
ImmunoCard STAT! <i>Giardia</i>	89 (0.76; 0.96)	100 (0.94; 1.00)	100 (0.90;1.00)	94 (0.85; 0.98)	96 (0.90; 0.98)
For <i>Cryptosporidium</i>					
Auramine-Rhodamine stain	67 (0.31; 0.91)	100 (0.96; 1.00)	100 (0.52; 0.98)	97 (0.92; 0.99)	98 (0.92; 0.99)
Ridascreen <i>Cryptosporidium</i>	78 (0.40; 0.96)	100 (0.96; 1.00)	100 (0.56;0.99)	98 (0.93; 1.00)	98 (0.94; 1.00)
ImmunoCardSTAT! <i>Cryptosporidium</i>	78 (0.40; 0.96)	100 (0.96; 1.00)	100 (0.56; 0.99)	98 (0.93;1.00)	98 (0.94;1.00)

^a CI, confidence interval; ^b PPV, positive predictive value; ^c NPV, negative predictive value; ^d DA, Diagnostic accuracy.

In the current study, 2 false negative specimens for *Cryptosporidium* were reported by the ImmunoCard STAT! , this may be because both ImmunoCardSTAT! and Ridascreen immunoassay detect only *Cryptosporidium parvum* [26] while, human Cryptosporidiosis is generally caused by the species *Cryptosporidium hominis* beside many different genotypes and subtypes of *Cryptosporidium* [14]. Also, both assays generally detect positive samples with >175 organisms/10 µl, they often fail to detect samples with small numbers of parasites that can be detected by staining more than one smear of same sample [25].

Regarding giardiasis, Ridascreen *Giardia* immunoassay proved to be the most sensitive method in the diagnosis of *Giardiasis*. In validating ImmunoCard STAT! *Giardia* against that CRS, 89% sensitivity, 100% specificity, 100% PPV and 94% NPV were recorded. In this study, ImmunoCard STAT! *Giardia* proved to be more sensitive than both Formalin-ethyl acetate concentration and Trichrome staining.

It was reported that the SIMPLE-READ *Giardia* rapid assay (Medical Chemical Corp.), which can also be used at the point of service like other rapid immunoassays currently available, have 97.2% sensitivity and 100% specificity, the PPV (predictive value of a positive test result) was 100%, and the NPV (predictive value of a negative test result) was 97.2% [27].

Using the results of the individual ELISAs as gold standards, the *Giardia/Cryptosporidium Check* assay had a sensitivity of 98.4%, 100% specificity, a positive predictive value of 98.7%, and a negative predictive value of 99.3%. ImmunoCard STAT! appears to have comparable sensitivities and specificities to those of the *Giardia/Cryptosporidium Chek* test [28, 29].

The limitation of the study was the relatively high cost of ImmunoCard STAT! *Crypt/Giardia* test and also, the limited number of specimens as only children suffering from chronic diarrhea illness not taking anti-parasitic treatment since three months were incorporated in the study.

Conclusion

The rapid ImmunoCard STAT! *Crypto/Giardia* immunochromatographic assay is simple to perform, objective, and time-saving. It can be used in integration with other diagnostic modality to confirm diagnosis or used as a rapid service assay when other methods are unavailable especially in waterborne outbreak situation. However, it's important to search for another available alternative with high degree of accuracy, sensitivity, and specificity and keeping both time and cost low.

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