

EVALUATION OF IMMUNOCHROMATOGRAPHIC ASSAY FOR DIAGNOSIS OF CRYPTOSPORIDIOSIS

By

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Abstract

Cryptosporidium is recognized globally as a major etiology of persistent and chronic diarrhea especially in immunocompromised patients and children with significant morbidity and mortality rates. Moreover, it is one of the most important pathogens causing waterborne outbreaks. Hence, rapid diagnosis is crucial. In this study, we tried to determine the diagnostic performance of Rida®Quick *Cryptosporidium* cassettes versus microscopy of the modified acid-fast stained smear. Forty fecal samples were collected from immunocompromised patients complaining of chronic diarrhea. All samples were examined by Rida®Quick *Cryptosporidium* cassettes for copro-antigen detection, and microscopically using concentration techniques and modified acid-fast staining. *Cryptosporidium* was detected in (45%) of immunocompromised patients. The sensitivity, specificity, PPV, and NPV of Rida®Quick *Cryptosporidium* cassettes were 88.9%, 95.5%, 94.1% & 91.3% respectively with an accuracy of 92.5%. There was no cross-reactivity with other intestinal parasites. Rida®Quick *Cryptosporidium* cassettes provide adequate sensitivity and specificity and give rapid results. Our results recommend its use as an alternative test in certain situations especially when screening large populations is needed urgently in a short time, as in outbreaks and epidemiological surveys.

Keywords: Acid-fast staining, *Cryptosporidium*, Rapid diagnosis, Rida®quick.

Introduction

Cryptosporidium is a worldwide enteric zoonotic protozoan infecting a wide range of hosts including mammals, birds, reptiles and fish (El-Badry *et al*, 2015; Zahedi *et al*, 2016). It is recognized globally as a major etiology of persistent and chronic diarrhea in immunocompromised patients and children leading to significant morbidity and mortality. Also, it is one of the most important pathogens causing waterborne outbreaks (Kotloff *et al*, 2013; Khurana and Chaudhary, 2018). To this date, more than 30 species and several genotypes were recognized in man and animals (Šlapeta, 2013; Zahedi *et al*, 2016). The zoonotic *C. parvum* and the anthroponotic *C. hominis* account for >90% of the human cases (Ryan and Hijjawi, 2015; Zahedi *et al*, 2016). Other species less commonly associated with human disease include *C. meleagridis*, *C. felis*, *C. canis* and *C. muris* (Ibrahim *et al*, 2016).

The main route of transmission is fecal-oral; via consumption of water or food contaminated with the infective sporulated thick-

walled oocysts (Burnet *et al*, 2014), and rarely by inhalation (Mor *et al*, 2010).

The main symptom is watery diarrhea with self-limiting in immunocompetent patients but in immunocompromised patients, the infection being more persistent and extra-intestinal manifestation as biliary tract disease, pancreatitis and respiratory tract disease can occur (Ryan *et al*, 2016; Florescu and Sandkovsky, 2016).

Diagnosis of cryptosporidiosis was usually achieved by microscopic detection of its oocysts in fecal specimens using different concentration and staining techniques. Microscopy was time-consuming and required experienced personnel to identify the oocysts. Besides, it must be performed on three fecal samples to increase sensitivity, which may lead to decrease the patient compliance and delay final diagnosis (Weitzel *et al*, 2006).

Therefore, a variety of commercially available copro-antigen assays were developed for diagnosis, which neither depend on microscopy skills nor special laboratory efficiency (Garcia and Shimizu, 2000). PCR is

the most sensitive and specific diagnostic method, but being expensive and required special equipment (Uppal *et al*, 2014; Abd-Elsalam *et al*, 2017).

Symptomatic treatment is vital in cryptosporidiosis (Abubakar *et al*, 2007; Sparks *et al*, 2015). Nitazoxanide is the only drug approved by the USFDA for the treatment of cryptosporidiosis in the immunocompetent adults and children (Carey *et al*, 2004; Bamaiyi and Redhuan, 2016). However, this drug can't be effective without a good immune response of the host so that, it did not use effectively in immunocompromised patients (Gargala, 2008).

The present study aimed to evaluate the rapid Rida[®]Quick *Cryptosporidium* in comparison with the conventional microscopic examination using modified acid-fast staining for the diagnosis of *Cryptosporidium*.

Materials and Methods

This descriptive analytical study was conducted from March 2019 to March 2020. All parasitological procedures were performed in the research laboratory of Medical Parasitology Department, Faculty of Medicine, Sohag University, Upper Egypt.

Fecal samples: Forty fecal samples were collected from immunocompromised patients complaining of chronic diarrhea and attended the outpatient clinics of pediatric, oncology and internal medicine departments in Sohag University Hospitals. Each sample was divided into two parts; the first part was examined freshly without preservatives by Rida[®]Quick (R-Biopharm, Germany) *Cryptosporidium* cassette, and the second one was preserved in sodium acetate-acetic acid-formalin (SAF) for concentration techniques, staining and microscopic examination (Tab. 1).

Comparison between their results revealed

Microscopy: The samples were submitted to Sheeters' flotation and formalin-ethyl acetate concentration techniques. Ten microliters of each concentrated specimen were smeared on a slide, allowed to air-dry, and then stained with modified Kinyoun's acid-fast

stain (Garcia, 2016).

Immunochromatographic assay: Fresh fecal samples were tested for *Cryptosporidium* copro-antigen using Rida[®]Quick *Cryptosporidium* cassettes (R-Biopharm, Germany). The test was carried out according to the manufacturer's instructions. In brief, the test procedure involved the addition of 100µl of the diarrheic stool to 1ml buffer in a test tube. The mixture was left for at least 3min at room temperature until a clear supernatant was formed. Next, 200µl (4 drops) of the clear supernatant of the stool suspension was added to the test window in the cassette, and the results were read after 5 min. The specimen was considered as positive when control (blue colored) and test (red colored) lines were visible (regardless of color intensity), as negative if only the control line showed blue band, and as invalid if no blue band was visible at the control line.

Statistical analysis: Data were analyzed by IBM SPSS Statistics for Windows version 25.0 and Medcalc version 15.8.0. Quantitative data were expressed as mean ± SD. Qualitative data were expressed as number and percentage. Chi-square (χ^2) test and Fisher's Exact Test were used for comparison regarding qualitative variables as appropriate. Sensitivity, Specificity, PPV, NPV and accuracy were calculated for the evaluated kits considering microscopy as the gold standard. For measuring the inter-rater agreement between each microscopy and Rida[®]Quick *Cryptosporidium*, Cohen's kappa test was done with level of significance set using the following criteria: ≤ 0 =poor, 0.01-0.20=slight, 0.21-0.40=fair, 0.41-0.60=moderate, 0.61-0.80=substantial and 0.81-1=almost perfect. *P*-value was considered significant if < 0.05 .

Ethical considerations: The study was approved by the Scientific Ethics Committee of the Faculty of Medicine, Sohag University. Consents were obtained from the patients or their guardians before data and sample collection with a brief explanation of the procedure and the purpose of the study. All infe-

cted patients were provided with appropriate treatment.

Results

Based on the microscopic examination of modified acid-fast stained smears, *Cryptosporidium* was detected in 18 fecal samples with a prevalence rate of 45%. Other intestinal protozoa (*Cyclospora*, *Giardia lamblia*, *Entamoeba* sp., *Blastocystis*) and helminths (*Hymenolepis nana*) were also detected in most fecal samples.

The results of Rida[®]Quick *Cryptosporidium* versus the modified acid-fast staining as that 16 samples were positive by both methods (true-positive), 2 samples were positive by staining but negative by the Rida[®]Quick

Cryptosporidium (false-negative), but one sample was positive by Rida[®]Quick *Cryptosporidium* only (false-positive). The relation was significant ($P < 0.0001$). Kappa value was (0.848), which means perfect agreement between both diagnostic methods.

The sensitivity, specificity, PPV, NPV of Rida[®]Quick *Cryptosporidium* cassettes were 88.9%, 95.5%, 94.1% & 91.3% respectively with accuracy of 92.5%.

Rida[®]Quick *Cryptosporidium* cassettes didn't reveal positivity for any fecal samples containing intestinal parasites other than the *Cryptosporidium* (Tab. 2). This indicated that there was no cross-reactivity with other parasites' copro-antigens.

Table 1: Efficacy of Rida[®]Quick *Cryptosporidium* cassettes using modified acid-fast staining as a gold standard.

Rida [®] Quick	Modified acid-fast stain (Gold standard)			Statistical values				
	Positive (%)	Negative (%)	Total (%)	Sensitivity	Specificity	PPV	NPV	Accuracy
Positive	16 (40%)	1 (2.5%)	17 (42.5%)	88.9%	95.5 %	94.1 %	91.3 %	92.5 %
Negative	2 (5%)	21 (52.5%)	23 (57.5%)					
Total	18 (45%)	22 (55%)	40 (100%)					
<i>P</i> value	< 0.0001*, K (CI ⁹⁵) 0.848 (0.682-1)							

PPV= Positive predictive value, NPV= Negative predictive value, *P* value calculated by Chi-square test., *Statistical significant. Key for Kappa: Poor agreement <0, Slight agreement 0.01-0.20, Fair agreement 0.21-0.40, Moderate agreement 0.41-0.60, Substantial agreement 0.61-0.8, perfect agreement 0.81-1.

Table 2: Microscopic examination versus RIDA[®]QUICK *Cryptosporidium*.

Microscopic examination	Rida [®] Quick <i>Cryptosporidium</i>	
	No. of positive samples	No. of negative samples
<i>Cryptosporidium parvum</i> (n=18)	16	2
<i>Cyclospora cayetanensis</i> (n=3)	0	3
<i>Blastocystis hominis</i> (n=3)	0	3
<i>Giardia lamblia</i> (n=2)	0	2
<i>Entamoeba coli</i> (n=2)	0	2
<i>Hymenolepis nana</i> (n=2)	0	2
<i>Entamoeba histolytica/dispar</i> (n=1)	0	1
Negative samples (n=9)	1	8

Discussion

Cryptosporidiosis is one of the commonest diarrheal infectious diseases in immunocompromised patients and children worldwide (Gebretsadik *et al*, 2018). Microscopy proved to be time-consuming, limiting early diagnosis, treatment and possible prevention of the severe life-threatening diarrhea caused by *Cryptosporidium* (Weitzel *et al*, 2006). The Rida[®]Quick *Cryptosporidium* cassette was designed to detect *Cryptosporidium* antigen in fecal specimens.

In the present study, prevalence of *Cryptosporidium* in the tested immunocompromised patients was (45%). This agreed with El-Hady *et al*. (2017) who reported the same

prevalence (45%) among Egyptian children on the chemotherapy in Sohag City. On the other hand, El-Nadi *et al*. (2015) reported much lower incidence of *Cryptosporidium* (5%) among diabetic patients in Sohag City. The major risk factor for *Cryptosporidium* infection is drinking water that was contaminated with oocysts. Moreover, oocysts can survive in chlorine used for water treatment (Latif and Rossle, 2015).

The high incidence rate of cryptosporidiosis in immunocompromised patients could be explained by suppression of cellular immunity (Gomez-Morales *et al*, 1995) which was the most important factor protecting against development of the disease and re-

duced the severity of infections which can be life-threatening in immunocompromised patients (Hoepelman, 1996).

The variability in prevalence rates may be attributed to the type of study population, variable sensitivity of diagnostic methods, intermittent shedding of *Cryptosporidium* oocysts, and disease seasonality (Thompson *et al.*, 2005).

The sensitivity, specificity, PPV, & NPV of Rida[®]Quick *Cryptosporidium* cassettes were (88.9%, 95.5%, 94.1% & 91.3%) respectively with no cross-reactivity with other intestinal parasites. Others showed various performance levels of Rida[®]Quick *Cryptosporidium* that might be due to different methodologies used and genetic diversity of *Cryptosporidium* with geographical regions. Weitzel *et al.* (2006) reported sensitivity (88.2%) and specificity of (100%), Chalmer *et al.* (2011) reported a sensitivity (84.9%) & (100%) specificity, and Agnamey *et al.* (2011) reported the lower sensitivity (62.4%) & specificity (98%). Whereas Regnath *et al.* (2006) reported (100%) sensitivity and specificity.

The decreased sensitivity in the present study might be explained by presence of two false-negative results due to presence of low parasite numbers which in turn led to a drop in the antigen levels below the detection limit of the rapid methods (Garcia *et al.*, 2003; Weitzel *et al.*, 2006). Liorente *et al.* (2002) and El-Hamshary *et al.* (2008) reported that the detection limit of *Cryptosporidium* oocysts by rapid immunoassays was above 540 oocysts/ml stool suspension. Another possible reason for false-negative samples might be the antigenic variability between the different species of *Cryptosporidium* (Garcia *et al.*, 2003). The used the rapid antigen test targeting zoonotic *C. parvum* and *C. hominis*. So, infections with species other than *C. parvum* and *C. hominis* may cause false-negative results (Llorente *et al.*, 2002; Agnamey *et al.*, 2011). On the other hand, the false-positive sample may be due to intermittent oocyst excretion in stool or due to persistent

antigen in recently cured patients (Shimelis and Tadesse, 2014).

The present study showed perfect agreement ($k=0.848$) between the modified acid-fast staining and Rida[®]Quick *Cryptosporidium*. This agreed with El-Helaly *et al.* (2012) with perfect agreement ($k=0.829$).

Microscopy of the modified acid-fast stained smear of other parasites such as *Cyclospora cayetanensis* and *Isospora belli* if was useful in diagnosing the active *Cryptosporidium* infection. But, the microscopy required special technician, time consuming and not ideal for implementation in epidemiological field study. The Rida[®]Quick *Cryptosporidium* cassette proved to be simple, short time (10min) and didn't require complicated laboratory equipment. Thus, the cassettes improved the laboratories efficiency by reducing time and resources. Also, it has great significance in screening large populations such as immunocompromised patients, children, and in outbreak situations and may successfully serve as the diagnostic option in situations where microscopy was not available. The present Rida[®]Quick *Cryptosporidium* with performance recorded could not replace microscopy. The need to confirm the negative Rida[®]Quick *Cryptosporidium* results with more sensitive tests in symptomatic patients should also be considered.

Conclusion

Rida[®]Quick *Cryptosporidium* is simple, rapid, good sensitivity and specificity test. Moreover, it did not require experienced personnel or special technical equipment. So, it can be used as an alternative test in certain situations where the microscopic diagnosis of *Cryptosporidium* is limited due to time constraints, lack of microscopy experts, and unavailability of appropriate equipment or when examining large populations as in outbreaks and epidemiological surveys.

Authors contribution: Shima Refaey Mohamed performed the laboratory works, collected data and wrote the manuscript, Amal Mostafa Ahmed helped with the laboratory analysis of samples, collection of data; Ha-

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