Immunochromatography Versus Microscopy For Diagnosis Of Amoebiasis In Sohag, Egypt

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Research Article

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Abstract

Amoebiasis; a parasitic infection caused by *E histolytica* protozoon, is still a major public health in developing countries. Delay in diagnosis and treatment may lead to fatality. So, rapid and accurate diagnosis plays a critical role in patient's management. Therefore, this study aimed to evaluate the efficacy of immunochromatographic test (ICT) using RIDA®QUICK *Entamoeba* test compared with traditional microscopic examination as gold standard for the diagnosis of *E histolytica /dispar* infection. A Cross-sectional study was conducted on 100 patients complaining of dysentery or diarrhea with an age range of 3-62 years, attending Sohag University Hospitals and Endemic Diseases Hospital. All stool samples were examined by direct wet smears, formalin ethyl acetate concentration techniques, staining of the smears using Wiegert's iron hematoxylin and Modified Ziehl-Neelsen method. Copro-antigen detection in stool sample was by using RIDA®QUICK *Entamoeba* test. Microscopic examination revealed 43 out of 100 patients (43%) were positives for *E histolytica/dispar*. The infection was significantly associated with residence (*P* value **<** 0.036) and family size (*P* value **<** 0.03) but not associated with gender (*P* value **<** 0.471) and age (*P* value **<** 0.344). The sensitivity, specificity, PPV and NPV of Rida®Quick *Entamoeba* tests were 97.67%, 96.49%, 95.45% and 98.21% respectively with an accuracy of 97%. There was no cross-reactivity with other intestinal parasites.

Conclusion: Rida® Quick *Entamoeba* test is a simple, rapid, convenient, sensitive and specific assay for the diagnosis amebiasis and is therefore very suitable for large-scale field applications.

Introduction

Entamoeba histolytica continues to be an important global health issue being the third leading cause of death from parasitic infections (Ghosh et al. 2019).

Nearly 10% of the world population are infected with *E histolytica*, 1% of which develop the invasive form of the disease with up to 100,000 annual deaths in the tropical areas and developing countries (Morf and Singh 2012).

The transmission occurs by feco-oral routes, ingestion of contaminated food or water, person to person, and zoonotic transmission (Thompson and Smith 2011).

In amebiasis, 90% of infected persons are asymptomatic, the others have symptoms of intestinal amebiasis ranges from colitis to dysentery and extraintestinal amebiasis (Fotedar et al. 2007).

The most common clinical manifestation of extraintestinal infection is amoebic liver abscess (ALA) and a delay in diagnosis and treatment may cause fatality (Zlobl 2001).

Laboratory diagnosis of *E histolytica/dispar* is usually achieved by microscopic detection of trophozoites or cysts in stool samples. However, it is time- consuming and requires an experienced technician to identify the organism. Furthermore, it must be performed on three stool samples to increase sensitivity leading to decreased patient compliance and delay in the final diagnosis (Carrero et al. 2020).

Therefore, Antigen detection assays such as rapid immunochromatographic tests for *E histolytica* have been developed for diagnosis of the infection which do not d epend on microscopy skills and increase laboratory efficiency by reducing time (Saidin et al. 2018).

This study aims to evaluate the efficacy of immunochromatographic test (ICT) using RIDA®QUICK *Entamoeba* test compared with traditional microscopic examination as gold standard for copro-antigen detection of amebiasis.

Materials & Methods

Study area

This study was carried out in Sohag Governorate, Upper Egypt. Sohag is located in the southern part of the country toward 467 km to the south of Cairo. It covers an extent of the Nile Valley with a total area of 1547 km^2, with estimated 5,510,510 people.

Ethical consideration

The study was approved by the Medical Research Ethical Committee (MREC) of the Faculty of Medicine, Sohag University, Egypt with IRB registration No. (Soh-Med-21-02-16). This study was registered at Clinical Trials.gov with registry No. (NCT04759937). Written informed consent was obtained from each patient after explaining the procedure and the purpose of the study.

Study Design

A cross-sectional study was conducted on 100 patients complaining of dysentery or diarrhea attending Sohag University Hospitals and Endemic Diseases Hospital during the period from March 2021 to January 2022. They had to fulfill the criteria; not taking amoebicidal drugs 2 weeks before sample collection. Patients were subjected to a survey questionnaire. The questionnaire included age, sex, residence and family size.

Sample Collection And Laboratory Processing

Fecal samples were collected in clean, dry, leak proof containers and send immediately to lab. Each collected sample was divided into two parts; the 1st part was examined macroscopically for consistency, color, odor, blood and mucus, the presence of adult worms or segments and microscopically by:

a. direct wet mount saline smear and Lugol's iodine smear (Garcia and Bruckner, 1997).

b. formalin ethyl acetate sedimentation and the slides were examined using a low-power objective (10×) and high-power objective (40×) respectively (Cheesbrough, 2009).
c. permanently stained smear using both Wiegert's iron hematoxylin & Modified Ziehl-Neelsen method (Garcia and Bruckner, 1997).

The 2nd part for rapid ICT Rida®Quick *Entamoeba* test (R-Biopharm AG, Darmstadt, Germany, LOT NO AL57.43) according to the manufacturer's instructions (Abo Sheishaa et al. 2021).

Immunochromatography Assay

Fresh fecal samples without preservatives were used to perform the test. The test procedure involved the addition of 100 µl (50 mg) of the diarrheic stool sample to 1 ml buffer in a test tube. The mixture was left for at least 3min at room temperature until a clear supernatant was formed. Then, 200 µl (4 drops) of the clear supernatant was placed into the round opening of the cassette and the result was read off after 5 minutes. 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 a blue band, and as invalid if no blue band was visible at the control line (Goñi et al. 2012).

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 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 *Entamoeba*. Cohen's kappa test was done with a 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 it was < 0.05.

Results

Demographic characteristics

A total of 100 fecal samples from patients was included in the study. Of those, 46% were males and the rest 54% were females. The age of the patients ranged from 3 to 62 years (Mean ± SD = 20.44 ± 1.61). 73% live in rural areas whereas 27% in urban areas. 65% belonged to a family size ≥ 5 members whereas 35% < 5 members.

Based on the microscopic examination, *E. histolytica / dispar* was the most frequent parasitic infection with a prevalence rate of 43% (43/100).

Single *E. histolytica / dispar* infection was found in 30 patients, coinfection of *E. histolytica / dispar* with *Giardia lamblia* was found in 7 patients, coinfection with *Blastocystis hominis* was found in 5 patients and coinfection with *E coli* was found in one patient.

Other intestinal parasites; *Cryptosporidium* (25%), *G lamblia* (16%), *Cyclospora* (5%), *B hominis* (5%), *Entamoeba coli* (3%), *Iodamoeba buetschilii* (2%), *Hymenolepis nana* (1%) *& Enterobius vermicularis* (1%) were also detected in most fecal samples.

The infection was found to be significantly associated with residence (*P* value < 0.036) and family size (*P* value < 0.03). However, there was no significant association between gender (*P* value < 0.471) and age (*P* value < 0.344) with the infection as shown in Table 1.

Table 1
Prevalence of *E. histolytica/dispar* infection among 100 outpatients according to sociodemographic risk factors in Sohag

	Positive *E. histolytica/dispar* (n = 43)	*Negative E. histolytica/dispar* (n = 57)	*P* value
Age			
< 20 years	**25 (58.14%)**	**36 (63.16%)**	**0.344**
20–40 years	**15 (34.88%)**	**12 (21.05%)**	
> 40–60 years	**4 (9.3%)**	**6 (10.53%)**	
> 60 years	**0 (0%)**	**2 (3.51%)**	
Gender			
Male	**18 (41.86%)**	**28 (49.12%)**	**0.471**
Female	**25 (58.14%)**	**29 (50.88%)**	
Residency			
Rural	**36 (83.72%)**	**37 (64.91%)**	**0.036***
Urban	**7 (16.28%)**	**20 (35.09%)**	
Family size			
< 5 members	**8 (18.6%)**	**27 (47.37%)**	**0.03***
≥ 5 members	**35 (81.4%)**	**30 (52.63%)**	
P **value was calculated by Chi-square test and Fisher's Exact Test * Significant association if** P **value (< 0.05).**			

The results of Rida®Quick *Entamoeba* test versus the microscopic examination revealed that 42 samples were positive by both methods (true-positive), one sample was positive by microscopic examination but negative by the test (false-negative) and two samples were positive by the test only (false-positive). The relation was significant (*P* value < 0.0001). Kappa value was 0.939, which means perfect agreement between both diagnostic methods. The sensitivity, specificity, PPV & NPV, Rida® Quick *Entamoeba* cassettes were 97.67%, 96.49%, 95.45% & 98.21% respectively as shown in Table2. Rida® Quick *Entamoeba* cassettes didn't reveal positivity for any fecal samples containing intestinal parasites other than the *E histolytica / dispar* (Table 3). This indicated that there was no cross-reactivity with other parasites' copro-antigens. Fig 1 depicted examples of positive and negative result on Rida®Quick *Entamoeba* test.

Table 2. Efficacy of RIDA®QUICK *Entamoeba test* in comparison to microscopic examination as “Gold standard”

Rida® Quick *Entamoeba* cassettes	Microscopy (Wiegert's iron hematoxylin stain)			Statistical values				
	Positive (%)	Negative (%)	Total	Sensitivity	Specificity	PPV	NPV	Accuracy
Positive	42	2	44	97.67%	96.49%	95.45%	98.21%	97%
Negative	1	55	56					
Total	43	57	100					
***P value* =** .0000*	**Kappa =** 0.939**							

P-value was calculated by Chi-square test and Fisher's Exact Test *Statistically significant *P*-value (< 0.05). **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 0.81-1.00. Accuracy: the overall probability that a patient is correctly classified. Accuracy = Sensitivity × Prevalence + Specificity × (1 − Prevalence).

Table 3
Microscopic examination versus RIDA® QUICK *Entamoeba*

Microscopic examination	Rida®Quick *Entamoeba*	
	No. of positive samples	No. of negative samples
E. histolytica/dispar (n = 43)	42	1
Cryptosporidium (n = 25)	0	25
Giardia lamblia (n = 16)	0	16
Cyclospora (n = 5)	0	5
Blastocystis hominis (n = 5)	0	5
Entamoeba coli (n = 3)	0	3
Iodamoeba buetscilii (n = 2)	0	2
Hymenolepis nana (n = 1)	0	1
Entrobius vermicularis (n = 1)	0	1

Discussion

Microscopic technique is still frequently practiced in many parasitology diagnostic laboratories, particularly in developing countries for the identification of *E. histolytica/E. dispar* and include wet preparation, concentration, and permanently stained smears (Fotedar et al. 2007).

However, several studies suggested that microscopy is labor-intensive, time-exhaustion, needs technician's experience, and sensitivity is low (Vanathy et al. 2017).

In addition, examination of three stool samples over not more than 10 days is recommended to increase the sensitivity of microscopy, as these organisms may be shedded intermittently (Varghese et al. 2021).

So, Immunochromatographic tests were designed to provide a solution to overcome these disadvantages and allow the detection of *Entamoeba* copro-antigen (Saad et al. 2015).

In the present study, the microscopic examination revealed that the prevalence of *E. histolytica/dispar* was 43% in participated patients. This was more or less close to those reported in Egypt; 40.77% among 130 patients complaining of chronic abdominal pain in Sohag (Omran and Mohammed 2015) and 44.4% out of 230 primary school children in Sharkia (Hussein et al. 2021).

While the prevalence of study was lower than 56% among 50 hemodialysis patients in Sohag University Hospitals (EL-Nadi and Taha 2004). This may be due to our study patients were immunocompetent.

On the other hand, the prevalence of study was higher than 8.3% out of 300 children in Assuit (Dyab et al. 2016), 13% among school children and 23.5% among 100 random fecal samples using the mini-FLOTAC in Sohag (EL-Nadi et al. 2017; EL-Nadi et al. 2019). This could be explained by the fact that our study conducted on symptomatic patients.

The present study showed the highest prevalence was observed among the age group less than 20 years (58.14%), followed by patients between 20–40 years (34.88%) and patients > 40–60 years (6.98%) however it revealed no significant relationship between age and the infection (*P* value = 0.344). The result agreed with El-Nadi et al. (2017) in Sohag and ESlam et al. (2017) in Libya who revealed that there was no significant correlation between the infection and age groups in school children (*P* < 0.425, *P* < 0.081 respectively). However, the result disagreed with a study by Hegazi et al. (2013) who found that the prevalence of the infection among infants under one year in Saudi Arabia was statistically significant (*P* value < 0.02) due to inadequate breastfeeding and bottle-feeding practices among cases.

The present study revealed *E. histolytica/dispar* infection among female patients (58.14%) was higher than males (41.86%), however, no significant association was found between the gender and the infection (*P* value < 0.471). This agreed with the study by El-Nadi et al. (2017) in Sohag who showed no statistically significant differences regarding gender (*P* value < 0.446). However, these results differed from the results by ESlam *et al.* (2017) who reported boys (6%) were significantly more infected with *E. histolytica/dispar* than girls (3%) in school children in Libya (*P* value < 0.05). This could be explained by the fact that boys were commonly affected because they were fully independent in using toilet and were more involved in outdoor activities.

In the present study, Patients in rural areas (83.72%) were at higher risk for *E. histolytica/dispar* infection than those living in urban areas (16.28%) and there was a significant association between the prevalence and residence (*P* value < 0.036). Also, Khan et al. (2019) in Pakistan reported that the prevalence of the infection among participants in rural areas was significantly higher than urban areas (*P* value < 0.05). However, Atia et al. (2016) in Zagazig, Egypt found that the prevalence of the infection was higher in patients living in rural areas, but without significant difference (p > 0.05).The high percentage of intestinal protozoan infections in rural areas may be due to poverty, poor living and hygienic conditions, drinking of underground water which is contaminated with sewage, compared to urban areas, also the extensive use of human and animal excreta as fertilizer in agriculture, the household wastewater is thrown in irrigation channels in addition to the close contact with animals (Pham-Duc et al. 2011).

In the present study, the patients from large households with family sizes of 5 members or more (81.4%) showed a significantly higher prevalence of the infection than those from smaller families (18.6%). Family size was a significant risk factor associated with the infection (*P* value = 0.03). The same results were met with the study by El-Nadi et al (2017) in Sohag who showed that intestinal parasitic infections among school children were significantly higher with large family sizes of more than 5 members compared with smaller families (*P* value < 0.006).

In the present study, the sensitivity, specificity, PPV, & NPV of the Rida®Quick Entamoeba test was 97.67%, 96.49%, 95.45% and 98.21% respectively with no cross-reactivity with other intestinal parasites. This agreed with Saad et al. (2015) who revealed sensitivity (100%) and specificity (97.4%) in addition to Atia et al. (2016) who reported sensitivity (100%) and specificity (100%). As these studies compared the ICT with microscopy in detection of the parasites in stool samples.

However, these results disagreed with a study by Goñi et al. (2012) who reported lower results in detection of *E histolytica* where sensitivity was 62.5% and specificity was 96.1% which might be due to the fact that they used PCR as standard reference. Also, Abu Sheishaa, (2021) found lower sensitivity (80.0%) and specificity (88%) as they used ELIZA as standard reference.

The false-negative samples may be attributed to the presence of low parasite numbers, which leads to a drop in the antigen levels below the detection limit of the rapid methods (Garcia et al.2003; Weitzel et al. 2006).

On the other hand, the false-positive samples may be due to intermittent parasite excretion in the stool or due to persistent antigen in recently cured cases (Shimelis and Tadesse 2014).

The limitations of the ICT test were high cost and inability to differentiate between the pathogenic *E. histolytica* and non-pathogenic *E. dispar*. Moreover, quantitative ICT models are required to measure the intensity of infection and monitor therapeutic success.

Conclusion

The Rida®Quick *Entamoeba* test is simple, rapid, and good sensitivity and specificity test. So, it can be used as an alternative test in certain situations where the microscopic diagnosis of *E. histolytica/dispar* is limited due to lack of microscopy experts, unavailability of appropriate equipment or when examining large populations as in outbreaks and epidemiological surveys.

Declarations

Authors contribution

Khoulood Zakaria Hashem performed the laboratory works, collected data and wrote the manuscript, Amaal Ahmed Abd El-Mawgood & Asmaa Kamal Abd Ellah reviewed practical work, wrote and reviewed manuscript; Amal Moustafa Ahmed proposed the idea and reviewed manuscript.

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Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

1. Abu-Sheishaa G A, Adel O H, Haytham M A (2021) Efficacy of triage parasite panel in diagnosis of *Entamoeba histolytica, Giardia lamblia*, and *Cryptospordium parvum* antigens in symptomatic children stool specimens. Egypt Vet Med Soc Parasitol J 17(1): 84-89.
2. Atia M M, Elsettawy M A, Fathy G M, Salama M A, Metally A (2016) SEM. comparison of immunochromatographic test and microscopy in the detection of some enteric protozoa in stool samples. J Egypt Soc Parasitol 46(3): 625-632.
3. Carrero J C, Reyes-Lopez M, Serrano-Luna J, Shibayama M, Unzueta J, Leon-Sicairos N, de la Garza M (2020) Intestinal amoebiasis: 160 years of its first detection and still remains as a health problem in developing countries. Inter J Med Microbiol 310(1): 151358.
4. Cheesbrough M (2009) In: District Laboratory Practice in Tropical Countries. 2nd Edition, New York, Cam-bridge University Press 195-216.
5. Dyab AK, El-Salahy M, Abdelmoneiem H, Mohammed MF (2016) Prevalence and risk factors associated with intestinal parasitic infection among children in Aswan, Egypt. J Bacteriol Parasitol.,1:23.
6. El-Nadi N A F, Omran E K, Ahmed N S, Fadel E F (2017) Current status of intestinal parasites among elementary school children in Sohag, Egypt. J Adv Parasitol 4(2): 33-40.
7. El-Nadi N A, Ahmed A M, Ahmed N S, Abd El-Laah A A (2019) Evaluation of mini-FLOTAC method for diagnosing intestinal parasitic infections. PUJ 12 (2): 147-152.
8. EL-Nadi N and Taha A (2004): Intestinal parasites detected among haemodialysis patients in Sohag University hospitals. EL-MINIA Med Bull 15 (2): 223-240.
9. ESalem RM, Gahgah SA, Ali A. Shrief SA (2017) Prevalence and risk factors associated with *E. Histolytica* infection among children in Sebha, Libya. Dent & Med Res 5(48).
10. Fotedar R, Stark D, Beebe N, Marriott D, Ellis J, Harkness, J. (2007) Laboratory diagnostic techniques for *Entamoeba* species. Clin Microbiol Rev 20(3): 511-532.
11. Garcia L S, Shimizu RY, Novak S, Carroll M, Chan F (2003) Commercial assay for detection of *Giardia lamblia* and *Cryptospordium parvum* antigens in human fecal specimens by rapid solid-phase qualitative immunochromatography. J Clin Microbiol 41(1): 209-212.
12. Garcia LS, Bruckner DA (1997) Diagnostic medical parasitology, 3rd ed. ASM Press, Washington DC 36–49.
13. Ghosh S, Padalia J, Moonah S (2019) Tissue Destruction Caused by *Entamoeba histolytica* Parasite Cell Death, Inflammation, Invasion, and the Gut Microbiome. Curr Clin Microbiol Rep 6(1):51-57.
14. Goni P, Martin B, Villacampa M, Garcia A, Seral C, Castillo F J, Clavel A (2012) Evaluation of an immunochromatographic dip strip test for simultaneous detection of *Cryptospordium spp., Giardia duodenalis* and *Entamoeba histolytica* antigens in human faecal samples. Eur. J Clin Microbiol Infect Dis 31:2077-2082.
15. Hegazi, M. A.; Patel, T. A. and El-Deek, B. S. (2013): Prevalence and characters of *Entamoeba histolytica* infection in Saudi infants and children admitted with diarrhea at 2 main hospitals at South Jeddah: a re-emerging serious infection with unusual presentation. Braz J Infect Dis 17(1): 32-40.
16. Hussein Y H H, Fahmy H H, Sewilam D E A (2021) Intestinal Parasitic Infections among Primary School Children in Al Qurain District, Sharkia Governorate. **EFMJ** 5(1): 68-81.
17. Khan B, Afshan K, Firasat S, Qayyum M (2019) Seroprevalence and associated risk factors of *Entamoeba histolytica* infection among gastroenteritis patients visiting the public healthcare system, Pakistan. J Pak Med

Assoc 69(12): 1777-1784.

18. Morf L, Singh U (2012) *Entamoeba histolytica*: a snapshot of current research and methods for genetic analysis. Curr Opin Microbiol; 15(4), 469-475.
19. Omran E, Mohammed A (2015) Intestinal parasites in patients with chronic abdominal pain. J Egypt Soc Parasitol 45(2): 389-396.
20. Pham-Duc P, Nguyen-Viet H, Hattendorf J, Zinsstag J, Dac-Cam, P, *et al* (2011) Risk factors for *Entamoeba histolytica* infection in an agricultural community in Hanam Province, Vietnam. Parasites Vectors 4:102-8.
21. Saad M Y, El-shahat M M, Khaled AT (2015) Qualitative immunochromatographic assay for *Cryptospordium parvum, Giardia lamblia* and *Entamoeba histolytica antigens* in Damietta Governorate, Egypt. AL-AZHAR Assuit Med J 13(2): 88- 92.
22. Saidin S, Othman N, Noordin R (2018) Update on laboratory diagnosis of amoebiasis. Eur J Clin Microbiol & Infect Dis 38: 15-38.
23. Shimelis T, Tadesse E (2014) Performance evaluation of point-of-care test for detection of *Cryptosporidium* stool antigen in children and HIV Infected Adults. Parasit Vectors 7: 227.
24. Thompson R, Smith A (2011) Zoonotic enteric protozoa. Vet Parasitol 182, 70-78.
25. Vanathy K, Parija SC, Mandal J, Hamide A, Krishnamurthy S, (2017) Detection of *Cryptosporidium* in stool samples of immunocompromised patients. Trop Parasitol 7: 41-46.
26. Varghese V, Kansal A, Bhardwaj S, Sharma A. (2021) Cerebral Amebiasis: An Uncommon Cerebral Abscess. Annals Ind Acad Neurol 24(3): 445-447.
27. Weitzel T, Dittrich S, Möhl I. Adusu E, Jelinek T (2006) Evaluation of seven commercial antigen detection tests for *Giardia* and *Cryptosporidium* in stool samples. Clin Microbiol Infect 12(7): 656-659.
28. Zlobl TL (2001) Amebiasis. Prim Care Update Ob Gyns 8:65–68.

Figures

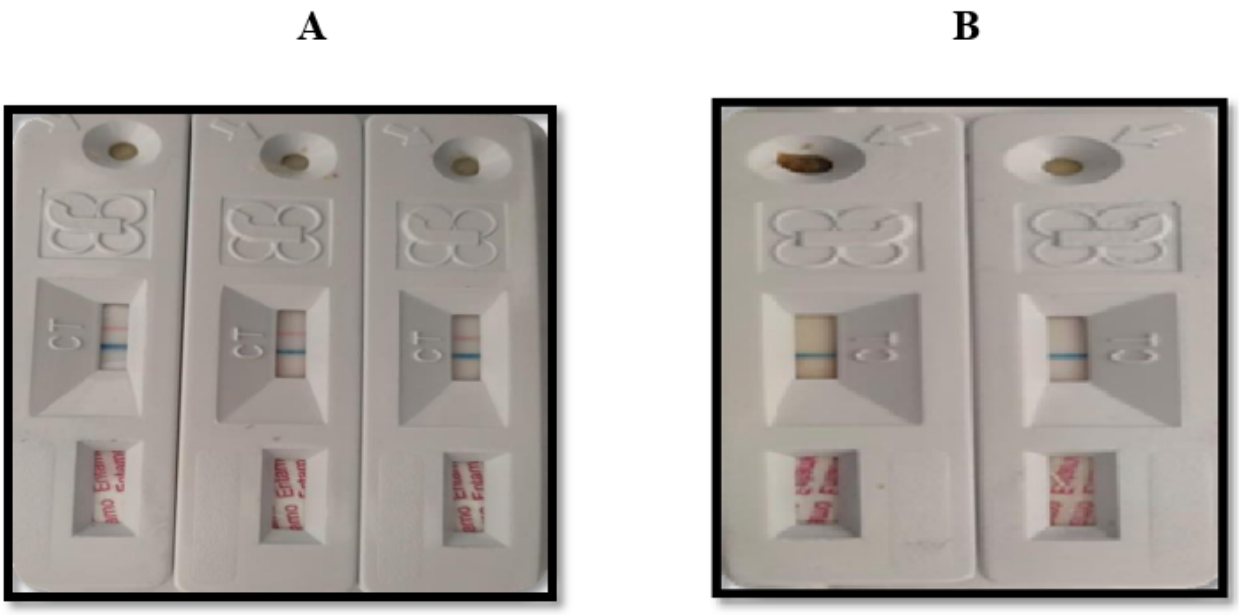

Figure 1

Results of Immunochromatographic Rida® Quick *Entamoeba* tests: A. Positive tests and B. Negative tests.