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Diagnosis of *Giardia* Species by Comparison of Two Enzyme-Linked Immuno-Sorbent Tests

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Abstract

Background: There was some trade produced enzyme-immuno-sorbent assays tests to diagnose antigens of *Giardia* spin stool specimens, like Rida Quick *Giardia* and Serazym ELISA *Giardia*® tests. In this study, we evaluated the performance of two commercially available EIA kits for detecting *Giardia* antigens.

Methods: A total of 96 stool specimens were collected from patients who attended for different Primary Health Care centers inAl-Qurna sector / Basrah Health Directorate, Ministry of Health/ Environment, Basrah, Iraq at period from 2nd of March 2018 to 28th of September 2018. All specimens were examined by conventional methodsof microscopic examination, andby both enzyme-immuno-sorbent assays tests.

Results: Serazym ELISA *Giardia*® had a sensitivity of 90.1%, a specificity of 100%, and an accuracy of 91.7%. The RidaQuick *Giardia*® showed a sensitivity of 79%, a specificity of 100%, and an accuracy of 82.3%.SerazymELISA *Giardia*® showed 10% discrepancy better than Rida Quick *Giardia*® which showed 8%, this was of a statistically significant difference (P<0.05).

Conclusion: Antigen detection by EIA has been established as a valuable tool to make parasite stool diagnostics more effective. Serazym ELISA *Giardia*® is more reliable than Rida Quick *Giardia*®.

Keywords: *Giardia*; Enzyme-linked immuno-sorbent; Rida Quick *Giardia*; Serazym ELISA *Giardia*

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Introduction

Giardia is one of the most common intestinal protozoan parasites, which is a world-wide distribution [1]. The poor sanitary conditions are represented the highest pool for infection. Its transmission by a feco-oral mode andis also by ingestion of contaminated water or food [1]. In developing countries, found the highest rates of *Giardia*sis as>20% in young children, whereas in developed Western countries, are occur mostly in homosexual men, and travellers who coming from highly endemic areas [1]. The protozoan flagellate *Giardia* was a single celled microscopic protozoan micro-organismthat cause intestinal enteritis in human and other mammals, which is reported globally [1,2]. Mostly, *Giardia*sis is asymptomatic, found in carrier persons, and may be manifest with different symptoms, as watery, foul smelling diarrheal dysentery, abdominal cramps, bloating and weight loss [1]. Its lifecycle consists of two different stages: the trophozoite and the cyst. The diagnosis of *Giardia* is frequently based on microscopic detection of the parasites in stool samples, yet this method is requiring an experienced staff and it is time consuming [3]. In addition this way of diagnosis have a low sensitivity reach up to 50% of *Giardia*sis, due to that *Giardia* characteristic by the intermittent shedding of the parasites, the vegetative stages can be missed because of delays in stool processing and/or low compliance with the request to submit multiple stool samples, as microscopy of stools forms the cornerstone of detection in clinical parasitology laboratories, for those reasons the microscopic examination requirethree consecutive stool specimens to get high sensitivity [1,4,5]. The rapid diagnostic tests, sensitive methods, and cost effective are mandatory to overcome these barriers. In last two decades Enzyme immuno-assays (EIAs) for diagnosis of the specific antigens in stools specimens have developed into enough technique methodin *Giardia* examination [4,5].

In this study we tested two trade mark available EIA kits that detect antigens of *Giardia* species in stool samples (Rida Quick *Giardia*®; manufactured by r-biopharm [Catalogue No: **N1103**], and Serazym ELISA *Giardia*®; manufactured by VIROTECH Diagnostic [Catalogue No: **TS/B-205-G**]).

Materials and Methods

Study design and setting

We enrolled 96 Iraqi participants who completed the study. A comparison study between 2 tests regarding their sensitivity, specificity, accuracy, and discrepancy.

Ethical approval

Written, informed consent from all study participants was obtained in accordance with the Helsinki Declaration of 1975, as revised in 2000, at the time of their visit to the clinic. The Medical Ethical Committee of the Training and Development Unite of Basrah Health Directorate approved this work (No. 592; date: 10/09/2012).

Participants and sample size

About 96 stool samples were collected from human cases for different age, sex, address, economic state and service level, who attended to Centers of Al-Qurnah sector for Primary Health Care, during period from 2 March to 28 September 2018.

Clinical samples collection

All samples were collected from participants complain of diarrhea, abdominal discomfort, and cramp, and all samples collected in sterilized cups and taken up to laboratory of above centers.

Clinical sample processing

Conventional microscopic methods were used in sample processing include direct smear methods either with normal saline or with Lugol′s iodine[1,6]. In addition, concentrated methods either floatation technique by zinc sulphatecentrifugal flotation and/or sedimentation technique by formol-ether (Ethyl Acetate) concentration, can of benefits for microscopic detection of *Giardia*spp [7-14].

Serazym ELISA *Giardia*®

Principle: It is based on polyclonal antibodies to *Giardia* cyst wall protein 1 (CWP-1). The first incubation stepfor 60 min at room temperature lead to diluted stool specimens, then the positive and negative controls reacts with the solid-phase adsorbed polyclonal antibodies, while the unbound components are removed by a subsequent washing step. This follow by the second incubation step for 30 min, at this point the solid-phase bound immune complexes react with the horseradish (HRP)-labeled polyclonal antibodies of the conjugate, whereas the unbound reagents are separated from the solid-phase antibody-antigen-antibody immune complexes by a further washing step. The HRP further converts the subsequently added colorless chromogenic substrate solution into a blue product. The enzyme reaction is terminated by sulphuric acid dispensed into the wells after 10 min incubation at room temperature turning the solution from blue to yellow [15]. The optical density (OD) of the solution read at 450/≥620 nm is directly proportional to the specifically bound amount of cyst wall protein. Referring to the cut-off value results are interpreted as positive or negative.

Preparation, collection and storage: Fresh, untreated stool samples stored at 2-8 immediately after collection and processed within 72 h. Then quickly thaw frozen samples. We warm samples to room temperature and mix well. Then pipette 1000 µl of sample diluent into a clean tube. By using a disposable stirring rod transfer about 200 mg (diameter about 4 - 6 mm) of faeces if solid or 200 µl if liquid into the tube and suspend thoroughly.Then mix sample thoroughly. We allow all components to reach room temperature prior to use in the assay. In addition, we allow the sealed plate to reach room temperature before opening. Then prepare enough wash solution by diluting the 10-fold concentrated wash buffer 1+9 with distilled or deionized water [15].

Qualitative evaluation and Cut-off determination - OD negative control+0.10: Samples with OD values equal with or higher than the cut-off is considered positive, and samples with OD values below the cut-off are considered negative for *Giardia* antigen [15].

Procedure: Dilution of samples with sample diluent (3) as1: 6, e.g. 200 mg or 200 µl faeces+1.0 ml sample diluent. There is no any time shift during dispensing of reagents and samples, and the wash buffer soak time is 5 seconds per wash cycle and residual fluid is completely drained in every single wash cycle. There was no light exposure of the TMB substrate solution. Then we warm all reagents to room temperature (RT) before use. Mixed allgently without causing foam. Pipetted 100 µl CONTROL+positive control (4)100 µl CONTROL-negative control (5)100 µl diluted sample. Covered plate and incubated for 60 min at RT. Decanted, then washed each well 5x with 300 µl wash solution (diluted from (2)) and tapped dry onto absorbent paper. Used 3 drops (or 100 µl) CONJ HRP HRP-conjugate (6) per well. Then, covered plate and incubated for 30 min at RT.Decanted, then washed each well 5x with 300 µl wash solution (diluted from (2)), and tapped dry onto absorbent paper. Then, 3 drops (or 100 µl) SUBSTR TMB substrate (7) per well. Incubated for 10 min at RT protected from light. Then, 3 drops (or 100 µl) STOP solution (8) per well, mixed gently. Finally, we read OD at 450 nm /≥ 620 nm with a micro-plate reader within 30 min after reaction stop [15].

Rida Quick *Giardia*®

Principle: This quick test is a single-step immuno-chromatographic lateral-flow test, where specific antibodies which are directed against both parasites attach themselves to red (*Giardia* specific) latex particles. Other specific antibodies against the two pathogens are firmly bound to the membrane. The stool sample is first suspended in the extraction buffer and then precipitated. The test strip is dipped into the clear supernatant of the sample. The sample then passes, with the colored latex particles to which the antigens are attached if the test is positive, through the membrane and bonds to the specific collection bands. A blue and/or red band appears, depending on the antigens present in the sample [16].

Preparation and collection: Stool samples collected in clean containers without any additives and stored at 2-8℃ before beginning the test. Placed 1 ml Extraction Buffer Diluent in the test tubes indicated. Then pipetted 100 µl of the sample with a disposable pipette Pipet (up to just above the second thickening) and suspended in the buffer placed in the tube beforehand. With solid stool samples, suspended 50 mg in the buffer then leaved sample to be well homogenized by repeated suction and ejection of the suspension using the disposable pipette Pipet or, alternatively, by mixing on a vortex mixer. Then allow the homogenized suspension to precipitated for at least 3 min until a clear supernatant is formed from whichat least 200 µl but at most 500 µl is then transferred into another clean tube (or uncoatedmicro-titer well). Removed the test strip from the tube and immersed it in the prepared sample. The test stripmust not be immersed any farther than the line indicated by the arrow. The test result can beread off after 5 min[16].

Qualitative evaluation: A maximum of three bands appeared in the following order, as seen from the sampleabsorptionsite: *Giardia* positive: Redand greenbands are visible [16].

Clinical sample analysis: If different results between the EIA, and microscopy were obtained, immunofluorescence microscopy was used for additional confirmation of true positive results. EIA results were compared with those obtained by conventionalmicroscopic examination, and immunofluorescencemicroscopy. The samples that had apositive result in CME and/or immunofluorescence microscopy

were considered true positives.

Statistical analyses: Data tabulation and input was performed using IBM©SPSS©V 24. The sensitivity, specificity, accuracy, and discrepancy of both EIAs tests were calculated and analyzed using Spearman's rank-correlation analysis (ρ) and test of agreement using Cohen's kappa coefficient (κ).

Results

The 96 specimens were examined by CME, IFM, and two EIAs. Comparing the Serazym ELISA *Giardia*® EIA against microscopy, 73 stool samples were positive, sensitivity of the test against CME was 90.1%, whilst the specificitywas 100%, with 91.7% test accuracy. With the Rida Quick *Giardia*® test, sensitivity was calculated at 79% (60 samples positive), with a specificity of 100%, and 82.3% test accuracy (Table 2).

When compared with immunofluorescence, Serazym ELISA *Giardia*® performed with a sensitivity of 86.4%, a specificity of 80%, and an accuracy of 85.4%, while Rida Quick *Giardia*® showed a sensitivity of 83.9%, a specificity of 66.7%, and an accuracy of 81.3% (Table 3).

Regarding discrepancy, the Serazym ELISA *Giardia*® showed 10% discrepancy better than Rida Quick *Giardia*® which showed 8%, this was of a statistically significant difference (P<0.05) (Table 4).

Discussion

When microscopy was used as the reference standard, the EIA kits both showed a specificity of 100%, while sensitivity varied a lot. Rida Quick *Giardia*® showed 79%, whilst Serazym ELISA *Giardia*® performed better with a sensitivity of 90.1%. It is important to see though that the use of IFM as gold standard for the evaluation of EIAs in this setting would have produced false low specificity results for both tests (66.7% for Rida, and 80% for Serazym, as opposed to the actual 100% specificities in both tests against CME). The reasons behind are low concentration of gene in stool samples that traditionally hamper IFM diagnostics from this material [4].

The microscopic examination of one single stool specimen has a low sensitivity [4]. Many parasitologists discovered that antigens of *Giardia* sppcan be detected by EIA even in the diminish of intact organisms (cysts or trophozoites) [3-5]. This give a greater sensitivity of EIA-tests compared with microscopyand therefore provide low specificity results when only one CME is used as a reference standard test. In a study conducted by Aldeen *et al.* [17], his results like our study

Table 1: Supplementary table.

WELLS	Microtitration plate coated with polyclonal anti-*Giardia* CWP-1 antibodies (sheep)	12 single breakable 8 well strips color coding white vacuum-sealed with desiccant
WASHBUF CONC 10x	Wash buffer 10-fold	100 ml concentrate for 1000 ml solution white cap
DIL	Sample diluent	100 ml - ready to use colored yellow black cap
CONTROL+	Positive control Recombinant *Giardia* lamblia Cyst wall proteins	2.0 ml - ready to use colored blue red cap
CONTROL–	Negative control *Giardia* lamblia negative sample	2.0 ml - ready to use colored blue green cap
CONJ HRP	HRP-conjugate HRP-labeled polyclonal anti-*Giardia* CWP-1 antibodies (sheep)	15 ml - ready to use colored green violet cap
SUBSTR TMB	Substrate 3,3',5,5'-Tetramethylbenzidine and hydrogen peroxide	15 ml - ready to use blue cap
STOP	Stop solution 0.25 M sulphuric acid	15 ml - ready to use yellow cap

Table 2: Results of Rida Quick *Giardia*® and Serazym ELISA *Giardia*®tests against conventional stool microscopy.

Antigen test		**Microscopy (CME)**	
		Positive	**Negative**
Serazym ELISA *Giardia*®	Positive	73	0
	Negative	8	15
Rida Quick *Giardia*®	Positive	60	0
	Negative	21	15
Sensitivity Serazym 90.1%		Sensitivity Rida 79 %	
Specificity Serazym 100%		Specificity Rida 100%	
Accuracy 91.7%		Accuracy 82.3%	
Positive predictive value Serazym 100%		Positive predictive value Rida 100%	
Negative predictive value Serazym 65.2%		Negative predictive value Rida 46.9%	

Table 3: Results of Rida Quick *Giardia*® and Serazym ELISA *Giardia*® tests against immunofluorescence.

Antigen test		**Immunofluorescence**	
		Positive	**Negative**
Serazym ELISA *Giardia*®	Positive	70	3
	Negative	11	12
Rida Quick *Giardia*®	Positive	68	5
	Negative	13	10
Sensitivity Serazym 86.4%		Sensitivity Rida 83.9%	
Specificity Serazym 80%		Specificity Rida 66.7%	
Accuracy 85.4%		Accuracy 81.3%	
Positive predictive value Serazym 95.9%		Positive predictive value Rida 93.2%	
Negative predictive value Serazym 52.2%		Negative predictive value Rida 43.5%	

Table 4: Association between results of cell block and tissue block.

Antigen test	ρ	κ	Discrepancy	P-value
Serazym ELISA *Giardia*®	0.774	0.749	10%	< 0.05
Rida Quick *Giardia*®	0.786	0.786	8%	
ρ: Spearman's rank-correlation coefficient PR				
κ: Cohen's Kappa				

results. He foundnine different immunoassay kits sensitivities ranged from 93% to 100% and the specificities in all EIAs was above 99% [17].

Conclusion

We found that the EIAs antigen tests evaluated are highly sensitive and specific for detection of *Giardia*spp. In our setting, Serazym ELISA *Giardia*® is more reliable than Rida Quick *Giardia*®. Those methods certainly have developed into a powerful method for increasing the efficacy of stool diagnostics. There are considerable differences in sensitivity and specificity between different commercial tests.

References

1. Luján HD, Svärd S (2011) *Giardia* a Model Organism. (1stedtn), Springer-Verlag/Wien, Austria.
2. Thompson RC, Monis PT (2004) Variation in *Giardia* implications for taxonomy and epidemiology. Adv Parasitol 58: 69-137.
3. Smith HV, Cacciò SM, Tait A, McLauchlin J, Thompson RA (2006)Tools for investigating the environmental transmission of Cryptosporidium and *Giardia* infections in humans. J Trends Parasitol 22: 160-167.
4. 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: 656-659.
5. Schunk M, Jelinek T, Wetzel K, Nothdurft HD (2001) Detection of *Giardia* lamblia and *Entamoeba histolytica* in stool samples by two enzyme immunoassays. Eur J Clin Microbiol Infect Dis 20: 389-391.
6. Markell EK, Jone DT, Krotoski WA (1999) Markell and Voge'smdicalparasitology. (8thedtn) Saunders, Philadelphia, USA.
7. Chatterjee KD (2009) Parasitology, protozoology and helminthology. (13thedtn), Thomson Press (India) Ltd, India.
8. Al Saad RK, AlEmarah GY (2014) Morphological descriptive study of *Giardialamblia*in man and cow at Basrah. Int. J Biol Res 2: 125-128.
9. Al-Emarah GY, Othman RM, Alsaad RK (2013) Determine the zoonotic *Giardia* between human and cows by nested PCR technique in Basrah, Iraq. J Inter Acad Resea Multidis 9:51-61.
10. Al Saad RK, AlEmarah GY (2014) Epidemiological comparative study of *Giardialamblia* between human and cowin Basrah, Iraq. Inter J Innov Appl Stud 7:843-848.
11. Alsaad RK, AlEmarah GY (2014) *Giardia*sis in cattle: clinical assessment of *Giardialamblia* in cattle at Basrah, Iraq. Inter J Scient Wor 2:80-83.
12. Al Emarah GY, Al Saad RK (2013) Experimental infection of calves by *Giardialamblia* cyst isolation from human. Bas J Vet Res2:159-167.
13. AlEmarah GY, Al Saad RK (2014) Symptomatic assessment of human *Giardia*sis in Basrah. J Infect Dis Ther 2:5.
14. Alsaad RK, AlEmarah GY (2014) Biological and Molecular Study of *Giardia* Parasite as a Zoonotic Potential in Human and Animal at Northern Basrah- Iraq. Zoonoses *Giardia*, LAP Lambert Academic publishing, Germany.
15. R-Biopharm AG (2012) RIDA®QUICK Cryptosporidium/*Giardia* Combi, Germany.
16. Seramun Diagnostica GmbH (2017) Serazym®*Giardia*, Germany.
17. Aldeen WE, Carroll K, Robison A, Morrison M, Hale D (1998) Comparison of nine commercially available enzyme-linked immuno-sorbent assays for detection of *Giardia* lamblia in fecal specimens. J Clini Microbiol 36: 1338-1340.