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Evaluation of Conventional Parasitological Assays, Antigen Detection and Rapid Kit for Diagnosis of Human Giardiasis

[1]Ibrahim Aly, [2]Karima M. Metwalley, [3]Faten Nagy and [3]Mohamed EL-Mohandes

[1]Department of Parasitology, Theodor Bilharz Research, Institute, Giza, Egypt
[2]Department of Zoology, Faculty of Science, Al-Azher University, Cairo, Egypt
[3]Department of Immunology, Theodor Bilharz Research, Institute, Giza, Egypt

Abstract: *Giardia* is the most common causes of protozoan diarrhea that lead to significant morbidity and mortality worldwide. The purpose of this study was to compare the conventional microscopy, coprological sandwich ELISA and rapid kit for diagnosis of human giardiasis. This cross-sectional study was conducted between May 2013 to January 2015. A total of 82 stool specimens were collected and examined for intestinal parasites using direct smear and merthiolate iodine formaldehyde concentration methods (MIF). The number of *Giardia* cysts/g of feces were calculated after MIFC. *Giardia* cyst and/or trophozoite were purified from human fecal samples. Purified *Giardia* antigen was characterized for determination of molecular weight range using discontinuous SDS-PAGE. Polyclonal Antibodies (pAb) against purified *Giardia* antigen was performed and used for detection of *Giardia* antigens in different stool specimens. Commercial rapid *Giardia* kit was used for detection of giardiasis. Among 42 of giardiasis fecal samples 26(61.9%) were diagnosed as *Giardia*-positive by direct smear, while the no. of *Giardia*-positive samples were 30 (71.4%) when using ordinary microscopy MIF method. Using rapid kit, the no. of *Giardia*-positive samples were 36 (85.7%). While the *Giardia*-positive samples were 40 (94.4%) when using the prepared pAb against *Giardia* antigen by sandwich ELISA. In conclusion, the direct microscopical examination is reliable in *Giardia* diagnosis as a first choice. In addition, our research study using the prepared pAb that was carried out for detection of *Giardia* antigen in stool samples of patients infected with *G. lamblia*, provides a well-established reference test for diagnosis of giardiasis.

Key words: Coprantigen ELISA · Diagnosis · Giardiasis · *Giardia lambila*

INTRODUCTION

Giardia nowadays is recognized as the most common parasitological cause of diarrhea, with 280 million infections per year. Giardiasis is a frequently diagnosed waterborne infection and a major concern to drinking water authorities. Because of the impact on socioeconomic development, as well as on domestic animals such as cattle and sheep especially in developing countries, *Giardia* is included in the ‘‘Neglected Disease Initiative’’ of the World Health Organization [1, 2]. Manifestations of giardiasis vary from an asymptomatic carrier state to severe malabsorption syndrome with chronic diarrhea [3]. Several extra-intestinal manifestations associated with giardiasis have been reported in literature, although infrequent. Reactive arthritis [4], dermatologic manifestations [5] and some ocular manifestations [6] have been correlated with giardiasis confirmed cases. In the last decade, *G. lamblia* trophozoites and cysts have been recovered from gall bladder and biliary tree of an immune-deficient patient suffering from cholecystitis [7] and from liver and pancreatic masses in another patient [8] where resolution in both was achieved after metronidazole therapy. There is no current gold standard diagnostic test for detecting giardiasis [9]. Clinical parasitology laboratories, in contrast to most other diagnostic laboratories, utilize many complex manual technical procedures that are subjected to individual variation and subjective interpretations [10]. Sensitivity is poor when only a single sample is analyzed, particularly

Corresponding Author: Ibrahim Aly, Department of Parasitology, Theodor Bilharz Research Institute (TBRI), Imbaba, Giza, Egypt. E-mail: ibrahimshalash@yahoo.com.

if there is low parasite density, insufficient microscopic quality, intermittent excretion of cysts or the probability of parasite hiding by bile pigments [11]. Microscopic examination must be performed on three stool samples to increase sensitivity [12]. Even if three specimens are examined correctly the parasite may not be recovered because the passage of cysts is often sporadic and intermittent and it does require a skilled technician [13]. This leads to problems concerning patient compliance and delays the final diagnosis [14]. The sensitivity of laboratory diagnosis of *Giardia lamblia* infection can be improved by including alternative diagnostic procedures which are more rapid and reliable [15, 16]. Immunodiagnostic assays for detection of *G. lamblia* antigen in stool are available such as enzyme immune assay (EIA) and indirect fluorescent assay [10]. Single antigen detection technique is able to detect 50% more infections than the stool examination. An alternative assay is a more invasive approach as duodenal biopsy, aspiration or collection of duodenal fluid with the string test [8]. The potential diagnostic value of ELISA by detecting specific antibodies has been reported in intestinal nematode infections [17] toxoplasmosis [18] invasive amoebiasis [19] Naegleria infection [20] and giardiasis [21]. Direct immunofluorescent antibody (DIF) test, based on binding of specific fluorescent monoclonal antibodies to *G. lamblia* cysts, was used for diagnosis with high sensitivity and specificity [22]. In addition, fecal concentration (FC) has been suggested as an effective method, with significant advantages. An optimized FC protocol for *G. lamblia* provides an accurate, fast, simple and automated detection method for clinical diagnosis and water analysis [10]. The aim of this study is to compare conventional microscopy, coprological sandwich ELISA and rapid kits as diagnostic methods for *Giardia* diagnosis.The present work aimed to detect *Giardia lamblia* antigen(s) in stool samples of infected individuals, as a promising non-invasive technique, to diagnose giardiasis. Moreover, this study is to compare conventional microscopy, coprological sandwich ELISA and rapid kits as diagnostic methods for *Giardia* diagnosis.

MATERIALS AND METHODS

This study was conducted in the period from May 2013 to January 2015. Eighty-four patients from outpatient clinics of Kasr EL-Ini Hospital and outpatients of Theodor Bilharz Research Institute (TBRI), Giza, were enrolled in this study: 46 males and 38 females, the mean age of examinees was 26.8 - 42.01 (Range: 29 years old). The purpose of the study was explained and verbal consents were obtained from parents of all participants.

Collection of Fecal Samples: Fecal samples were collected in clean wide mouth containers and examined by direct smear and merthiolate iodine formaldehyde concentration methods (MIF). One gram from each sample was then transferred into each of two tubes containing 5 ml of 10% buffered formalin as a preservative to prepare suspensions. These tubes were labeled according to ID number and date of collection and kept refrigerated at 4–8 °C until subsequent immunological analysis.

Parasitological Examination: The samples were processed immediately without preservation. Two types of direct wet film preparation were done for each sample at the same time, 1 slide using normal saline (0.85%) for detecting the actively motile trophozoites and Lugol's iodine (5%) for demonstrating structures. All samples were examined microscopically by the same researchers using10×and40×power lenses) for the presence of cysts and/or trophozoites of *G. lamblia* and for detection of other parasites. The microscopic examination was done 3 times on each sample for confirmation. The criteria for positive *Giardia* were active motile flagellated trophozoites and thick hyaline wall of cyst stages.

Direct Smear: A piece of stool was taken and emulsified in normal saline, One drop of fecal suspension was transferred to a microscope slide with a cover slip. Each slide was then examined as a direct mount at 40 magnification and presence or absence of *G. lamblia* cysts was recorded.

Merthiolate iodine formaldhyde Concentration Technique (MIFC): One gram of faecal specimen was added to 5ml MIF solution, mixed well and filtrated in other cup. This was followed by the addition of 7ml ether. The prepared specimen was centrifuged for 5 min at 3000 g. A drop of mixed sediment was placed on a slide, covered and examined under light microscope [23] (MIF solution is a mixture of 2 solutions with ratio 4:1. solution A composed of 0.1% merthiolate, 36-40% formaldehyde, glycerin and distilled water; solution 2 B composed of potassium iodide, iodine and dist. H_2O).

Cyst Count: The number of *Giardia* cysts/g of feces were calculated after MIFC using the formula N = S/(V W); while N is the number of cysts/g of feces, S

is the number of cysts counted on the slide, V is the volume of sample examined and W is the stool weight in grams [24].

***Giardia*-Strip Rapid Assay:** The Becton Dickinson *Giardia*-Strip rapid assay is a rapid immunoassay for the qualitative detection of *Giardia* specific antigens in aqueous extracts of fecal specimens. The rapid assay was used in accordance with the manufacturer's directions. In brief, add 0.5mL or 15 drops of the dilution buffer solution to each tube. Plunge the inoculating loop containing the stool sample into the tube. The dilution ratio must be at most 4% w/v. For liquid samples, take 2 loops of 10 µL, for solid samples, take 1 loop. Stir to homogenize the solution and let stand for 1-2 minutes. Discard the inoculating loop and immerse the sensitized strip in the direction indicated by the arrow. Let react for 15 minutes. Results must be read on wet strips after 15 minutes incubation.

Preparation and Purification of *Giardia* Antigen: Preparation of Giardia cyst and/or trophozoite inocula - Giardia cysts were purified from human fecal samples. Trophozoites were obtained from experimentally infected gerbils following the procedure described by Belosevic and Faubert [25]. Their concentration was independently adjusted to 1x106 per 1 ml saline [26]. Freeze (-80°C) and thaw (4°C) cycles were carried out. These solutions were submitted to ultrasound sonication [27].

Characterization of Antigen: Purified *Giardia* antigen was characterized for determination of molecular weight range using discontinuous SDS-PAGE in 12% slab gels (1 mm thick), under reducing conditions (+2-mercaptoethanol) and stained with silver stain [28]. Molecular weight standards were electrophoresed on the same gel to calculate the relative molecular weights of the examined antigens.

Preparation of Polyclonal Antibodies (pAb) Against Purified *Giardia* Antigen (PGA): Just before immunization, rabbits' sera were assayed by ELISA for *Giardia* antibodies and cross-reactivity with other parasites. Rabbits were injected intramuscularly (IM), with 1mg of PGA mixed 1:1 in complete Freund adjuvant (CFA). Then, two booster doses were given at 1 week intervals after the primary injection, each was 0.5 mg antigen emulsified in equal vol. of incomplete Freund adjuvant (IFA) [29]. One week after the last booster dose, the rabbits sera were obtained and pAb fraction was purified by 50% ammonium sulfate precipitation method Odutayo and Alina [30]. More purification of pAb was performed by 7% caprylic acid method Mckinney and Parkinson [31]. The IgG was produced with high degree of purity, but a trace of serum protein contaminants. Partially purified pAb was further adsorbed with fetal calf serum (FCS) to eliminate any non-specific binding with bovine antigen.

Preparation of Fecal Samples: Individual fecal samples were processed by mixing the fecal material in a 1:5 proportion with PBS-formalin 5%. The samples were mixed using a vortex to form a slurry and centrifuged at 3,000 xg for 30 minutes at 25°C. The supernatant was recovered, aliquoted in 1.5-mL vials and stored at 4°C until used. On the day of use, vials containing fecal supernatants were vortex mixed and re-centrifuged at 3,000 xg for 15 minutes before use. Morrison and Franklin [32].

Sandwich ELISA: After several optimization trials, the following sandwich ELISA originally described by Espino and Finlay [33], was performed. Microtitration plates (Dynatech) were coated with 10 µg/ml of purified anti-PGA pAb in 0.1 M carbonate buffer, pH 9.6 dispensed as 100 µl/well and left overnight at room temperature e. Plates were blocked by adding 200ul/well of 3% fetal calf serum/PBS/Tween for 1 hour at 37°C (3% FCS/PBS/T was used as diluting buffer and PBS/T as washing buffer). A hundred µl of fecal supernatant samples was added to each well in a duplicate. Plates were re-sealed and incubated for 1 hour at room temperature on an orbital shaker. Plates were washed with washing buffer. One hundred µl/well of 1:1000 dilution of peroxidase-conjugated pAb (5µg/ml) were added and incubated for 2 hours at room temperature and then plates were washed as before. The reaction was visualized by the addition of 100 µl/well of O-phenylene diamine (OPD) substrate solution for 30 minutes in the dark at room temperature. The reaction was stopped by adding 50 µl/well of 8 N H2SO4 and plates were read at 492 nm using ELISA microplate reader (Bio Rad).

Statistical Analysis: Statistical analysis data entry and analysis were performed using SPSS (Ver. 17 for Windows) and Medcalc software. The agreement between each test and the gold standard test was determined based on the calculated j (Kappa) value gradation [34]. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of each test were calculated and compared to the gold standard. The results

of P < 0.05 were considered statistically significant. The cut off value was calculated as the mean OD readings of negative controls + 2 standard deviations of the mean. Sensitivity (%)= A/(A+C)x100, specificity (%) = D/(B+D)x100, PPV(%) = A/(A+B)x 100 and NPV(%)= D/(C+D)x 100, where A=True positive, B=False positive, C=False negative and D=True negative.

RESULTS

Purification *of G. lamblia.* The purified *G. lamblia* antigen was analyzed by 12% SDS-PAGE under reducing condition and stained with silver stain showed four major bands at 65, 50, 40 and 25 kDa (Fig. 1).

Rabbit anti-*G. lamblia* Serum: Detection of specific anti-*G. lamblia* antibodies by indirect ELISA had demonstrated an elevation of IgG polyclonal antibody level one week after the first booster dose and it was remarkably higher when detected three days after the 2nd booster dose (Fig. 2).

Purification of Rabbit anti-*G. lamblia* Serum IgG pAb: The total protein content of anti-*G. lamblia* pAb was measured before (Crude) and after different purification steps including ammonium sulfate precipitation method followed by 7% caprylic acid precipitation method. It was 12.5 mg/ml, 5.9 mg/ml and dropped to 3.1 mg/ml, respectively. The purity of IgG after each steps of purification was assayed by 12% SDS-PAGE under reducing condition. The purified IgG pAb was represented by H- and L-chain band at 53 and 31 kDa, respectively (Fig. 3).

Conjugation of Purified Rabbit Anti-*G. lamblia* IgG pAb: Rabbit anti- *Giardia lamblia* IgG pAb in amount of 7 mg was conjugated with HRP. IgG antibody was assessed against purified *G. lamblia* antigen in ELISA assay. The 5000 pg/ml of the conjugate gave the highest OD reading against *G. lamblia* antigen (Fig. 4).

Specificity of anti-*G. lamblia* serum IgG pAb Against Purified *Giardia* Antigen: Reactivity of anti-*G. lamblia* pAb against purified *Giardia* antigen and other parasite antigens (*Blastocysts* and *Toxoplasma*) was determined by indirect ELISA. Anti-*G. lamblia* IgG pAb diluted 1/100 gave a strong reactivity to purified *Giardia* antigen. The OD reading for *G. lamblia* was 2.84 compared to 0.462 and 0.281 for *Blastocysts* and Toxoplasma, respectively, at 492 nm.

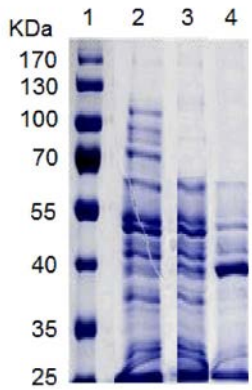

Fig. 1: 12% SDS-PAGE of *G. Lamblia* antigen under reduced conditions (Stained with coomasie blue stain)
1-Standard
2,3- crude *Giardia* cysts
4- Purified Giardia antigen

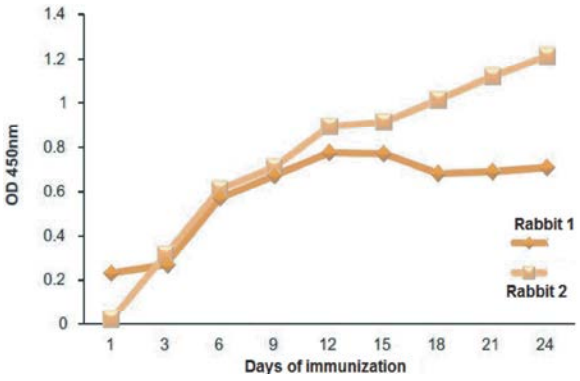

Fig. 2: Reactivity of raised rabbit anti-*Giardia* antibodies (diluted 1/100) against purified *Giardia* antigen by indirect ELISA.

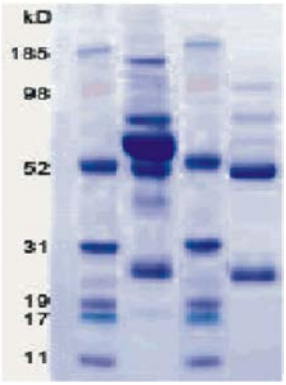

Fig. 3: 12% SDS-PAGE of anti-*G. lamblia* IgG pAb.
1- Standard.
2- Before purification
3- After ammonium sulfate
4 After Caprylic acid

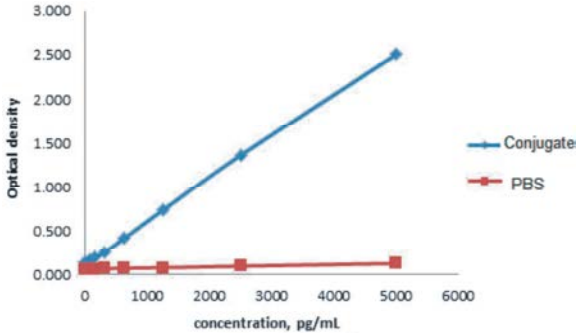

Fig. 4: Shows the results of titration, from which the 5000 pg/ml concentration of the conjugate gave the highest OD reading against purified *Giardia* antigen

Table 1: Detection of *G. lamblia* cyst in stool samples of infected human

	Positive cases		Negative cases	
Groups	*Direct smear*	*MIFC method*	*Direct smear*	*MIFC method*
Healthy control (n= 20)	--	--	20	20
Giardiasis group *(n=42)*	26	30	12	3
			6	4
Blastocysts (n= 12)	--	--	12	12
Toxoplasma (n= 8)	--	--	8	8

Table 2: Key features of parasitological tests

Detection method	Sensitivity	Specificity	PPV	NPV
Direct smear	61.9%	100%	100%	73.5%
MIFC method	71.4%	100%	100%	80.6%

PPV is denoted for positive predictive value;NPV is denoted for negative predictive value.

Table 3: Detection of *Giardia* antigen in stool samples of infected human using ELISA

	Positive cases		Negative cases	
Groups	*No.*	*X±SD*	*No.*	*X±SD*
Healthy control (n= 20)	--		20	0.29±0.07
Giardia (n= 42)	38	1.07±0.22	4	0.211±0.08
Blastocysts (n= 12)	3	0.77±0.19	9	0.44±0.17
Toxoplasma (n= 8)	1	--	7	0.38±0.19

Parasitological Examination: According to stool analysis by direct smear 26 patients from 42 giardiasis group were positive with *G. lamblia* cyst while by MIFC method 30 patients were positive. The twenty normal and 20 patients infected with other parasites (12 with *Blastocysts* and 8 with *Toxoplasma*) all are negative by both methods (Table 1).

Both direct smear and MIFC methods gave 100% specificity and positive predictive value (PPV), yet the MIFC method recorded higher sensitivity (71.4%) and negative predictive value (NPV) (80.6%) than those of direct smear (61.9% & 73.5% respectively; (Table 2).

Sandwich ELISA for Detection of *G. Lamblia* Antigen in Stool of Patients: Table 3 shows the results of detection *G. lamblia* antigen in stool samples among different studied groups. Cut off value for positivity was 0.43 as mean +2 SD. The OD value of *G. lamblia* infected group was significantly higher than that of both healthy control and other parasite groups. Four out of 42 *G. lamblia* infected samples from giardiasis patients showed false negative results and the sensitivity of the assay was 90.5%. All the 20 negative controls were below the cut off value while 4 out of 20 of other parasites groups were at the border line of the cut off value giving 90% specificity. The PPV and NPV were 93.9 and 87.5%, respectively (Table 4, 5).

Rapid Test for Detection of *G. Lamblia* Antigen in Stool of Infected Human: Table 4 showed the results of detection *G. lamblia* antigen in stool samples among different studied groups using rapid kits *(Giardia*-Strip rapid assay). Sex out of 42 *G. lamblia* infected samples from giardiasis patients showed false negative

Positive Negative

Table 4: Detection of *Giardia* antigen in stool samples of infected human using Rapid Test

	Positive cases		Negative cases	
Groups	*No.*	*Score*	*No.*	*Score*
Healthy control (n= 20)	--	-	20	-
Giardia (n= 42)	36	+++	6	-
Blastocysts (n= 12)	3	++	7	-
Toxoplasma (n= 8)	2	++	6	-

Table 5: Sensitivity, specificity, PPV and NPV of sandwich ELISA and Rapid Test for detection of *Giardia* antigen in human stool samples

Detection method	Sensitivity	Specificity	PPV	NPV
ELISA	94.4%	92.0%	95.7%	88%
Rapid Test	85.7%	87.5%	83.7%	85.4%

results and the sensitivity of the assay was 85.7%. All the 20 negative controls were below the cut off value while 5 out of 20 of other parasites groups were at the border line of the cut off value giving 87.5% specificity. The PPV and NPV were 83.7% and 85.4%, respectively. (Table 4, 5).

DISCUSSION

One of the most common intestinal protozoan parasites is *G. lamblia*; about 200 million people in Asia, Africa and Latin America have symptomatic infections [35] with 280 million infections per year [36]**.** Since it has a fecal-oral transmission cycle and is contracted by ingestion of contaminated water or food or by person-to-person contact, the highest disease burden is found in areas where sanitary conditions are poor. The highest rates of infection are therefore encountered in developing countries 10–30% in young children [37]. Because of the impact on socioeconomic development, as well as on domestic animals such as cattle and sheep especially in developing countries, Giardia is included in the ‘‘Neglected Disease Initiative’’ of the World Health Organization [1, 2]. After ingestion of infective *Giardia* cysts with as few as 10 to 25 cysts, 5–15% pass cysts without symptoms. When the parasite causes symptoms, they may last for 5 to 7 days with diarrhea (89%), malaise (84%), flatulence (74%), abdominal cramps (70%), bloating (69%), nausea (68%), anorexia (64%) and weight loss (64%). About 25–50% develop acute self-limiting diarrhea. Chronic infection may produce steatorrhea (copious light-colored, fatty stools) and full-blown malabsorption syndrome [37]. At the time of stool collection, 48.6% (137/282) abnormal stool consistency (Loose, mushy, mucous, loose-watery/watery) were observed. However, the prevalence of giardiasis from previous reports in asymptomatic children of all age groups in Thai-orphanages ranged from 14.3% to 37.7% [38-40].

In the published methods, some chemical materials like formalin, iodine, mercury and ethers were used for the isolation of protozoa cysts that could affect physical and biological properties of cysts. Methods like sucrose or percoll-sucrose gradient tests had gained suspensions of cysts that contained surplus fecal particles. A special one-phase sucrose gradient method was used in a study to isolate purified *Giardia* cysts antigen that we used that procedure in the current study as efficient method of separation of purified *Giardia* cysts antigen

By using 12% SDS-PAGE technique under reducing condition, the purified *Giardia* antigen showed four major bands at 65, 50, 40 and 25 kDa. Several studies aimed to purify antigens from cysts and/or trophozoites of *G. lamblia*, in order to be used in diagnosis, Kaur *et al.* [41] and Palm *et al*. [42] characterized a 58 kDa excretory–secretory product (ESP) of *G. lamblia*. The ESP was purified over 508-fold by a combination of ammonium sulphate precipitation and sequential chromatography on affinity matrix and a gel filtration column. The homogeneity of the purified protein was established by SDS-PAGE (*M*r, 58 kDa) and analytical isoelectrophoresis (pI 4.75). The purified protein was recognized by the pooled sera of *G. lamblia*-positive patients as well as an antiserum raised against crude *Giardia* extract, thus indicating it to be an immunodominant parasite product.

The diagnosis of giardiasis is frequently based on microscopic examination of stool samples by visualizing the organism, either the trophozoites or the cysts [42]. Diagnosis via microscopical examination of a single stool specimen has a low sensitivity and may therefore miss up to 50% of *Giardia* infections [34, 40], particularly if there is a low parasite density, insufficient microscopic quality, intermittent excretion of cysts or the probability of parasite hiding by bile pigments [41]. Microscopic examination of three consecutive stool specimens is required to reach a sensitivity of over 90% [42, 43]. The study was conducted on 42 *G. lamblia* infected patients, 20 other parasites infected patients and 20 healthy controls. Parasitological examinations by direct smear and MIFC gave 100% specificity and positive predictive value (PPV). The MIF method showed a sensitivity of 71.4% and negative predictive value (80.6%). While in direct smear method, the sensitivity and the NPV were 61.9%and 73.5%, respectively.

Given these difficulties the development of sensitive, cost-effective and rapid diagnostic methods is of the most importance [44]. ELISA is a rapid, sensitive and economic method for detection of specific antigens in stools and confirmation of certain infection. Coproantigens of a parasite could be traced and diagnosed even if the live parasite is absent in the fecal samples [44, 45].

The ELISA for antigen detection in human fecal eluates will improve diagnosis of *Giardia* infection. When applied under field conditions the test will be useful in determining the prevalence of *Giardia* infection and in making a timely diagnosis of infected individuals, particularly children who suffer from recurrent diarrhea, chronic abdominal pain, malabsorption and stunting as a consequence of infection. Currently, studies concerning the relationship between ELISA results, severity of disease and gastrointestinal symptoms as well as the application of the test in clinical follow-up, are being planned [46].

Our work has demonstrated that using sandwich ELISA assay as a trial for diagnosis of human giardiasis which confer a higher sensitivity for detecting *G. lambilia* in stool samples compared with Rapid test because sandwich ELISA assay has potential larger surface area. Purified *Giardia* antigen was used with complete and incomplete Freund's adjuvants for immunization of rabbits in order to prepare anti-*Giardia* polyclonal antibodies. This was in agreement with Duque-Beltrán *et al*. [47] who purified *Giardia* cysts from human fecal samples by sucrose and percoll gradients. Gerbils (Merionesunguiculatus) were infected to obtain trophozoites. Rabbits were inoculated with either cyst or trophozoite antigens of 14 Colombian *Giardia* isolate to develop antibodies against the respective stages. The anti-*G.lamblia* pAb IgG was purified by sequential caprylic acid and ammonium sulfate precipitation. A portion of these pAb was linked to alkaline phosphatase Conjugate. The optimal concentration of pAb for antigen capture was 2000 pg/ml and the optimal conjugate dilution was 2000 pg/ml.

The reactivity of pAb was determined by indirect ELISA and showed a strong reactivity to *Giardia* antigen till dilution 1:1000. Optimization of various reagents was assayed by sandwich ELISA. The optimum concentration of purified IgG pAb was 10µg/ml, whereas conjugated IgG pAb was 3.3µg/ml. This yield of pAb was reasonable in comparison with the yield of purified immunoglobulin from any biological fluid following similar purification procedures. The reactivity of the purified pAb was tested by indirect ELISA. It is worthy to know that ELISA has been described as a valid test for detection of rabbit antibodies to *Giardia* antigen and is the technique receiving most attention in immunodiagnosis of various parasitic infections [48]. Antigen detection assay in stool is generally performed by sandwich ELISA [49-51]. The specificity estimated for sandwich ELISA was comparable to that obtained with the rapid test when the same samples were tested. However, sandwich ELISA offered the potential advantage on improving the sensitivity of the assay. The sandwich ELISA are of high binding capacity (As a solid phase) and rapid reaction kinetics of solutions (With the simple separation of bound and unbound materials on the solid phase); thus, they provide the chance of enhancing the sensitivity of antigen detection [52] in the immunoassay. Detection of *Giardia* antigen in the stools of tested groups proved 38 out of 42 giardiasis cases with positive results and 4 were negative when examined by ELISA. In other words, a sensitivity of 94.4% was obtained by that assay. On the other hand, *Giardia* antigen detection by rapid test in the same stool samples revealed a sensitivity of 85.7%. In conclusion, our research study using the prepared pAb that was carried out for detection of *Giardia* antigen in stool samples of patients infected with *G. lamblia*, provides a well-established reference test for diagnosis of giardiasis.

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