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Original Article

Effective Diagnosis of Giardiasis (*Giardia lamblia*) by Immunomagnetic Bead ELISA technique using Paramagnetic Nanoparticles

Eglal A. Koura[1], Ibrahim R. Aly[2], Nashwa I. El-deeb[1] and Manal A. Shahin[1]

[1]Department of Zoology, Girls College for Arts, Science and Education, Ain-Shams University, Cairo, Egypt.
[2]Department of Parasitology, Theodore Bilharz Research Institute, Giza, Egypt

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ABSTRACT

Giardiasis is considered to be endemic in all regions of the world. *Giardia lamblia* (*G. lamblia)* cysts are spread in Egypt via the faecal-oral route, through cyst ingestion or with contaminated food or water. The most important clinical symptoms of giardiasis are malabsorption and diarrhea. Although microscopy has the advantage of low cost and ability to simultaneously identify other gastrointestinal parasite, the limitation of this method is that *G. lamblia* cysts are small and similar to many pseudoparasites. The presnt study aimed to detect the potential specificity and sensitivity of paramagnetic nanoparticles based-ELISA in diagnosing of giardiasis in stool of infected individuals. *Giardia lamblia* antigen in stool samples was detected by using the coproantigen ELISA, incorporated with conjugated anti-purified *G. lamblia* cysts antigen (PGA) with paramagnetic nanoparticles, compared to the traditional sandwich ELISA. Sandwich ELISA achieved sensitivity of 88%, specificity of 92 %, positive predictive value (PPV) of 84.61% and negative value of 93.87% while immunomagnetic bead ELISA with paramagnetic nanoparticles achieved sensitivity, specificity, PPV and NPV of 92%, 94%, 88.46% and 95.91% respectively. In conclusion, this research provides that immunomagnetic bead ELISA is a well-established reference test for giardiasis diagnosis more than traditional sandwich ELISA.

Key words: Immunomagnetic bead ELISA technique – Paramagnetic nanoparticles - Coproantigen – Diagnosis- Giardiasis – *Giardia* purified antigen PGA

INTRODUCTION

Giardiasis constitutes a significant public health problem in Egyptians. Contamination of the Nile River with faecal materials containing viruses and pathogenic protozoa still represents an environmental health hazard in Egypt, especially in rural areas **(1).** The world health organization (WHO) considers diarrheal disease as the second most common cause of morbidity and mortality in children in the developing world **(2).** It is the most common nonviral nonbacterial cause of diarrhea worldwide with a prevalence range from 2-7% in developed countries to 20-30% in most developing countries **(3).**

Approximately 200 million people experience symptomatic giardiasis in Asia, Africa, and Latin America and about 500,000 new cases are evaluated each year according to the World Health Organization (WHO) **(4,5).** *Giardia* cyst can be transmitted directly, through the faecal-oral route, or indirectly, through ingestion of contaminated water or food **(6).**

Diarrhea and malabsorption are the most important clinical symptoms of giardiasis **(7).** Acute giardiasis is characterized by nausea, anorexia and sometimes low-grade fever and chills followed by explosive, watery and foul-smelling diarrhoea. Upper or mid-epigastric cramps may also occur. Chronic infection may develop which may last for 2 or more years. Chronic giardiasis is associated in early childhood with poor cognitive function and failure to thrive **(8).**

Direct examination of faeces or intestinal tissue samples for cysts or trophozoites of parasite is the most trusted diagnostic test **(9).** Although microscopy has the advantage of low cost and ability to simultaneously detect other gastrointestinal parasite, the disadvantages of this method is that *G. lamblia* cysts are small and similar in appearance to many pseudoparasites such

***Corresponding Author Address:** Manal A. Shahin, Department of Zoology, Girls College for Arts, Science and Education, Ain-Shams University, Cairo, Egypt. E-mail: manal.shahin88@gmail.com*

as yeast. Also, the trophozoites break up rapidly in the stool, so cannot be used to measure the severity of infection **(10).** Moreover, the sensitivity of routine examination of a single stool specimen for cysts is approximately 50 to 70% (**11,12**), so two or three specimens collected on different days should be analyzed **(13,14).** Detection of *Giardia* antigens by counter immunoelectrophoresis or enzyme-linked immunosorbent assay, is reported to be more sensitive than detection of cysts and is less labor intensive than microscopic examination **(15).**

Recently, ELISA has been considered as cost effective diagnostic method which can detect small quantities of coproantigens of parasite, even in mild infection, and diagnosed even if the live parasite is absent in the feacal sample **(16,17,10).** The use of nanotechnology in clinical diagnosis (Nanodiagnostics), meet the demands for increased sensitivity and early detection in less time. Nanomaterials have large surface area which enables attachment of large number of target-specific molecules of interest for ultra-sensitive detection. Conventional methods are limited to achieve this ultra-sensitivity. In addition, nanomaterials have unique properties could allow rapid (as short as few minutes) and real-time detection of the pathogens. Also relatively small sample volumes **(18).**

The use of nanoparticles as labels or tags allows for the detection of infections agents in small volumes directly in a very specific, sensitive and rapid format at lower costs than current in-use technologies. This advance in early detection enables accurate and prompt treatment **(19).**

The present study aimed to detect *Giardia lamblia* antigen in stool of infected individuals through raising anti-*G. lamblia* immunoglobulin G polyclonal antibody (IgG pAb) using parasitological examination by (direct smear and merthiolate iodine formaldehyde concentration methods, MIFC) and to compare these methods with immunomagnetic bead ELISA with paramagnetic nanoparticles and traditional sandwich ELISA.

MATERIAL AND METHODS

Animal: New Zealand white male rabbits, weighing approximately 2.0 Kg and about 2 months age, purchased from rabbit research unit (RRU), Agriculture Faculty, Cairo University. They were examined before the start of the experiments and found free from *G. lamblia* and other parasitic infection and used in the production of the antibodies **(20).** They housed in the animal house in (TBRI) and kept for 4 weeks.

Preparation of parasite antigen: This study was conducted in the period from February 2014 to March 2015. Seventy patients from outpatient clinics of Kasr EL-Ini Hospital and outpatients of (TBRI), Giza, were enrolled in this study. 25 patients were infected with *G. lamblia*, 30 patients infected with other parasites (17 *E. histolytica*, 8 *Blastocyste* sp. and 5 *E. coli*). In addition, 20 individuals of the medical staff at TBRI served as parasite free-healthy negative controls.

Collection of faecal samples: Faecal samples were collected in clean wide mouth containers and examined by direct smear and merthiolate iodine formaldehyde concentration methods (MIFC). 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 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 method: A specimen of approximately 2 mg of stool was taken and simply emulsified in a drop of normal saline, placed on a dry clean glass slide, using wooden applicator and a cover slip is applied to make a thin film free of air bubbles. The preparation is examined under low power microscope and presence or absence of *G. lamblia* cysts was recorded **(21)**.

Merthiolate-iodine-formaldehyde concentration method (MIFC): Approximately 1 g of faecal specimen is emulsified in a tube containing 5.0 ml of merthiolate iodine formaldehyde (MIF) 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 3500g. A drop of mixed sediment was placed on a slide, covered and examined under light microscope solution (MIF solution is a mixture of 2 solutions with ratio 4:1. A composed of 0.1% merthiolate, 36-40% formaldehyde, glycerin and distilled water; solution B composed of potassium iodide, iodine and distilled water) **(22,23)**.

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

Purification and Characterization of parasite antigen: *Giardia* cyst antigen was purified using Parasep filter faecal concentrator tubes system

according to (25,26) as it traps rejected particles and debris in stool samples, preventing their extrusion into the sedimentation cone during centrifugation. Thus improving the clarity results in an increased accuracy of diagnosis during microscopy. Then protein content was estimated by a Bio-Rad protein assay kit (Bio-Rad, Richmond, CA, USA) as shown by **(27)**. Finally, molecular weight of PGA purified *Giardia* antigen was determined by SDS PAGE according to **(28,29,30).**

Assessment of Reactivity of *Giardia* Purified Antigen PGA by Indirect ELISA: This method was performed, with some modifications from the original method of **(31)**.

Production and Purification of Polyclonal Antibodies Against
Purified *Giardia* Antigen (PGA): 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) **(32).**

Then, two booster doses were given at 1 week intervals after the primary injection, each was 0.5 mg antigen emulsified in equal volume of incomplete Freund adjuvant (IFA). One week after the last booster dose, the rabbits sera were obtained and pAb fraction was purified by 50% ammonium sulfate precipitation method **(33).** More purification of pAb was performed by 7% caprilic acid method **(34).** The purified pAb was further adsorbed with fetal calf serum (FCS) to eliminate any non-specific binding with bovine antigen. The reactivity of anti-*G. lamblia* IgGpAb against PGA was assessed using indirect ELISA **(31).**

Labeling of Rabbit anti-*G. lamblia* Serum IgGpAb with Horseradish Peroxidase (HRP) (Periodate Method): According to **(35)** and **(36),** 5 mg HRP (Sigma) was resuspended in 1.2 ml distilled water; followed by the addition of 0.3 ml freshly prepared sodium periodate and incubation at room temperature for 20 min. HRP solution was dialyzed against 1 mM sodium acetate buffer (pH 4) at 4°C with several changes overnight. IgGpAb solution (5 mg/ml in 0.02M carbonate buffer, pH 9.6) was prepared. The HRP was removed from dialysis tubing and was added to 0.5 ml of antibody solution. The mixture was incubated at room temperature for 2 hr. 100 µl sodium borohydride was added and the solution was incubated at 4°C for 2 hr. The HRP conjugate pAb was dialyzed with several changes against 0.01 M PBS (pH 7.2).

Detection of *Giardia* Antigen in Patient's Stool by Sandwich ELISA: positive stool samples with *Giardia* or other parasites and free non-infected samples were individually diluted 1:3 with PBS. According to **(37)** Polystyrene microtitre plates were coated with 100 µl/well of purified pAb (1/100 for IgG diluted in 0.06 M carbonate buffer, pH 9.6) and incubated over night at room temperature. The plates were washed 3 times with washing buffer 0.1 M PBS/T, pH 7.4.

The free sites were blocked with 200 µl/well of blocking buffer (0.1% BSA in 0.1 M PBS/T) and incubated for 2 hr at 37°C. The plates were washed with washing buffer 3 times. 100µl/well of faecal supernatant samples were added to each well and incubated for 2 hr at 37°C and the plates were washed 3 times with washing buffer. 100µl/well of peroxidase-conjugate pAb of a dilution 1/50 for IgG was added into each well and plates were incubated for 1 hr at 37°C.

The plates were washed 5 times with washing buffer. Hundred µl of substrate solution [one tablet of O-phenylene diamine dihydrochloride (OPD) (Sigma)] dissolved in 25 ml of 0.05 M phosphate citrate buffer, pH 5 with peroxidase H_20_2 (Sigma)] were added to each well and the plates were incubated in the dark at room temperature for 30 min., 50 µl/well of 8 N H_2SO_4 were added to stop the enzyme substrate solution. The absorbance was measured at 492 nm using ELISA reader (Bio-Rad microplate reader, Richomond, Co.).

Detection of *Giardia* Antigen in Patient's Stool by Immunomagnetic beads ELISA with Paramagnetic Nanoparticals: sandwich ELISA using conjugated anti-PGA with paramagnetic nanoparticles as the capture antibodies and anti-PGA conjugated with HRP as the conjugated antibodies. The above sandwich ELISA procedure was repeated by using peroxidase-conjugated nano-pAb.

Statistical analysis: The data are presented as mean ± standard deviation of mean (X ± SD). The mean values of each group were calculated from the mean values of individual patients. The mean groups were compared by analysis of variance **(38)**. This may be accomplished by changing the selection of the reference value (i.e. cut-off) for the particular test **(39)**. 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

Estimation of total protein content of *Giardia lamblia* antigen: The crude antigen obtained from positive *Giardia* stool samples contains 8 mg/ml of total protein as measured by Bio-Rad Protein assay while it was 4.5 mg/ml after purification by Parasep.

Purification of *Giardia* antigen of *G. lamblia* cysts:

Collection of samples: According to stool analysis by direct smear 18 from 77 patients were positive with *G. lamblia* cyst, while by MIFC method, 21 were positive **(Table1).**

Cyst count of stool samples: According to MIFC method, the stool samples were classified to heavy, moderate and light samples.10 of the samples were heavy infection, 8 moderate while 7 were light infection **(Table2).**

Characterization of *Giardia* antigen by SDS-Gel electrophoresis: The purified protein resulted from the purification method by Parasep technique was analyzed by 12.5% SDS-PAGE under reducing condition and stained with Coomassie Blue. Protein bands were appeared at 4 different bands at 47.5, 17.0, 14.0 and 12.5 kDa representing purified *Giardia* antigen **(Fig.1).**

Reactivity of PGA by Indirect ELISA: The antigenicity of the purified target antigen was tested by indirect ELISA technique. Stool samples from human infected with *G. lamblia* gave a strong reaction against *Giardia* antigen and no cross reactions were recorded with samples of animals or patients infected with other parasites e.g., *E. histolytica*, *E. coli* and *Blastocyste* sp. **(Table 3).**

Production and Purification of Polyclonal Antibodies: Test blood samples were withdrawn from New Zealand white rabbit before the injection of each immunizing dose. They were tested for the presence of specific anti-*Giardia* antibodies (Abs) by indirect ELISA. An increasing antibody level started one wk after the first booster dose. Three days after the 2nd booster dose, immune sera gave a high titre against *Giardia* antigen with OD of 1.297 **(Fig. 2).**

The total protein content of crude rabbit serum containing anti- *G. lamblia* pab was 11 mg/ml. The yield of purified anti-*Giardia* IgG pAb following each purification step was determined by the assessment of protein content. Using the 50% ammonium sulfate precipitation method **(33),** the protein content was 6.2 mg/ml, while following 7% caprylic acid precipitation method **(34)**, the content dropped to 3.5 mg/ml.

Characterization and reactivity of anti-*G. lamblia* IgGpAb: The purity of anti-*G. lamblia* IgG pAb after each steps of purification was assayed by 12.5%SDS-PAGE under reducing condition. The purified pAb IgG was represented by H- and L-chain bands at 50 and 31 kDa respectively. The pAb appears free from other proteins **(Fig.3)**. Reactivity of anti-*Giardia* antibodies against *Giardia* antigen and other parasitic antigens (*E. histolytica*, *E. coli* and *Blastocyste*) was determined by indirect ELISA. The produced anti-*Giardia* antibody IgG pAb diluted 1/100 in PBS/T buffer gave strong reactivity to *Giardia* antigen. The OD readings at 492 nm for *Giardia* were 1.14 compared to 0.223, 0.217, and 0.170 for *E. histolytica*, *E. coli* and *Blastocyste* sp., respectively (**Table4**).

Conjugation of Purified rabbit anti-*G. lamblia* IgGpAb: Seven mg of rabbit anti-*G. lamblia* IgGpAb was conjugated with HRP. IgG antibody was assessed against PGA in ELISA assay. 1/250 µg/ml of the conjugate gave the highest OD reading against PGA **(Fig. 4,5).**

Study population

Parasitological examination in patients: According to stool analysis by direct smear 18 from 25 patients were positive with *G. lamblia* cyst, while by MIFC method 21 were positive. 20 normal and 30 patients infected with other parasites (17 *E. histolytica*, 8 *Blastocyste* sp. and 5 *E. coli*) **(Table 5).** Both direct smear and MIFC methods gave 100% specificity and positive predictive value (PPV), yet the MIFC method recorded higher sensitivity (84%) and negative predictive value (NPV) (92.59%) than those of direct smear 72% and 87.71%, respectively. (**Table 6).**

Sandwich ELISA for detection of *G. lamblia* antigen in stool of patients: Table (7 and 8) show the results of OD value of human stool samples group. The cut off values for positivity was 0.27. The OD values of *G. lamblia* infected group (0.993± 0.203) were significantly higher than both the healthy control group (0.212 ± 0.029) and the other parasites groups (0.132 ± 0.048). Three out of 25 *G. lamblia* infected patient showed false negative results and the sensitivity of the assay was 88%. All healthy control group were below the cut off value while 4 out of 30 patient infected of other parasites were above the cut off value recording a 92% specificity. PPV and NPV were 84.61% and 93.87%, respectively.

Detection of *G. lamblia* antigen in human stool using immunomagnetic bead ELISA with paramagnetic nanoparticals: Table 9 shows the results of OD value of the human stool samples

group. The cut off values for positivity was 0.23. The OD values of *G. lamblia* infected group (1.103± 0.172) were significantly higher than both the negative control group (0.199 ± 0.02) and the other parasites groups (0.207 ± 0.07). Two out of 25 *Giardia* infected patient showed false negative results and the sensitivity of the assay was 92%. All healthy control group were below the cut off value while 3 out of 30 patient infected of other parasites were above the cut off value recording 94% specificity. PPV and NPV were 88.46% and 95.91%, respectively **(Table10).**

DISCUSSION

G. lamblia is considered to be one of the most common intestinal protozoan parasites, about 200 million people in Asia, Africa and Latin America experience symptomatic giardiasis **(40)** with 280 million infections per year **(41)**. Faeco-oral transmission cycle of *Giardia* is the major route of transmission of giardiasis **(42).**

Young children and toddlers are the most susceptible population to the infection, as the highest case rate of symptomatic giardiasis was recorded from 0–4 years' group of age. Giardiasis is frequently reported in immigrants and returning travellers from endemic regions **(43,44).** This parasite has been included in the "Neglected Diseases Initiative"of the World Health Organization WHO because of its diffusion in these regions of the world **(45-47,41,11).** *Giardia* is found to be the most frequent enteroparasite in coprositological surveys presented in developing and developed countries **(48).**

This study was conducted on 25 *G. lamblia* infected patients in addition to 30 other parasites infected patients and 20 healthy controls. Parasitological examinations by both direct smear and MIFC gave 100% specificity and positive predictive value (PPV). The MIFC method showed a sensitivity of 84% and NPV was 92.5%, whereas, direct smear method achieved sensitivity of 72 % and NPV was 87.7%. MIFC showed higher results than direct smear. This was in agreement with **(49).**

In the current study, several steps were carried out for *Giardia* antigen detection including the preparation of *Giardia* antigen, production of polyclonal antibodies, purification and labeling of rabbit anti-*G. lamblia* IgG pAb. Stool samples were collected to obtain, purify and analyze *Giardia* antigen. Antigen purification from stool samples was done by using Parasep (Midi Faecal Parasite Concentrator), According to **(50)** and in agreement with **(26).** Then the total protein content of *G. lamblia* antigens was estimated, the crude antigen obtained from positive *Giardia* stool samples found to be 8 mg/ml as measured by Bio-Rad Protein assay while it was 4.5 mg/ml after purification by Parasep. By using 12.5% SDS-PAGE technique under reducing condition, the PGA showed four major bands at 47.5, 17.0, 14.0 and 12.5 kDa. The antigenicity of the crude antigen was tested by indirect ELISA.

Giardia antigen was used for immunization of rabbit for preparation of rabbit anti-*G. lamblia* IgG pAb according to **(51),** with complete and incomplete Freund's adjuvants for immunization of rabbits. Adjuvants are usually used in animals' immunization protocols for many reasons. They can provide a depot for the immunogens at the site of injection allowing for slow, prolonged release of the immunogen in the animal and more important, they provide a mean of enhancing the immune response to the antigen. This was in agreement with **(52).**

The purification steps followed in this study were satisfactory, for IgG pAb two purification methods undertaken, ammonium sulfate precipitation and 7% caprylic acid according to **(53)**. The purity of IgG pAb was assayed by 12.5% SDS-PAGE and the purified IgG pAb was represented by H- and L-chain bands at 50 and 31 kDa respectively, indicating that, the purified pAb appears free from other proteins.

The yield of pAb as protein content by these methods was 3.5 mg/ml IgG from starting protein content of 11 mg/ml. these yields were reasonable in comparison with immunoglobulin purified yield from any biological fluid following similar purification procedure **(54,55).**

The reactivity of the purified anti-*G. lamblia* IgGpAb against *Giardia* and other parasites antigens (*E. histolytica*, *E. coli* and *Blastocyste* sp.) was determined by indirect ELISA. The purified pAb was further used as a primary capture to coat ELISA plates. The secondary capture of pAb was by Horse-Raddish Peroxidase enzyme (HRP) conjugation.

In the present study, the purified anti-*G. lamblia* IgG pAb was labeled with HRP according to (**36).** The optimum dilution of purified IgG pAb as a coating layer was 1/25 where as a peroxidase conjugated layer was 1/250. This was in agreement with **(52)** and also with **(16).**

This study demonstrated comparison between the ordinary parasitological methods and both types of ELISA, immunomagnetic bead ELISA and the traditional sandwich ELISA.

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 faecal samples **(41,56). (57,58,59)** who supposed that ELISA is a practical, simple and highly specific diagnostic test for detection of *Giardia* antigens. They stated that ELISA technique can be used instead of conventional microscopic techniques, and it does not require the observation of intact organism. In the current study, sandwich ELISA was adopted using pAbs against *Giardia* antigen, anti- *G. lamblia* IgG pAb and peroxidase-conjugated IgG polyclonal antibodies.

The cut-off value for positivity in sandwich ELISA for *Giardia* antigen was equal to 0.27. All values equal to or above these cut-off values were considered positive. On detection of *G. lamblia* antigen by sandwich ELISA in human stool samples, 22 out of 25 giardiasis cases gave positive results with 88% sensitivity and 92% specifity, but PPV and NPV were 84.61%, 93.87% respectively.

The above results were in agreement with **(60)** who used ELISA in the detection of *G. lamblia* antigen in stool specimens. Another study of **(61)**, ELISA has detected higher number of positive samples than conventional microscopy, and when several samples are needed to be examined, microscopy appears to be more time-saving.

The present study aimed at developing pAb-based antigen detection assay through paramagnetic nanoparticles conjugation in order to increase sensitivity of antigen detection assays, hence early and light *Giardia* infections could be easily detected.

The current work has demonstrated that the immunemagnetic beads ELISA conferred a higher sensitivity (92%) in detecting *G. lamblia* in stool samples compared with sandwich ELISA 88%. Also, the nano-sandwich ELISA gave higher specificity, PPV and NPV 94%, 88.64 % and 95.91 %, respectively.

23 out of 25 *G. lamblia* infected patients showed positive results. All the 20 healthy control showed negative results and the sensitivity in healthy control was 100% while 27 out of 30 of other parasites group were found to be below cut-off value, giving an overall specificity 92%. Similar findings were obtained by **(49).**

In conclusion, immunemagnetic bead ELISA with paramagnetic nanoparticles appear to be sufficiently sensitive assays for the detection of human giardiasis than sandwich ELISA.

Group (no. of samples)	**Positive cases for *Giardia***		**Negative cases for *Giardia***	
	Direct smear	**MIFC method**	**Direct smear**	**MIFC method**
Collected stool samples (n=77)	18	21	59	56

Table (1): Parasitological detection of *G. lamblia* cysts in stool samples.

Stool samples	Heavy infection	Moderate infection	Light infection
No. of samples	10	8	7
No. of cyst per field	9-12 cysts	5- 7 cysts	1-3 cysts

Table (2): Shows heavy, moderate and light stool samples.

Stool samples	**OD readings at 492 nm ± SD**
Giadia lambila	**0.99 ± 0.102**
Entamebea histolytica	**0.101 ± 0.011**
Entamebea coli	**0.211 ± 0.032**
***Blastocyste* sp.**	**0.159 ± 0.029**

Table 3: Reactivity of purified target antigen by indirect ELISA.

Parasitic antigen	**OD readings at 492 nm**
Giadia lambila	**1.14**
Entamebea histolytica	**0.223**
Entamebea coli	**0.217**
***Blastocyste* sp.**	**0.170**

Table 4: Reactivity of rabbit anti-*Giardia* antibodies against different parasitic antigens by indirect ELISA (OD reading= 492 nm). OD= optical density; SD= standard deviation.

Groups (no. of samples)	Positive cases		Negative cases	
	Direct smear	MIFC method	Direct smear	MIFC method
Healthy control (n= 20)	__	__	20	20
G. lamblia (n= 25)	18	21	7	4
E. histolytica (n= 17)	__	__	17	17
Blastocyste sp. (n= 8)	__	__	8	8
E. coli (n= 5)	__	__	5	5

Table (5): Parasitological detection of *G. lamblia* in stool samples of infected human.

Detection method	Sensitivity	Specificity	+ve predictive value(PPV)	-ve predictive value(NPV)
Direct smear	72%	100%	100%	87.71%
MIFC method	84%	100%	100%	92.59%

Table (6): The sensitivity, specificity, PPV and NPV of direct smear and MIFC method.

Group (no. of samples)	Positive cases		Negative cases	
	No.	X± SD	No.	X± SD
Healthy control (n= 20)	__		20	0.212 ± 0.029
G. lamblia (n= 25)	22	0.993± 0.203	3**	0.192± 0.030
E. histolytica (n= 17)	*2	0.49 ± 0.143	15	0.132 ± 0.048
E. coli (n= 5)	__	__	5	0.079 ± 0.038
Blastocyste sp. (n= 8)	2	0.523 ± 0.032	6	0.163 ± 0.031

Table 7: Detection of *G. lamblia* antigen in stool of human subjects infected with *Giardia* or other parasites in comparison to healthy control. X= mean; SD=standard deviation. * False +ve results, ** False –ve results.

Giardia antigen detected in stool samples	Sensitivity	Specificity	PPV	NPV
	88%	92%	84.61%	93.87%

Table 8: Sensitivity, specificity, PPV and NPV of sandwich ELISA.

Group (no. of samples)	Positive cases		Negative cases	
	No.	X± SD	No.	X± SD
Healthy control (n= 20)	__		20	0.199 ± 0.02
G. lamblia (n= 25)	23	1.103± 0.172	2	0.209 ± 0.06
E. histolytica (n= 17)	2	0.709 ± 0.211	15	0.207 ± 0.07
E. coli (n= 5)	1	__	4	0.138 ± 0.01
Blastocyste sp. (n= 8)	---	0.523 ± 0.032	8	0.159 ± 0.05

Table 9: Detection of *Giardia* antigen in stool samples of infected human using immunomagnetic bead ELISA.

Giardia antigen detected in stool samples	Sensitivity	Specificity	PPV	NPV
	92%	94%	88.46%	95.91%

Table 10: Sensitivity, specificity, PPV and NPV of immunomagnetic bead ELISA with paramagnetic nanoparticles.

Giardia antigen detected in stool samples		Sensitivity	Specificity	PPV	NPV
Parasitology	Direct smear	72%	100%	100%	87.71%
	MIFC method	84%	100%	100%	92.59%
Sandwich ELISA		88%	92%	84.61%	93.87%
Sandwich ELISA with paramagnetic nanoparticles		92%	94%	88.46%	95.91%

Table (11): Comparison between data resulted from parasitological examination, sandwich ELISA and immunomagnetic bead ELISA with paramagnetic nanoparticles.

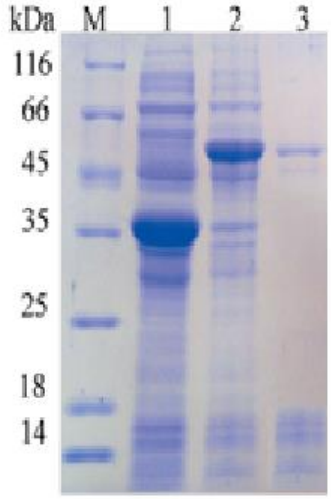

Fig. 1: 12.5% SDS-PAGE of target antigen before and after purification (stained with Coomassie Blue).
Lane M: Molecular weight of standard protein.
Lane 1: Crude *Giardia* antigen.
Lane 2: Crude *Giardia* antigen
Lane 3: Purified target antigen from Parasep.

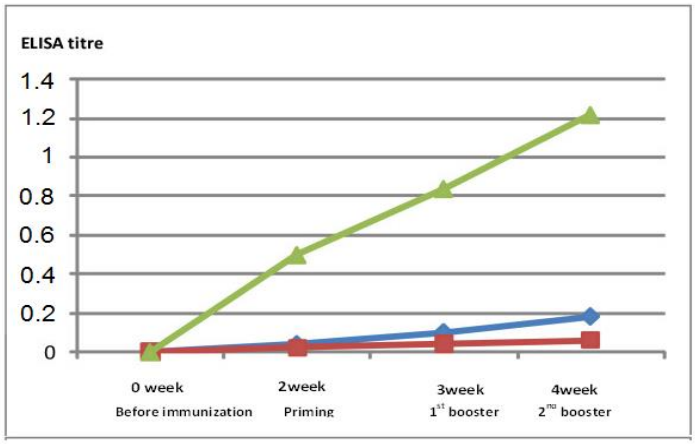

Fig. 2: Reactivity of raised rabbit anti-*Giardia* antibodies IgG pAb against *Giardia* antigen by indirect ELISA.

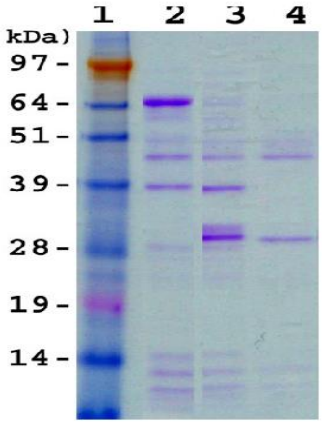

Fig. 3: 12.5% SDS-PAGE of anti- *G. lamblia* IgG pAb before and after purification (stained with Coomassie Blue).
Lane 1: Molecular weight of standard protein; **Lane 2**: Anti- *G. lamblia* IgG pAb before purification. **Lane 3**: Purified pAb IgG after 50% ammonium sulfate treatment; **Lane 4**: Purified pAb IgG after 7% caprylic acid treatment.

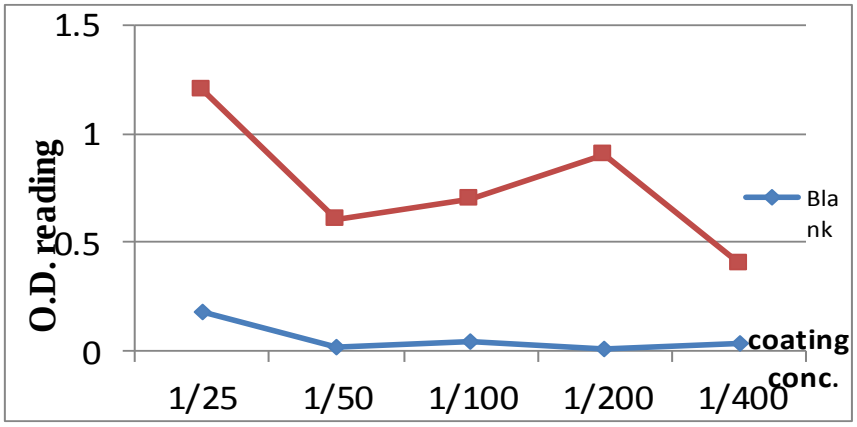

Fig. 4: Determination of the optimum concentration of purified anti-*G. lamblia* IgG pAb as a coating layer in sandwich ELISA.

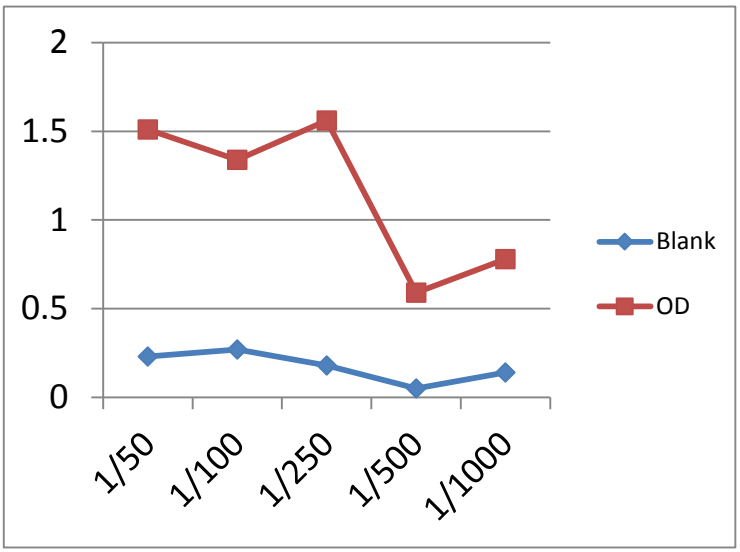

Fig. 5: The titration curve of conjugate conc. versus the O.D reading

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