Diagnostic Potential of Target *Giardia lamblia* Specific Antigen for Detection of Human Giardiasis Using Coproantigen Sandwich ELISA

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**Abstract:** Giardiasis is endemic in all regions of the world. *Giardia lamblia* (*G. lamblia*) cysts are spread in Egypt via the fecal-oral route, through ingestion of the cyst with contaminated food or water. The symptoms of giardiasis vary from the asymptomatic passage of cysts to chronic diarrhea, malabsorption and weight loss. Although microscopy has the advantage of low cost and ability to simultaneously detect other gastrointestinal parasite, the limitation of this method is that *G. lamblia* cysts are small and similar to many pseudoparasites. This study aimed to verify the advantages and disadvantages of enzyme-linked immunosassaying versus microscopy for diagnosing *G. lamblia* in human. The 65 kDa glycoprotein *Giardia* specific antigen (*GSA*-65 kDa) is considered to be an antigen of interest. It is shared by cysts and trophozoites of *G. lamblia* as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis and immunoblotting and very specific to this parasite. Sandwich ELISA was more sensitive (95.12%) than ordinary parasitological diagnosis (78.05% for direct smear or 85.36% for MIFC method). Also, the NPV of sandwich ELISA (95.12%) is higher than that of parasitological procedure (82.35% for direct smear or 87.5% for MIFC method). On the other hand, the specificity (92.85%) and PPV (92.85%) of sandwich ELISA is lesser than that of parasitological procedure (100% and 100%, respectively). Sandwich ELISA, using purified anti-GSA-65 kDa IgG pAb, is recommended as a diagnostic test for *Giardia spp.* diagnosis more than ordinary microscopy methods.

**Key words:** *Giardia lamblia* · Diagnosis · Coproantigen · Sandwich ELISA

**INTRODUCTION**

The World Health Organization (WHO) ranks diarrheal diseases as the second most common cause of morbidity and mortality in children in the developing world [1]. Giardiasis is a diarrheal illness infects the small intestine and caused by a small flagellate protozoan called *G. lamblia*. It is the most common nonviral nonbacterial cause of diarrhea worldwide with a prevalence range from 2-7% in developed countries and increased to 20-30% in most developing countries [2]. The most trusted diagnostic test is direct examination of feces or intestinal tissue samples for cysts or trophozoites of parasite [3]. Although microscopy has the advantage of low cost and ability to simultaneously detect other gastrointestinal parasite, the limitation of this method is that *G. lamblia* cysts are small and similar in appearance to many pseudoparasites such as yeast. Also, the trophozoites break up rapidly in the stool, so cannot be used to measure the severity of infection. Other limitation is that its sensitivity is quite low because *Giardia* has periodic expulsion in alternative days or various hours of day and its quantity will be decreased when the disease becomes chronic [4].

Direct immunofluorescence microscopy using monoclonal antibody to detect giardia antigen offer greater sensitivity compared to light microscopy, but they are not available in all parasitology laboratories due to the high cost and limited access to the required equipments. Recently, enzyme-linked immunosorbent assay (ELISA) has been considered as cost effective diagnostic method which can detect small quantities of coproantigens of parasite even in mild infections and if the live parasite itself is absent in the fecal samples [4-6]. It can detect
different soluble antigens dispersed in fecal matter rather than detecting cysts, trophozoites, or antigens on the surfaces of these morphologic forms.

Most studies have approached the antigenic characterization of trophozoites of *Giardia* strains. Studies conducted in order to characterize the cysts have expanded our knowledge of the antigenic composition of the parasite and have permitted the identification of specific antigens for the immunologic diagnosis of giardiasis. Specific antigens present in the cysts such as proteins with a molecular weight 66-103 kDa are recognized by antibodies produced in rabbits [7-9]. The 65 kDa glycoprotein (GSA 65) is considered the most efficient and important antigen for diagnosis of *G. lamblia*. It is shared by cysts and trophozoites of *G. duodenalis* as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis and immunoblotting and very specific to this parasite [9,10]. GSA 65 possesses the qualities of a diagnostically ideal stool antigen. (i) It is specific to *G. lamblia*. (ii) It is stable in the host gastrointestinal tract. (iii) It is stable in standard laboratory fixatives. (iv) It is present in immunologically detectable quantities [11,12]. Furthermore, GSA 65 specifically occurs in *Giardia* and is the major antigen in the feces of individuals with giardiasis. The antigen is employed in the immunodiagnosis of giardiasis and show both increase in the sensitivity and specificity of immunoenzymatic assays.

This Study aimed to verify the advantages and disadvantage of ELISA versus microscopy for diagnosing *G. lamblia*.

**MATERIALS AND METHODS**

**Animals:** 4 month old New Zealand white rabbits (~3 Kg) from Theodor Bilharz Research Institute (TBRI). Animals were free from *G. lamblia* and other parasitic infections after examination. Animals were kept at TBRI for 5 weeks.

**Preparation of Parasite Antigens:** Fecal samples were collected from heavy infected patient from the laboratory in TBRI. *G. lamblia* cysts were isolated from fecal samples according to O’Handley et al. [13]. The (GSA-65 kDa) was purified according to Sheehan and Gerald [14], in brief Giardia lamblia cysts isolated from fecal samples were concentrated using an Amicon 8400 ultrafiltration unit with membrane (3000 Da cut-off). The concentrated giardia lamblia cysts were applied to a 120 ml DEAE-Sephadex G-25-ion exchange chromatography equilibrated in 0.1 M Tris-HCl, pH 7. Fractions of 5 ml were collected, after the void volume (110 ml) had been passed. The column elute is monitored at 280 nm. Four distinct fractions were obtained. *Giardia* specific antigen (GSA-65 kDa) was collected from fraction IV, concentrated, dialyzed and its protein content was determined by using protein assay kit (Bio-Rad, Richmond, CA, USA) [15].

**Sodium dodecyl-sulfate Polyacrylamide Gel Electrophoresis (SDS PAGE):** Molecular weight was determined by SDS PAGE according to Harlow and Lane [16]; Myers [17] and Thaumaturgo et al. [18].

**Immunization of Rabbits for Production of Polyclonal Antibodies (pAb) According to Yinghai et al., [19]:** Rabbit anti-*G. lamblia* antibodies were obtained by immunization of New Zealand white rabbits with GSA-65 kDa. The rabbit received 1 mg of GSA-65 kDa, as the priming dose by intramuscular (i.m) injection at four sites. Two week later, two booster doses (1 mg / rabbit) were given at weekly intervals.

**Purification of Rabbit anti-*G. lamblia* Serum (IgGpAb):** Purification steps of rabbit anti-*G. lamblia* serum (IgGpAb) were based on ammonium sulphate precipitation method [20] and caprylic acid purification method [21]. At the end of the two purification procedures, protein content was measured according to Bradford [15].

**Labeling of Rabbit anti-*G. lamblia* Serum IgGpAb with Horseradish Peroxidase (HRP) (Periodate Method):** According to Nakane and Kawaoi [22] and Tijssen and Kurstak [23], 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 2 hr. 100 µl sodium borohydride was 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).

**Application of Rabbit anti-*G. lamblia* Serum IgGpAb Study Population:** This study was conducted on 41 *G. lamblia* infected out patients clinic and 22 patients infected with other parasites (*E. histolytica* and Blastocyst). In addition, 20 individuals were served as parasite free-healthy negative control.
**Parasitological Examination:** Faecal samples were collected in clean, wide-mouthed covered containers and examined by direct smear and merthiolate iodine formaldehyde concentration methods (MIF).

**Direct Smear:** A piece of stool was taken and emulsified in normal saline, then examined using the x10 and x40 magnification of the ordinary light microscope.

**Merthiolate-iodine-formaldehyde Concentration Technique (MIFC):** 1 gm 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 centrifuge for 5 min at 3000 g. A drop of mixed sediment was placed on a slide, covered and examined under light microscope [24] (MIF solution is a mixture of 2 solutions with ratio 4:1. Solution A composed of 0.1% merthiolate, 36-40% formaldehyde, glycerin and dist. H2O. Solution B composed of potassium iodide, iodine and dist. H2O).

**Detection of G. lamblia Antigens in Patient's Stool by Sandwich ELISA:** positive stool samples with giardia or other parasites (*E. histolytica* and Blastocyst) and free non-infected samples were individually diluted 1:3 with PBS [25,26].

The microtitration plates were coated with 100 µl/well of purified anti-*G. lamblia* IgGpAb, (1/100 for IgG in carbonate buffer 0.06 M pH 9.6) and incubated overnight at room temperature. Plates were washed 3 times with 0.1 M PBS/T (pH 7.4). The remaining sites in the wells were blocked by 200 µl/well of 0.1% BSA/PBS/T and incubated for 2 hr at 37°C and were washed trice with PBS/T. 100µl of serum samples was pipetted into each well in duplicate and incubated for 2 hr at 37°C and then wells were washed 3 times. 100µl/well of peroxidase-conjugated anti-*G. lamblia* IgGpAb diluted 1/50 was then added and incubated for 1 hr at room temperature. The assay was completed according to De Jonge et al. [25] and Qiu et al. [26].

**Statistical Analysis:** The data are presented as mean ± standard deviation of mean (X ± SD). Correlation coefficient (r) was used to find the relation between the ELISA O.D and parasitological data according to Snedecor and Cochran (1981) [27]. The data were considered significant if P values were equal to or less than 0.05. Statistical analysis was performed with the aid of the SPSS computer program (version 6.0 windows).

**RESULTS**

**Purification of G. lamblia GSA-65 kDa by DEAE-Sephadex G-25-ion Exchange Chromatography:** The eluted antigen was represented by a single peak with maximum OD value equal to 0.695 at fraction number (Fig.1). The eluted protein fractions resulted from the purification method was analyzed by 12.5% SDS-PAGE under reducing condition and showed only one band at 65 kDa which representing GSA-65 kDa (Fig. 2).

**Estimation of Total Protein Content of G. lamblia Antigens:** The crude antigen obtained from positive *G. lamblia* stool samples contains 7mg/ml of total protein as measured by Bio-Rad Protein assay while it was 3.5mg/ml after purified by DEAE-sephadex G-25 ion-exchange chromatography.

**Reactivity of Target Antigen by Indirect ELISA:** The antigenicity of the purified antigen was characterized by indirect ELISA. Stool samples from *G. lamblia* infected patients gave a strong reaction against GSA-65 kDa with mean OD reading equal to 1.309 and no cross reactions were recorded with sample of animals or patients infected with other parasites e.g., *E. histolytica* and blastocyste.

**Reactivity and Specificity of anti-*G. lamblia* Serum:** IgGpAb against GSA-65 kDa rabbit blood samples were withdrawn before each immunizing dose and tested for the presence of specific anti-*G. lamblia* antibodies by indirect ELISA. An increasing antibody level started 1 wk after the 1st booster dose (Fig. 3). Three days after the 2nd booster dose, immune sera gave a high titer against GSA-65 kDa with the highest optical density at 1/100 dilution.

**Specificity of Anti-*G. lamblia* Serum IgGpAb against GSA-65 kDa:** Reactivity of anti-*G. lamblia* pAb against GSA-65 kDa and other parasite antigens (*E. histolytica* and Blastocyste) was determined by indirect ELISA. Anti-*G. lamblia* IgGpAb diluted 1/100 gave a strong reactivity to 65KDa GSA. The OD readings at 492 nm, for *G. lamblia*, was 2.84 compared to 0.462 and 0.281 for *E. histolytica* and Blastocyste, respectively.

**Purification of Rabbit Anti-*G. lamblia* Serum IgGpAb:** 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.
Fig. 1: Elute profile of 65kDa GSA on DEAE sephadex G-25

Fig. 2: SDS-PAGE of target antigen eluted from ion exchange chromatography column

Fig. 3: Reactivity of raised rabbit anti-Giardia antibodies (diluted 1/100) against 65kDa GSA by indirect ELISA.

The purity of IgG after each steps of purification was assayed by 12.5% SDS-PAGE under reducing condition. The purified IgGpAb was represented by H-and L-chain band at 53 and 31 kDa, respectively (Fig. 4). The pAb appears free from other proteins.

Table 1: Reactivity of purified target antigen by indirect ELISA

<table>
<thead>
<tr>
<th>Parasitic antigen</th>
<th>OD readings at 492 nm ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. lambila</td>
<td>(1.309 ± 0.342)</td>
</tr>
<tr>
<td>E. histolytica</td>
<td>(0.264 ± 0.201)</td>
</tr>
<tr>
<td>Blastocyste</td>
<td>(0.182 ± 0.082)</td>
</tr>
</tbody>
</table>

* OD= optical density, SD= standard deviation.

Table 2: Reactivity of rabbit anti-Giardia antibodies against many parasitic antigens by indirect ELISA (OD reading= 492 nm)

<table>
<thead>
<tr>
<th>Parasitic antigen</th>
<th>OD readings at 492 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. lambila</td>
<td>2.84</td>
</tr>
<tr>
<td>E. histolytica</td>
<td>0.462</td>
</tr>
<tr>
<td>Blastocyste</td>
<td>0.281</td>
</tr>
</tbody>
</table>

* OD= optical density

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 65 kDa GSA in ELISA assay. 1/50 µg/ml of the conjugate gave the highest OD reading against GSA-65 kDa (Fig. 5).
Table 3: Detection of *G. lamblia* cyst in stool samples of infected human

<table>
<thead>
<tr>
<th>Groups</th>
<th>Positive cases</th>
<th>Negative cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Direct smear</td>
<td>MIFC method</td>
</tr>
<tr>
<td>Healthy control (n= 20)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Giardia (n= 41)</td>
<td>32</td>
<td>35</td>
</tr>
<tr>
<td>Entameaba Histolytica (n= 17)</td>
<td>--</td>
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</tr>
<tr>
<td>Blastocyst (n= 5)</td>
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</tbody>
</table>

Table 4: Key features of parasitological tests

<table>
<thead>
<tr>
<th>Detection method</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct smear</td>
<td>78.05 %</td>
<td>100 %</td>
<td>100 %</td>
<td>82.35%</td>
</tr>
<tr>
<td>MIFC method</td>
<td>85.36 %</td>
<td>100 %</td>
<td>100 %</td>
<td>87.5 %</td>
</tr>
</tbody>
</table>

PPV: Positive Predictive Value, NPV: Negative Predictive Value.

Table 5: Detection of GSA-65 kDa in stool samples of infected human

<table>
<thead>
<tr>
<th>Groups</th>
<th>Positive cases</th>
<th>Negative cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. X± SD</td>
<td>No. X± SD</td>
</tr>
<tr>
<td>Healthy control (n= 20)</td>
<td>--</td>
<td>20 0.29 ± 0.07</td>
</tr>
<tr>
<td>Giardia (n= 41)</td>
<td>39 1.98 ± 0.35</td>
<td>2 0.36± 0.032</td>
</tr>
<tr>
<td>Entameaba Histolytica (n= 17)</td>
<td>3 0.51 ± 0.14</td>
<td>14 0.31 ± 0.10</td>
</tr>
<tr>
<td>Blastocyst (n= 5)</td>
<td>--</td>
<td>5 0.27 ± 0.16</td>
</tr>
</tbody>
</table>

Study Population

Parasitological Examination: According to stool analysis by direct smear, 32 patients from 41 patients were positive with *G. lamblia* cyst while, by MIFC method 35 patients were positive. 20 normal and 22 patients infected with other parasites (17 *E. histolytica* and 5 Blastocyst) all are negative by both methods (Table 3).

Both direct smear and MIFC methods gave 100% specificity and positive predictive value (PPV), while the sensitivity of MIFC method (85.36%) was higher than that of direct smear (78.05%). Again, MIFC method recorded high negative predictive value (NPV) (87.5%) than direct smear (82.35%) (Table 4).

Standardization of Sandwich ELISA Used for Detection of *G. lamblia* Antigen: The optimum concentration of coating anti-*G. lamblia* IgGpAb displays in the highest OD reading. It was found to be 1/100 (Fig.6).

Application of sandwich ELISA for detection of *G. lamblia* Antigen (GSA-65 kDa) in Stool of Infected Human: In order to measure the incidence of positivity for *G. lamblia* in stool samples. Table (5) shows the results of 65 kDa GSA detection among different studied groups. Cut off value for positivity was 0.428 as mean±2 SD. The OD values of *G. lamblia* infected group (1.98 ± 0.35) was significantly higher than both the healthy control group (0.29 ± 0.07) and other parasite groups (0.31 ± 0.10 and 0.27 ± 0.16).

Two out of 41 *G. lamblia* infected samples showed false negative results and the sensitivity of the assay was 95.12%.

All the 20 negative controls were below the cut off value while 3 out of 22 of other parasites groups were at the border line of the cut off value giving 92.85 % specificity. The PPV and NPV were 92.85 % and 95.12 %, respectively (Table 6).
Table 6: Key features of ELISA test

<table>
<thead>
<tr>
<th>Detection method</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td>95.12 %</td>
<td>92.85 %</td>
<td>92.85 %</td>
<td>95.12 %</td>
</tr>
</tbody>
</table>

Table 7: Summarizes the sensitivity, specificity, PPV and NPV of parasitological and sandwich ELISA results used for detection of GSA-65 kDa in stool samples of human

<table>
<thead>
<tr>
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<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
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<tr>
<td>Parasitological</td>
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<td>85.36 %</td>
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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 [28], with 280 million infections per year [29]. 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 [30]. 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 [29-31].

The diagnosis of giardiasis is frequently based on microscopic examination of stool samples by visualizing the organism, either the trophozoites or the cysts [30]. Diagnosis via microscopical examination of a single stool specimen has a low sensitivity and may therefore miss up to 50% of *Giardia* infections [30,33,34], particularly if there is low parasite density, insufficient microscopic quality, intermittent excretion of cysts or the probability of parasite hiding by bile pigments [29,35]. Microscopic examination of three consecutive stool specimens is required to reach a sensitivity of over 90% [30, 34].

Given these difficulties the development of sensitive, cost-effective and rapid diagnostic methods is of the most importance [30]. ELISA for detection of the specific antigens in stools is a rapid, sensitive and economic method to confirm infection and coproantigens of that parasite which could be traced and diagnosed even if the live parasite is absent in the fecal samples [4,6].

In the present study, stool samples were collected in order to purify and analyze GSA-65 kDa. It was purified from stool samples by DEAE sephadex G-25 ion exchange chromatography in which 65 kDa GSA appeared as a single band at 65 kDa by reducing SDS-PAGE. Then, the total protein content of crude *G. lamblia* in positive giardia stool sample was 7 mg/ml, while it was 3.5 mg/ml after purification with DEAE-sephadex G-25 ion exchange chromatography.

Recently, Barazesh *et al.* [36] introduced a rapid, simple and economical method to purify *G. lamblia* cysts from fecal samples of the patients suffering from giardiasis. They introduced and run a modified method that in fact is a mixture of various purification methods like one-and two-phase sucrose gradient isolation, percoll-sucrose gradient isolation and a modified two-phase method run by 0.85 and 1.5 M sucrose with some changes.

In our study, anti-*G. lamblia* IgGpAb was prepared by immunization schedule of rabbits with GSA-65 kDa according to Tendler *et al.* [37]. Two purification steps were processed, ammonium sulfate and 7% caprylic acid precipitation methods. The purity of IgGpAb was assayed by 12% SDS-PAGE under reducing condition. The purified anti-*G. lamblia* IgGpAb was represented by H- and L-chain bands at 53 and 31 kDa, respectively; indicating that the pAb is free from other proteins. The protein content of pAb was 3.1 mg/ml IgG refering to the crude one (12.5 mg/ml). These yields were reasonable in comparison with the other purified immunoglobulin from any biological fluid following similar purification procedures [38,39].

Then, the reactivity of the purified anti-*G. lamblia* IgGpAb against GSA-65 kDa and other parasite antigens (*E. histolytica* and *Blastocystes*) was determined. The purified HRP-labeled IgGpAb was used for the detection of 65 kDa GSA in stool (coproantigen) of infected patients by sandwich ELISA. The optimization of various reagents used in sandwich ELISA was done. The optimum dilution of purified IgGpAb as a coating layer was 1/100 where as a peroxidase conjugated layer was 1/50.
This was in agreement with Duque-Beltrán et al. [40] 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* IgGpAb 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 40 μg/ml and the optimal conjugate dilution was 1:100.

Later on, Barazesh et al. [4] purified giardia parasite from faeces of patients and injected into rabbit to extract serum pAb. Then the produced rabbit anti-*G. lamblia* IgGpAb was purified by ion-exchange chromatography and its identity was confirmed by SDS-PAGE. The purified pAb was conjugated by periodate method. This pAb was used to design direct and indirect ELISA kits to measure conjugation titer. In both ELISA methods, ODs were 1 by the specifcity was 100%.

We compared between the ordinary parasitological examination methods and sandwich ELISA in the diagnosis of giardiasis. The study was conducted on 41 *G. lamblia* infected patients, 22 other parasites infected patients and 20 healthy controls. According to parasitological examination by MIFC method, 35 from 41 patients were positive with *G. lamblia* cyst with a sensitivity of 85.36%, a specificity of 100%, a PPV of 100% and a NPV of 87.5 %. While, according to sandwich ELISA, 39 patients were positive giving higher sensitivity than parasitological examination. It had a sensitivity of 95.12%, a specificity of 92.85%, a PPV of 92.85% and a NPV of 95.12%.

Doğruman et al. [41] evaluate the value of the direct fluorescent antibody (DFA) techniques reported to have high sensitivity and specificity and the ELISA test used to determine antigens in stool samples in the routine diagnosis of *G. intestinalis*. When 44 stool samples in which *G. intestinalis* cysts and/or trophozoites had been seen during native Lugol examination were investigated, positivity detected with the trichrome staining, monoclonal ELISA and DFA methods were found to be 37 (84.0%), 39 (88.6%) and 35 (79.5%), respectively. DFA detected *Cryptosporidium parvum* cysts in addition to *G. intestinalis* in one sample. Twenty-seven (61.4%) of the samples were positive with all three methods. When compared with the DFA method, the ELISA method had a sensitivity of 88.6%, a specificity of 88.8%, a PPV of 79.5% and a NPV of 20.0% while the trichrome staining method had a sensitivity of 85.7%, a specificity of 77.8%, a PPV of 81.1% and a NPV of 22.2%. There was no statistically significant difference between the DFA and ELISA tests and between the DFA test and the trichrome staining method for diagnosing *G. intestinalis*.

Akav et al. [42] compared the results of a EIA test (to detect 65 kDa GSA) for the diagnosis of giardiasis with the microscopic examination. 280 patients with diarrhea and 60 controls were included in the study. 78 of 280 samples were positive by microscopy, whereas only 74 of 280 samples gave positive reaction by the EIA assay. EIA sensitivity exceeded 92% and its specificity was 99%.

Al-Saeed and Issa [43] used ELISA in the detection of *G. lamblia* antigen in stool specimens. Of 84 stool samples, 42 were positive and 42 were negative for *Giardia* spp. stages by using microscopic stool examination. Samples that were positive by microscopy were positive by ELISA test and 13 samples that were negative by microscopy were positive by ELISA test. The sensitivity of the ELISA test was therefore 76.4% and the specificity was 100%.

Gutiérrez-Cisneros et al. [44] assessed and compared the performance of two immunochromatographic tests for the simultaneous detection of *G. duodenalis* and *Cryptosporidium spp.* in faeces. 254 faeces samples were tested using the two immunochromatography strips Cryto-Giardia (CerTestBiotec) and Stick Crypto-Giardia (Operon). In the diagnosis of *G. duodenalis*, the sensitivity and specificity of the kits were 97% and 100%, respectively for the CerTest; and 97% and 95% for Operon. In the diagnosis of *Cryptosporidium spp.* CerTest strip rendering a sensitivity of 100%, compared to with a sensitivity of 92% using Operon.

Jelinek and Neifer [30] evaluated two EIAs assays to detect antigens in stool specimens, *Ridascreen Giardia* and *G. lamblia Serazym Giardia*. Every specimen was examined by a conventional microscopic examination (CME), PCR and tested by both EIA kits. When microscopy was used as the reference standard, the *Ridascreen Giardia* showed a sensitivity of 72.9% and a specificity of 100%. *Serazym Giardia* had a sensitivity of 93.8% and a specificity of 100%.

In conclusion, sandwich ELISA was more sensitive (95.12%) than ordinary parasitological diagnosis procedure. Also, the NPV of sandwich ELISA (95.12%) is higher than that of parasitological procedure. On the other hand, the specificity and PPV of sandwich ELISA is lesser than that of parasitological procedure. Moreover sandwich ELISA, using purified anti-65 kDa GSA IgGpAb, is recommended as a diagnostic test for *Giardia* spp. diagnosis more than ordinary microscopy methods.
REFERENCES


