Evaluation of Different Immunological Techniques for Diagnosis of
Schistosomiasis haematobium in Egypt

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Abstract: The detection of Soluble Egg Antigen (SEA) in serum and urine could be more valuable in diagnosis; hence early treatment would be applied before irreparable damage occurs. In this study, Schistosoma (S.) eggs were isolated from the intestine of infected hamsters and purified by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). The purified SEA was injected in rabbits to raise specific polyclonal antibodies (pAb) against S. haematobium. The purified pAb was further used as a primary capture to coat ELISA plates. The secondary capture of pAb was by conjugation with Horse-Radish Peroxidase (HRP). According to parasitological examination, this study included 150 S. haematobium infected patients, 50 other parasites infected patients and 30 negative control samples. Latex Agglutination Technique (LAT) was performed for both serum and urine in comparison to sandwich and dot-ELISA on 150 infected individual. Comparison was evaluated between LAT, sandwich and Dot-ELISA in serum samples, it showed 92.98 and 98.66% sensitivity and 92.50, 96.25 and 98.75% specificity, respectively, while in urine samples showed 88.66, 90.66 and 94.66% sensitivity and 91.25, 93.75 and 96.25% specificity, respectively. It was clear that, the sensitivity of LAT in urine was significantly higher than the parasitological examinations. From all data in this study and with consideration to sandwich and Dot-ELISA assays, LAT assay have an important value as an applicable, fast and accurate diagnostic technique for schistosomiasis in the field.

Key words: S. haematobium, sandwich ELISA, Dot-ELISA, LAT, diagnosis

INTRODUCTION

Schistosomiasis is one of the most prevalent parasitic diseases in the world, second behind malaria (WHO, 2005). It affects 207 million of the world’s poorest people through 74 countries in several parts of the world (King, 2009). 85% of them live in sub-Saharan Africa (Chitsulo et al., 2004). It is estimated that schistosomiasis causes about 200,000 deaths per year. There are five main Schistosoma species (S. sp.) that affect humans which are: S. haematobium, S. mansoni, S. japonicum, S. mekongi and S. intercalatum (Chitsulo et al., 2000, Chitsulo et al., 2004).

During schistosome infection, many of the eggs laid by the female worms become trapped in the tissues. The liver is particularly affected in S. mansoni and S. japonicum infections, while, the bladder and ureters are the main organs of egg deposition by S. haematobium worms. As the major factor in the pathogenesis of schistosomiasis is the host granulomatous response to antigens secreted from the trapped eggs in host tissues (Pearce, 2005). Furthermore, early diagnosis is not possible because eggs are not found in feces and urine until flukes reach maturity (Armour et al., 1997).

Despite advances in control via snail eradication and large-scale chemotherapy, the level of incidence has shown no significant decrease and continues to spread to new geographic areas particularly in sub-Saharan Africa (Patz et al., 2000; Siddiquis et al., 2005). So, early diagnosis is necessary for prompt treatment before irreparable damage to the liver occur (Hillyer et al., 1992). Schistosomiasis was diagnosed by many ways or methods as parasitological methods such as microscopic detection of eggs (Van Lieshout et al., 2000). But these methods, however, are labor-intensive, time consuming and somewhat messy due to low worm burden and/or high day to day fluctuation in egg counts (Corachan, 2002).

Several immunological tests using crude or purified egg and adult worm antigens have been developed in the last decades to detect anti-S. haematobium antibodies (Chen and Mott, 1989; Feldmeier, 1993). Therefore, several immunodiagnostic methods have been developed for the diagnosis of light infections, which developed on either

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detection of antibodies specific to schistosome antigens or the presence of schistosome Circulating Antigens (CSA) in patients’ serum or urine (Salah et al., 2006). Commonly used assays is Enzyme Linked Immunosorbent Assay (ELISA) (Whitty et al., 2000; Amorosa et al., 2005), Western Blotting (WB), or immunofluorescence (Thors et al., 2006). Although ELISA typically is a laboratory-based tool useful for large-scale operations. Its application in the field is difficult (Xue et al., 1993).

The Latex Agglutination Test (LAT) is one of the simplest agglutination tests available in a diagnostic parasitology laboratory. LAT has been used to detect antibodies in a variety of parasitic diseases such as visceral leishmaniasis (Arya, 1997; Bagchi et al., 1998), toxoplasmosis (Mazumder et al., 1988), Schistosomiasis japonicum (Wang et al., 2006) and Echinococcus granulosus (Barbieri et al., 1993). Ibrahim et al. (2010) used LAT in detecting CSA in urine and serum samples of S. mansoni infected patients, the sensitivity was 90 and 87.1% and specificity of the assay was 88.7 and 93.5%, respectively.

This study aimed at the development of pAb-based LAT as a simple, rapid and field applicable screening test for SEA in serum and urine samples of human Schistosoma haematobium.

**MATERIALS AND METHODS**

**Animals:** New Zealand white male rabbits, weighing approximately 1.5 kg and about 1.5 months age, were examined before the experiments (free from Schistosoma and other parasitic infections) and maintained at the Schistosome Biological Supply Program, Theodor Bilharz Research Institute, Giza, Egypt (SBSP/TBRI). They were kept under standard laboratory care (at 21°C, 45-55% humidity), filtered drinking water, 24% protein and 4% fat diet. Animal experiments have been carried out according to the internationally valid guidelines and ethical conditions (Nessim et al., 2000).

**Patients’ samples:** This study was conducted on 230 individuals from highly endemic areas in Fayoum Governorate and from out patients clinic and hospital at TBRI and El-kaser El-Aine University Hospital. By parasitological examination (urine analysis) they were divided into 150 S. haematobium infected patients with the mean age±SE was 38±11.7 years, 50 infected with other parasites (S. mansoni, Fasciola, Echinococcus, Ankylostoma and Ascaris) with mean age±SE equal to 28±10.1 years, in addition, 30 individuals of the medical staff at TBRI served as parasite free-healthy negative control with mean age±SE as 33±9.9 years. Urine and blood samples were collected from all cases and sera were separated, aliquoted and kept at -70°C until used.

According to the intensity of infection, S. haematobium infected group (the number of ova count/10 mL urine) was subdivided into light, moderate and high infection using Neucleopore technique.

**Light infection:** Included 50 patients with egg count ranging from 10-90 egg/10 mL urine with mean of 54.1±20.8.

**Moderate infection:** Included 30 patients with egg count ranging from 100-400 egg/10 mL urine with mean of 209.5±79.3.

**High infection:** Included 70 patients with egg count ranging from 200-1000 egg/10 mL urine with mean of 738.5±176.2.

**Antigen preparation:** Viable S. haematobium adult worms were purchased from the SBSP/TBRI. S. haematobium (Egyptian strain) SEA was prepared as previously described by Deelder et al. (1976) and used for ELISA standard curves. Antigen was identified by 12% SDS-PAGE (1 mm) under reducing condition according to Bio-Rad Lab. Model 595, Richmond, CA, USA manufacturer.

**Reactivity and specificity of S. haematobium SEA by indirect ELISA:** ELISA test based on the original method of Engvall and Perlmann (1971) was used with some modifications. Wells of polystyrene microtiter plates (Costar, Corporate Headquarters, Cambridge, MA, USA) were coated with 1 µg/well of S. haematobium SEA in coating buffer (carbonate-bicarbonate buffer), then incubated overnight at 4°C. Plates were washed 5 times with the washing buffer, blocked by dispensing 200 µL/well of 1% Bovine Serum Albumin (BSA) in PBS and left for 1 h at room temperature. Following washing the wells 5 times, 100 µL/well labeled primary antibody diluted in the washing buffer was added and then incubated for 1 h at room temperature. Then, the plates were washed 5 times with the washing buffer and 100 µL/well of the diluted conjugate (secondary antibody) was dispensed with incubation for 1 h at 37°C. After 5 times washing, 100 µL of the freshly prepared substrate solution was added in each well till color appearance. After that, stopping buffer 50 µL/well was added to stop the over enzyme-substrate reaction. The absorbance was measured at 492 nm in case of peroxidase conjugates or 405 nm in case of alkaline phosphatase conjugates.

**Production and purification of polyclonal antibody (pAb):** One mg of S. haematobium SEA product was mixed with an equal volume of Complete Freund's Adjuvant (CFA) and injected intramuscularly (i.m.) into each of 2 rabbits
according to Guobadia and Fagbemi, 1997. Booster doses [0.5 mg mixed with an equal vol. of incomplete FA (IFA)] were i.m. administered at week (wk) 2, 3 and 4 after the initial dose according to Fagbemi and Guobadia (1995). Blood samples were examined from the rabbit's ear before injection and before each boosting injection to detect the titer of antibodies produced. When the titer became high (~4 days post last injection), the animals were sacrificed and blood samples were collected. Antisera were pooled and heat-inactivated then stored as aliquots at -20°C till used (Pelley and Hillyer, 1978). Proteins in solutions form hydrogen bonds with water which increase its solubility, so ammonium sulfate precipitation methods was used to remove these water molecules (Harlow et al., 1988). The gamma protein was further purified from serum proteins (IgG) by caprylic acid treatment (Mckimney and Parkinson, 1987; Sheehan and Fitz Gerald, 1996). Protein content was estimated after each purification according to Bradford (1976). The purity of the produced IgG was identified by 12% SDS-PAGE (1 mm) under reducing conditions (Laemmli, 1970).

Testing for reactivity and specificity of pAb to *S. haematobium* SEA by indirect ELISA: As described above, microtiter plate was coated overnight at 4°C with 30 µg ml⁻¹ SEA in carbonate coating buffer, blocked with 0.1% BSA in PBS then 100 µl/well of serially diluted pAb (1:50 to 1-3200) in washing buffer was added. Hundred µl/well of anti-rabbit IgG peroxidase conjugate (Sigma) diluted in washing buffer (1/1000) was dispensed. Fifty µl/well of 8N H₂SO₄ was added to stop the enzyme substrate reaction. The absorbance was measured at 492 nm using ELISA reader (Bio-Rad microplate reader, Richmond, Ca). After each step, there were washing 5 times and the incubation was 1 h at 4°C.

Reactivity of *S. haematobium* pAb SEA in sera and urine by Sandwich ELISA: Labeling of pAb with HRP was performed by peridate method according to Tijsen and Kustak (1984). Sandwich ELISA, originally described by Engvall and Perlman (1971), was performed. Wells of microtiter plates were coated with 100 µL/well of purified (2.5, 5, 10, 20 and 30 µg ml⁻¹) pAb IgG in 0.06 M carbonate buffer, pH 9.6. The plates were washed 3 times with washing buffer 0.1 M PBS/T, pH 7.4. Then blocked with 200 µL/well, 2.5% Fetal Calf' Serum (FCS) (Sigma)/0.1 M PBS/T for 2 h and incubated at 37°C. The plates were washed with washing buffer 3 times. Hundred microliter/well of peroxidase-conjugated IgG antibodies of dilution 1/50, 100, 250, 500 and 1000 was dispensed and plates were incubated for 1 h at 37°C and then were washed 5 times with washing buffer. Color appearance was done by addition of 100 µL/well substrate buffer and the plates were kept in dark at room temperature for 30 min, then the enzyme reaction was stopped by 50 µL/well of 8N H₂SO₄. The absorbance was measured at 492 nm using ELISA reader.

**Dot-ELISA (antigen detection assay):** Dot-ELISA was performed according to Boctor et al. (1987), the pre-wetted NC membrane was transferred to the Bio-Dot apparatus and washed once with 0.6 carbonate coating buffer for 5 min. After removing the excess solution, by suction, the membrane coated with 10-50 µL/well IgG pAb diluted in carbonate buffer (1/250, 500 and 1000), from original concentration (8 mg ml⁻¹), then incubated for variable times. Excess solution was removed and then membrane was washed 3 times with 100 µL PBS-T/well. Then blocking solution was applied (10-50 µL/well), incubated at room temperature for 15-45 min. Positive and negative control reference samples were added diluted 1/1-1/32 in the diluent-blocking buffer then incubated for variable times (15-45 min) and washed 3 times with 100 µL PBS-T/well. HRP conjugated pAb was used in 3 dilutions (1/100, 250 and 500) diluted in the diluent-blocking solution and incubated for variable times, then the NC membrane was removed from the Bio-Dot apparatus and washed 5 times with 100 µL PBS-T/well each time, followed by 2 times washing with PBS only. DAB substrate was applied by immersing NC membrane in substrate solution. The reaction was stopped, just after development of the color, with cold dist H₂O.

**Latex agglutination test (LAT):** One percent standardized polystyrene latex suspension (0.81 µm; Sigma, St. Louis, MO) was prepared by mixing 0.1 mL of latex suspension with 9.9 mL of 0.02 M Glycine-Buffered Saline (GBS), pH 8.4. This was stored at 4°C until used. One milliliter of 1% latex suspension was mixed with 1 mL of purified pAb (1.0 mg ml⁻¹). The mixture was incubated at 37°C for 2 h in a water bath. After incubation, antibody-sensitized latex particles were washed two times with GBS, pH 8.4 and centrifuged at 3000 x g for 5 min. The pellet of pAb-sensitized latex particles was emulsified with 1% BSA/GBS, pH 8.4 to make a 2% suspension. The particles were stored at 4°C until used. Latex particles coated with normal rabbit serum were used as negative control.

The test was performed on a clean two halves slide. A drop of test serum or urine (50 µL) was placed on each half of the slide. An equal volume of sensitized latex
reagent was added to the serum or urine on one half. The same volume of control latex suspension was added to the serum or urine on the other half as a negative control. The slide was then manually rotated for two min then inspected. Agglutination with sensitized latex reagent and not with the control latex reagent was considered a positive result. Appropriate controls were examined in parallel in each test.

**Interpretation of results:** According to the intensity of agglutination accumulated around the edge of the reaction zone, the positivity was classified into high (+++), moderate (++), and low (+). When no agglutination was seen, the result was considered negative (-).

**RESULTS**

**Characterization of antigen:** The SEA products were found to contain 8 mg mL\(^{-1}\) of total protein as measured by Bio-Rad protein assay.

**Antigen profile:** The eluted protein gained from the different purification methods was analyzed by 12% SDS-PAGE under reducing conditions showing different bands ranging from 18.5-106 kDa (Fig. 1).

**Reactivity of target antigen by indirect ELISA:** The antigenicity of the purified SEA was tested by indirect ELISA technique. Serum samples from S. haematobium human-infected gave a strong reaction against S. haematobium SEA with OD reading equal to 1.31 and no cross reactivity was recorded with sera of patients infected with other parasites e.g., fascioliasis, echinococcosis, ancylostomiasis and ascariasis (Table 1).

**Characterization of pAb:** The total protein content of crude rabbit serum containing anti S. haematobium SEA antibody was 12.5 and 5.9 mg mL\(^{-1}\) after 50% ammonium sulfate precipitation method, while following 7% caprylic acid precipitation method the content dropped to 3.1 mg mL\(^{-1}\). Finally, the protein content of highly purified anti-S. haematobium SEA IgG pAb subjected to ion exchange chromatography method (DEAE Sephadex A-50 ion exchange column chromatography) was 2.3 mg mL\(^{-1}\).

**pAb profile:** The purity of IgG pAb after each purification step was assayed by 12% SDS-PAGE under reducing conditions. Analysis of 50% ammonium sulfate precipitated proteins showed several bands. While, the purified IgG pAb after 7% caprylic acid was represented by only 2 bands, L and H-chain bands at 31 and 53 kDa, respectively. The pAb appears free from other proteins (Fig. 2).

**Fig. 1:** SDS-PAGE of SEA eluted from affinity chromatography columns. Lane 1: Low molecular weight standard, Lane 2: Crude SEA product and Lane 3: Purified SEA

**Fig. 2:** 12% gel (1 mm) under reducing condition of anti S. haematobium SEA IgG antibody before and after pAb purification stained with coomassie blue. Lane 1: Molecular weight of standard protein; Lane 2: Crude anti-S. haematobium SEA IgG pAb; Lane 3: Precipitated proteins after 50% ammonium sulfate treatment and Lane 4: Purified IgG antibodies after 7% caprylic acid treatment

**Reactivity of pAb against S. haematobium SEA:** The sera of rabbit injected with S. haematobium SEA were tested for the presence of specific anti-S. haematobium SEA antibodies by indirect ELISA. An increasing antibody level started 1 wk after the 1st booster dose. Three days after the 2nd booster dose, immune sera gave a high titer against with OD of 2.97 at 1/200 dilution (Fig. 3).

These sera were also found to be strongly reacting to S. haematobium SEA compared to other parasitic antigens (Table 2).
Detection of *S. haematobium* SEA in human serum and urine by Sandwich ELISA: In order to measure the incidence of positivity for *S. haematobium* SEA in the studied sera and urine, the cut off point for positivity was measured as mean OD reading of negative controls±2SD. Tested samples showing OD values more than cut off value were considered positive for *S. haematobium* SEA. In sera of schistosomiasis group, the highest mean OD readings were observed in 70 cases with heavy intensity infection out of 70 (100% positivity), followed by moderate (29 cases out of 30, 96.7% positivity), while the lowest readings were observed in those of light intensity of infection (48 cases out of 50, 96% positivity) (Table 3).

In other parasites-infected groups, the highest positivity was observed with fascioliasis (2 cases out of 15, 13.3% positivity), followed by echinococcosis group, the SEA was detected in only 1 case out of 15 (6.6% positivity). While, ancylostomiasis and ascariasis groups were completely negative (0% positivity).

The results in urine of schistosomiasis infected group were the same as those in sera, where the mean OD hence the percent of positivity was directly proportional increased with the intensity of infection.

In other parasites-infected groups, fascioliasis and echinococcosis cases showed a highest positivity, followed by ancylostomiasis group. On the other hand, ascariasis group recorded 0% positivity.

**Dot-ELISA:** Using Dot-ELISA for identification of SEA in sera of schistosomiasis group that classified according to the intensity of infection and experimental color intensity score range into heavy, moderate and light infection groups. In schistosomiasis group, the color intensity score was ranged from 2 to 4.

The color intensity score was directly proportional to the intensity of infection, where the score was 4, 3 and 2 in sera of heavy, moderate and light infection group, respectively. In other parasites groups, the fascioliasis gave the highest positivity (6.6%), while echinococcosis, ancylostomiasis and ascariasis groups were completely negative (0% positivity).

The same results were obtained in urine of schistosomiasis infected group while in other parasites-infected group, SEA was detected in 13.3% of the fascioliasis and decreased to only 6.7% in echinococcosis.

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### Table 1: Reactivity of purified *S. haematobium* SEA by indirect ELISA

<table>
<thead>
<tr>
<th>Serum samples</th>
<th>OD readings at 492 nm (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schistosomiasis</td>
<td>1.51±0.342</td>
</tr>
<tr>
<td>Fascioliasis</td>
<td>0.26±0.201</td>
</tr>
<tr>
<td>Echinococcosis</td>
<td>0.11±0.094</td>
</tr>
<tr>
<td>Ancylostomiasis</td>
<td>0.18±0.082</td>
</tr>
<tr>
<td>Ascariasis</td>
<td>0.20±0.105</td>
</tr>
</tbody>
</table>

**OD:** Optical density, **SD:** Standard deviation

### Table 2: Specificity of rabbit anti- *S. haematobium* SEA pAb against different parasitic antigens by indirect ELISA

<table>
<thead>
<tr>
<th>Parasitic antigen</th>
<th>OD readings at 492 nm (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. haematobium</em></td>
<td>2.98±0.21</td>
</tr>
<tr>
<td>Fascioliasis</td>
<td>0.24±0.13</td>
</tr>
<tr>
<td>Echinococcosis</td>
<td>0.19±0.11</td>
</tr>
<tr>
<td>Ancylostomias</td>
<td>0.31±0.14</td>
</tr>
<tr>
<td>Ascariasis</td>
<td>0.41±0.10</td>
</tr>
</tbody>
</table>

### Table 3: Detection of *S. haematobium* SEA in human sera and urine infected with *S. haematobium* and other parasites-infected groups by sandwich ELISA in comparison to healthy control sera

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sera</th>
<th>Urine</th>
<th>Negative cases (OD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control (n = 30)</td>
<td>-----</td>
<td>-----</td>
<td>0.215±0.02</td>
</tr>
<tr>
<td><em>S. haematobium</em> (n = 150)</td>
<td>0.764±0.22</td>
<td>0.811±0.24</td>
<td>0.355±0.12</td>
</tr>
<tr>
<td>Light infection (n = 50)</td>
<td>1.155±0.26</td>
<td>1.020±0.21</td>
<td>0.435±0.18</td>
</tr>
<tr>
<td>Moderate infection (n = 30)</td>
<td>2.411±0.19</td>
<td>2.121±0.23</td>
<td>0.459±0.12</td>
</tr>
<tr>
<td>Heavy infection (n = 70)</td>
<td>-----</td>
<td>-----</td>
<td>0.21±0.13</td>
</tr>
<tr>
<td>Other parasites (n = 50)</td>
<td>0.444±0.22</td>
<td>0.521±0.39</td>
<td>0.19±0.11</td>
</tr>
<tr>
<td>Fascioliasis (n = 15)</td>
<td>0.379±0.11</td>
<td>0.429±0.17</td>
<td>0.20±0.20</td>
</tr>
<tr>
<td>Echinococcosis (n = 15)</td>
<td>-----</td>
<td>0.413±0.18</td>
<td>0.19±0.21</td>
</tr>
<tr>
<td>Ancylostomiasis (n = 10)</td>
<td>-----</td>
<td>-----</td>
<td>0.210±0.09</td>
</tr>
<tr>
<td>Ascariasis (n = 10)</td>
<td>-----</td>
<td>-----</td>
<td>0.233±0.13</td>
</tr>
</tbody>
</table>

Values are as Mean±SD. Cut off value: 0.312, m: Mean, **OD:** Optical density, **SD:** Standard deviation
group and still completely absent in urine of ancylostomiasis and ascariasis groups (0% positivity) (Table 4).

**LAT:** Agglutination was detected in 68 cases out of 70 of heavy infected patients, while in the moderate and light infection group was recorded in 27 cases out of 30 and 43 out of 50, respectively. On the other hand, 20, 13.3, 10 and 0% patient sera infected with fascioliasis, echinococcosis, ancylostomiasis and ascariasis were positive.

*S. haematobium* SEA was detected in the urine of 65 cases out of 70 heavy infected patients, in the moderate infection group it was detected in 26 cases out of 30 and in light infection group 42 out of 50. In the groups infected with other parasites, the fascioliasis and echinococcosis gave the highest positivity (20%), whereas, ancylostomiasis group gave only 10% positivity and ascariasis was completely negative (0% positivity) (Table 5).

**Sensitivity and specificity:** Table 6 summarizes the sensitivity, specificity, PPV and NPV of sandwich ELISA, Dot-ELISA and LAT which are used for detection of *S. haematobium* SEA in human sera and urine. It was found that, the sensitivity of LAT in comparison to sandwich ELISA and Dot-ELISA in human sera, were 92, 98 and 98.66%, respectively, while the specificity are 92.50, 96.25 and 98.75%, respectively. On the other hand, in human urine the sensitivity were 88.66, 90.66 and 94.66%, respectively, while the specificity were 91.25, 93.75 and 96.25%, respectively. Thus, from the above data we can conclude that, the LAT and Dot-ELISA specificity and sensitivity were much close to that of sandwich ELISA.

For all techniques (sandwich ELISA, Dot-ELISA and LAT), detection of SEA in urine recorded a slightly lower results than that detected in serum.

But, these detectable values have a highly satisfied sensitivity, specificity, PPV and NPV which are respective and reasonable values for diagnosis of *S. haematobium* in all degrees of infection.

**DISCUSSION**

Early diagnosis of schistosomiasis is necessary for prompt treatment before irreparable damage to the liver occurs (Hillyer et al., 1992). So that, development of early sensitive, specific as well as low-cost immunodiagnostics for detection of infected individuals would be an important step towards reaching the goal in schistosomiasis. Detection of *S. haematobium* ova in urine of infected individuals remains the leading routine method for direct diagnosis of the disease. However, a homogeneous distribution of *S. haematobium* ova in urine is difficult to achieve (Braun-Munzinger and Southgate, 1992) but due to many obstacles, it is not of valuable sensitivity (Hillyer, 1998). Sensitivity of all fecal examination methods is found to be poor and immunodiagnosis is considered essential for correct
diagnosis (Agrawal, 2004). Many attempts have been made to identify the egg antigens which are responsible for inducing those reactions and which proved also to be useful immunodiagnostic reagents (McManus and Loukas, 2008).

In this study, S. haematobium eggs were isolated from the intestine of 8-wk infected hamster and SEA was purified by affinity chromatography through SDS-PAGE with 8 mg mL⁻¹ total protein by Bio-Rad protein assay. The purified SEA was reasonable in comparison with that of purified antigen from any biological fluid following similar purification procedures Ibrahim et al. (2010).

The antigenicity of the purified SEA was tested by indirect ELISA, detecting the highly antigenicinity as the major factor in the pathogenesis of schistosomiasis. The pathology of schistosomiasis reflects the host granulomatous response to antigens secreted from the trapped eggs in host tissues (Pearce, 2005). Bosompem et al. (1996) precipitated proteins in urine of S. haematobium infected individuals and found that such antigen could be used to elicit specific antibodies which could bind SEA, so, it will be useful in S. haematobium diagnosis.

The present study was carried out for detection of SEA in both urine and serum samples of a group of S. haematobium infected patients, patients with other parasites and healthy individuals group using a pAb based-latex agglutination test comparing its results with pAb-based sandwich ELISA and Dot-ELISA as a well established reference tests for SEA assay.

So male New Zealand white rabbits were immunized with purified S. haematobium SEA and the reactivity of anti-S. haematobium SEA pAb against SEA of S. haematobium and other parasites (Fasciola, Echinococcus, Ancylostoma and Ascaris) were determined by indirect ELISA. The IgG fraction of rabbit anti-S. haematobium SEA pAb was purified using ammonium sulphate precipitation method followed by 7% caprylic acid treatment and finally by using ion exchange chromatography method (DEAE Sephadex A-50) according to Goding (1986).

The sensitivity and specificity of LAT for the detection of S. haematobium SEA were evaluated in both human sera and urine. In serum, the sensitivity and specificity were 92 and 92.50%, respectively, compared to 98 and 96.25% by sandwich ELISA. While in urine, they recorded 88.6 and 91.25%, respectively, compared to 90.66 and 93.75% by sandwich ELISA.

Our results are in agreement with those of Demerdash et al. (1995) who used anti-S. mansoni SEA mAb in sandwich ELISA for detection of CSA in serum and urine samples and reported a sensitivity of 90 and 97%, respectively, while in mixed S. mansoni and S. haematobium infection, it was 91% in sera and 100% in urine samples. The overall specificity of the assay was 98%. On the other hand, Hanallah et al. (1995) who used different mAb and reported a sensitivity and specificity of 90.0 and 94.8% in urine, while in serum it was 97.0 and 98.4%, respectively. Also, El-Bassicouy et al. (2005) used a pair of mAb and found 96.7% sensitivity and 92% specificity, respectively.

A considerable degree of cross reactivity was revealed in the present study between S. haematobium and other parasites with varying degrees. This was obvious in case of detection of human serum by sandwich ELISA, where cross reactivity show 13.3% positivity in fasciolasiss and 6.6% in echinococcosis while in detection of human urine, the cross reactivity show 13.3% positivity in echinococcosis and 10% in ancylostomiasis, so that, the best sensitivity and specificity obtained from using serum in detection of S. haematobium SEA in case of sandwich ELISA.

However, patients infected with parasites other than Schistosoma (3 patients in serum and 5 patients in urine) showing detectable levels of SEA were coming from endemic areas of S. haematobium infection and missed urine diagnosis of light infection is a possibility.

The negative results observed in ELISA were found in patients with low number of egg/10 mL urine and this could be due to the possibility that the intact ova of S. haematobium may release only small undetectable amounts of antigen into the circulation. Another possibility is that the antigen released from the parasite form immune complexes with circulating antibodies (Carlier et al., 1983; Nash, 1984). Additionally, the disappearance of CSA could be due to the effect of successful chemotherapy denoting the reliability of CSA assay as a cure monitor (Van Lieshout et al., 1993; Demerdash et al., 1995).

Although, the sandwich ELISA was specific and sensitive method but Dot-ELISA was more sensitive and specific technique than sandwich ELISA (El-Misiry et al., 1990; Shaheen et al., 1994; Parija, 1998; Montenegro et al., 1999; El-Amir et al., 2008).

In accordance, the sensitivity and specificity of Dot-ELISA assay in the present study for the detection of S. haematobium SEA in serum and urine were 98.66, 98.75, 94.66 and 96.25%, respectively. These results were also confirmed by Rokni et al. (2006), who used Dot-ELISA in detection of E/S antigens of F. hepatica and found the sensitivity, specificity, PPV and NPV were 96.8, 96.1, 96.8 and 96.1%, respectively.

Moreover, in this study, a significant correlation was observed between the level of SEA detected by ELISA
and LAT in both serum and urine and the number of eggs excrated in urine of schistosomiasis patients denoting the reliability of SEA detection as an indication for intensity of infection. These results were in parallel with those of Hendawy et al. (2006).

In conclusion, the use of LAT for SEA assay could be a valuable applicable screening diagnostic technique in field survey. A confirmatory sandwich ELISA for SEA assessment in sera is recommended for query false negative results. At the same time, more studies have to be performed to improve the sensitivity and specificity of LAT and hence encourage its use on a large scale for diagnosis of multiple parasitic infections in field surveys.

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