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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-Raddish 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; Siddiqui *et al*., 2005). So, early diagnosis is necessary for prompt treatment before irrepairable 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 slide 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 *Echinococcosis 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 *Schistosomiasis 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 main age±SE was 38±11.7 years, 50 infected with other parasites (*S. mansoni*, *Fasciola*, *Echinococcus*, *Ancylostoma* 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 Perlmanm (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 from 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 (Mckinney 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 microtitre plate was coated overnight at 4°C with 30 ug mL^{-1} SEA in carbonate coating buffer, blocked with 0.1% BSA in PBS then 100 uL/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 H_2SO_4 was added to stop the enzyme substrate reaction. The absorbance was measured at 492 nm using ELISA reader (Bio-Rad microplate reader, Richomond, 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 Sandwitch ELISA: Labeling of pAb with HRP was performed by periodate method according to Tijssen and Kurstak (1984). Sandwich ELISA, originally described by Engvall and Perlmanm (1971), was performed. Wells of microtitre 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 micriliter/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 8 NH_2SO_4. 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 dilutent-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 dilutent-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 H_2O.

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 ranged 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).

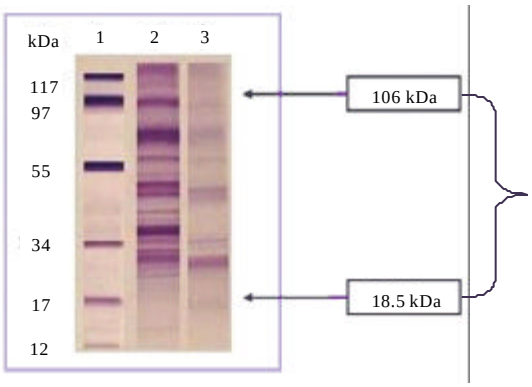

Fig. 1: SDS-PAGE of SEA eluted from affinity chromatography columns. Lane 1: Low molecular weight standard; Lane 2: Crude SEA product and Lane 3: Purified SEA

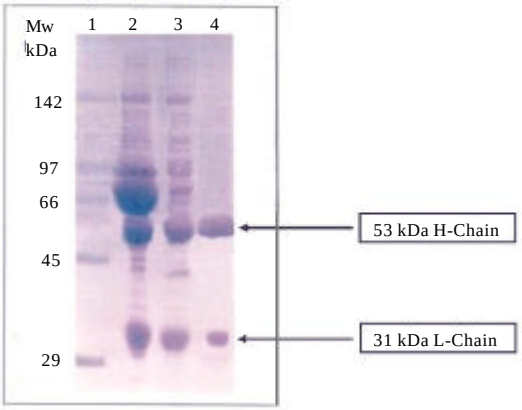

Fig. 2: 12% gel (1 mm) under reducing condition of anti *S. haematobium* SEA IgG antibody before and after pAb purification stained with coomassie blue. Lane 1: Molecular weight of standard protein; Lane 2: Crude anti-*S. haematobium* SEA IgG pAb; Lane 3: Precipitated proteins after 50% ammonium sulfate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

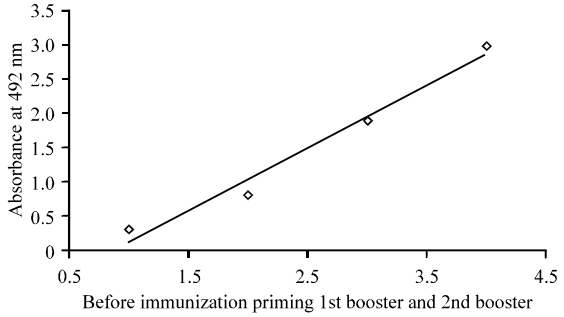

Fig. 3: Reactivity of immunized rabbit anti *S. haematobium* SEA antisera (diluted 1/250) against *S. haematobium* SEA by indirect ELISA

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

Serum samples	OD readings at 492 nm (Mean±SD)
Schistosomiasis	1.31±0.342
Fascioliasis	0.26±0.201
Echinococcosis	0.11±0.094
Ancylostomiasis	0.18±0.082
Ascariasis	0.20±0.105

OD: Optical density, SD: Standard deviation

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

Parasitic antigen	OD readings at 492 nm (Mean±SD)
S. haematobium	2.98±0.21
Fasciola	0.24±0.13
Echinococcus	0.19±0.11
Ancylostoma	0.31±0.14
Ascaris	0.41±0.10

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

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

	Positive cases (OD)		Negative cases (OD)	
Groups	Sera	Urine	Sera	Urine
Healthy control (n = 30)	-----	-----	0.215±0.02	0.287±0.03
***S. haematobium* (n = 150)**				
Light infection (n = 50)	0.764±0.22	0.811±0.24	0.355±0.12	0.371±0.14
Moderate infection (n = 30)	1.155±0.26	1.020±0.21	0.435±0.18	0.411±0.12
Heavy infection (n = 70)	2.411±0.19	2.121±0.23	-----	0.459±0.12
Other parasites (n = 50)				
Fascioliasis (n = 15)	0.444±0.22	0.521±0.30	0.211±0.13	0.191±0.11
Echinococcosis (n = 15)	0.379±0.11	0.429±0.17	0.178±0.23	0.201±0.20
Ancylostomiasis (n = 10)	-----	0.413±0.18	0.199±0.29	0.193±0.21
Ascariasis (n = 10)	-----	-----	0.210±0.09	0.233±0.13

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 anclystomiasis 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, anclystomiasis group gave only 10% positivity and ascariasis was completely negative (0% positivity) (Table 5).

Table 4: Detection of *S. haematobium* SEA in human sera and urine infected with *S. haematobium* and other parasites-infected groups by Dot-ELISA in comparison to healthy control

	Positive cases	
	Score of the color range	
Groups	Sera	Urine
Healthy control (n = 30)	-	-
***S. haematobium* (n = 150)**		
Light infection (n = 50)	++	++
Moderate infection (n = 30)	+++	+++
Heavy infection (n = 70)	++++	++++
Other parasites (n = 50)		
Fascioliasis (n = 15)	+	+
Echinococcosis (n = 15)	-	-
Ancylostomiasis (n = 10)	-	-
Ascariasis (n = 10)	-	-

Table 5: Detection of *S. haematobium* SEA in human sera and urine infected with *S. haematobium* and other parasites-infected groups by LAT in comparison to healthy control

	Positive cases		Negative cases	
	Score of the agglutination		Score of the agglutination	
Groups	Sera	Urine	Sera	Urine
Healthy control (n = 30)	-	-	-	-
***S. haematobium* (n = 150)**				
Light infection (n = 50)	++	++	+	+
Moderate infection (n = 30)	+++	+++	++	++
Heavy infection (n = 70)	++++	++++	+++	+++
Other parasites (n = 50)				
Fascioliasis (n = 15)	+	+	-	-
Echinococcosis (n = 15)	+	+	-	-
Ancylostomiasis (n = 10)	+	+	-	-
Ascariasis (n = 10)	-	-	-	-

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 irrepairable 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

Table 6: Sensitivity, specificity, PPV and NPV percentage of LAT versus sandwich ELISA and Dot-ELISA for detection of *S. haematobium* SEA in sera and urine

	Sensitivity (%)		Specificity (%)		PPV (%)		NPV (%)	
Type of experiment	Serum	Urine	Serum	Urine	Serum	Urine	Serum	Urine
Sandwich ELISA	98	90.66	96.25	93.75	96.08	87.74	92.77	79.78
Dot-ELISA	98.66	94.66	98.75	96.25	98.01	92.81	96.34	87.50
LAT	92	88.66	92.50	91.25	88.46	84.71	80.43	75.25

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 then 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 antigenicity 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-Bassiouny *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 fascioliasis 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-Missiry *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 excreted 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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REFERENCES

Agrawal, M.C., 2004. Problems of human *schistosomiasis* in India-status and strategy. Abstracts and Program, 3rd Global Meeting on Parasitic Diseases, Bangalore, India, PL 1, 2-3.

Amorosa, V., D. Kremens, M.S. Wolfe, T. Flanigan and K.M. Cahill *et al*., 2005. *Schistosoma mansoni* in family 5 years after safari. Emerg. Infect. Dis., 11: 339-341.

Armour, J., G.M. Urqhart and J.L. Ductan, 1997. Veterinary Parasitology. 2nd Edn., Blackwell Science Ltd, Oxford, New York, Pages: 64.

Arya, S.C., 1997. Latex agglutination test for the visceral *Leishmaniasis* (letter). Trans. R. Soc. Trop. Med. Hyg., 91: 366-366.

Bagchi, A., S. Tiwari, S. Gupta and J. Katiyar, 1998. Latex agglutination test. Standardization and comparison with direct agglutination and dot ELISA in the diagnosis of visceral *leishmaniasis* in India. Ann. Trop. Med. Parasitol., 92: 159-163.

Barbieri, M., S. Steria, J. Battisoni and A. Nieto, 1993. High performance latex reagent for hydatid serology using an *Echinococcus granulosus* lipoprotein antigen purified from cyst fluid in one step. Int. J. Parasitol., 23: 565-572.

Boctor, F.N., M.J. Stek, J.B. Peter and R. Kamal, 1987. Simplification and standardization of dot-ELISA for human *Schistosomiasis mansoni*. J. Parasitol., 73: 589-592.

Bosompem, K.M., T. Arishima, T. Yamashita, I. Ayi, W.K. Anyan and S. Kojima, 1996. Extraction of *Schistosoma haematobium* antigens from infected human urine and generation of potential diagnostic monoclonal antibodies to urinary antigens. Acta Trop., 62: 91-103.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Braun-Munzinger, R.A. and B.A. Southgate, 1992. Repeatability and reproducibility of egg counts of *Schistosoma haematobium* in urine. Trop. Med. Parasitol., 43: 149-154.

Carlier, Y., H. Nzeyimana, D. Boul and A. Capron, 1983. Evaluation of circulating antigens by a sandwich radioimmunoassay and of antibodies and immune complex, in *Schistosoma mansoni*-infected African parturients and their newborn children. Am. J. Trop. Med. Hyg., 29: 74-81.

Chen, M.G. and K.E. Mott, 1989. Progress in the assessment of morbidity due to *Schistosoma haematobium* infections: in a review of the recent literature. Trop. Dis. Bull., 48: 2643-2648.

Chitsulo, L., D. Engels, A. Montresor and L. Savioli, 2000. The global status of schistosomiasis and its control. Acta Trop., 77: 41-51.

Chitsulo, L., P. Loverde and D. Engels, 2004. *Schistosomiasis*. Nat. Rev. Microbiol., 2: 12-23.

Corachan, M., 2002. *Schistosomiasis* and international travel. Clin. Infect. Dis., 35: 446-450.

Deelder, A.M., H.T.M. Klappe, G.J.M.J. van den Aardweg and E.H.E.M. van Meerbeke, 1976. *Schistosoma mansoni* demonstration of two circulating antigens in infected hamsters. Exp. Parasitol., 40: 189-197.

Demerdash, Z., S. Mahmoud, Z. Shaker, S. Hassan and M. Mansour *et al*., 1995. Detection of circulating schistosome antigens in serum and urine of schistosomiasis patients and assessment of cure by a monoclonal antibody. J. Egypt Soc. Parasitol., 25: 471-484.

El-Amir, A., I. Rabee, N. Kamal and S. El-Deeb, 2008. Evaluation of the diagnostic potential of different immunological techniques using polyclonal antibodies against *Fasciola gigantic* excretory/secretory antigens in sheep. Egypt. J. Immunol., 15: 65-74.

El-Bassiouny, A., F. Salah, M. Kamel and I. Rabia, 2005. Effective diagnosis of active schistosomiasis by a pair of IgG monoclonal antibodies against *Schistosoma mansoni* soluble egg antigen. Egypt J. Med. Sci., 26: 369-382.

El-Missiry, A.G., A.O. El-Serougi, M.M. Salama and A.M. Kamal, 1990. Evaluation of dot ELISA technique in the serodiagnosis of *schistosomiasis* in Egypt. J. Egypt Soc. Parasitol., 20: 639-645.

Engvall, E. and P. Perlmanm, 1971. Enzyme-linked immunosorbent assay. Immunochemistry, 8: 871-874.

Fagbemi, B.O. and E.E. Guobadia, 1995. Immunodiagnosis of *fascioliasis* in ruminants using a 28-kDa cysteine protease of *Fasciola gigantic* adult worm. Vet. Parasitol., 57: 309-318.

Feldmeier H., 1993. Diagnosis. In: Human *Schistosomiasis*, Jordan, P., G. Webbe and R.F. Sturrock (Eds.). CAB Internat., Oxon, pp: 217-304.

Goding, J.W., 1986. Monoclonal Antibodies: Principles and Practice. Alden Press, Oxford, London and Northampton, pp:112-116.

Guobadia, E.E. and B.O. Fagbemi, 1997. The isolation of *F. gigantic*-specific antigens and their use in the serodiagnosis of *fascioliasis* in sheep by the detection of circulating antigens. Vet. Parasitol., 68: 269-282.

Hanallah, S.B., Z.A. Shaker and M.A. Kaddah, 1995. Monoclonal antibodies specifically directed to urinary schistosomal antigens detect antigenaemia and antigenuria in human *Schistosomiasis mansoni*. Egypt J. Bilh., 17: 27-49.

Harlow, E., E. Harlow and D. Lane, 1988. Monoclonal Antibodies. In: Antibodies. A Laboratory Manual, Harlow, E. and D. Lane (Eds.). Cold Spring Harbor Laboratory, U.S.A.

Hendawy, M.A., S.H. Mohamed and R.A. Ibrahim, 2006. Nitrocellulose cassettes (NCC) as a field applicable technique for rapid and effective diagnosis of *schistosomiasis* and *fascioliasis*. New Egypt J. Med., 35: 86-92.

Hillyer, G.V., S.M. de Galanes, J. Rodriguez-Perez, J. Bjorland, S.M. de Lagrava, S.R. Guzman and R.T. Bryan, 1992. Use of the Falcon assay screening test-enzyme-linked immunosorbent assay (FAST-ELISA), the enzyme linked immunoelectrotransfer blot (EITB) to determine the prevalence of human *Fascioliasis* in the Bolivian Altiplano. Am. J. Trop. Med. Hyg., 46: 603-609.

Hillyer, G.V., 1998. Immunodiagnosis of Human and Animal Fasciolosis. In: Fasciolosis, Dalton, J.P. (Ed.). CABI Publishing, Oxon, Wallingford, UK., pp: 435-448.

Ibrahim, R.B., S.H. Mohamed, Z.A. Demerdash, T.M. Diab, K. Maher, W. Safwat and Z.A. Shaker, 2010. Anti *Schistosoma mansoni* mAb-based latex agglutination: A reliable field applicable immunodiagnostic test for screening of active Human *Schistosomiasis*. J. Amer. Sci., 6: 19-27.

King, C.H., 2009. Toward the elimination of *schistosomiasis*. N. Engl. J. Med., 360: 106-109.

Laemmli, U.K., 1970. Cleavage of structural proteins during assembly of head of bacteriophage T4. Nature, 227: 680-685.

Mazumder, P., H.Y. Chuang and M.W. Wentz and D.L. Wiedbrauk, 1988. Latex agglutination test for the detection of antibodies to *Toxoplasma gondii*. J. Clin. Microbiol., 26: 2444-2446.

McManus, D.P. and A. Loukas, 2008. Current status of vaccines for schistosomiasis. Clin. Microbiol. Rev., 21: 225-242.

Mckinney, M.M. and A. Parkinson, 1987. A simple, non-chromatographic procedure to purify immunoglobulins from ascites fluid. J. Immunol. Meth., 96: 271-278.

Montenegro, S.M., P. Miranda, S. Mahanty, F.G. Abath and K.M. Teixeira *et al*., 1999. Cytokine production in acute versus chronic human *Schistosomiasis mansoni*: The cross-regulatory role of interferon-gamma and interleukin-10 in the responses of peripheral blood mononuclear cells and splenocytes to parasite antigens. J. Infect. Dis., 179: 1502-1514.

Nash, T.E., 1984. Immune complex size to determine the clearance rate of circulating antigen in schistosome infected mice. Am. J. Trop. Med. Hyg., 33: 621-626.

Nessim, N.G., S.I. Hassan, S. William and H. El-Baz, 2000. Effect of the broad spectrum anthelmintic drug flubendazole upon *Schistosoma mansoni* experimentally infected mice. Arzneimittelforschung, 50: 1129-1133.

Parija, S.C., 1998. Urinary antigen detection for diagnosis of parasitic infections. Parasitol. Today., 14: 5-6.

Patz, J.A., T.K. Graczyk, N. Geller and A.Y. Vittor, 2000. Effects of environmental changes on emerging parasitic diseases. Int. J. Parasitol., 30: 1395-1405.

Pearce, E.J., 2005. Priming of the immune response by schistosome egg. Parasite Immunol., 27: 265-270.

Pelley, R.P. and G.V. Hillyer, 1978. Demonstration of a common antigen between *Schistosoma mansoni* and *Fasciola hepatica*. Am. J. Trop. Med. Hyg., 27: 1192-1194.

Rokni, M.B., A. Samani, J. Massoud and M.B. Nasr, 2006. Evaluation of Dot-ELISA method using excretory-secrotory antigens of fasciola hepatica in laboratory diagnosis of human fascioliasis. Iran. J. Parasitol., 1: 26-30.

Salah, F., A. El Bassiouny, I. Rabie, Z. Demerdash, M. Roshdy and Z. Shaker, 2006. Human *schistosomiasis haematobium*: Effective diagnosis of active infection using a pair of monoclonal antibodies against soluble egg antigen. Parasitol. Res., 99: 528-533.

Shaheen, H., M. al Khafif, R. Farag and K.A. Kamal, 1994. Serodifferentiation of human fascioliasis from *Schistosomiasis*. Trop. Geogr. Med., 46: 326-327.

Sheehan, D. and R. Fitz Gerald, 1996. Ion-exchange chromatography. Methods Mol. Biol., 59: 145-150.

Siddiqui, A.A., J.R. Pinkston, M.L. Quinlin, V. Kavikondala, K.A. Rewers-Felkins, T. Phillips and J. Pompa, 2005. Characterization of protective immunity induced against *Schistosoma mansoni* via DNA priming with the large subunit of calpain (Sm-p80) in the presence of genetic adjuvants. Parasite, 12: 3-8.

Thors, C., P. Holmblad, M. Maleki, J. Carlson and E. Linder, 2006. *Schistosomiasis* in Swedish travelers to sub-Saharan Africa: Can we rely on serology? Scand. J. Infect. Dis., 38: 794-799.

Tijssen, P. and P. Kurstak, 1984. Highly efficient and simple methods for the preparation of peroxidase and active peroxidase-antibody conjugates for enzyme immunoassays. Anal. Biochem., 136: 451-457.

Van Lieshout, L., N. De Jonge, M.M. Mansour, S. Bassily, F.W. Krijger and A.M. Deelder, 1993. Circulating cathodic antigen levels in serum and urine of schistosomiasis patients before and after chemotherapy with praziquantel. Trans. R. Soc. Trop. Med. Hyg., 87: 311-312.

Van Lieshout, L., A.M. Polderman and A.M. Deelder, 2000. Immunodiagnosis of *Schistosomiasis* by determination of the circulating antigens CAA and CCA, in particular in individuals with recent or light infections. Acta Trop., 77: 69-80.

Wang, H., Y. Zhang, B. Yan, L. Liu, S. Wang, G. Shen and R. Yu, 2006. Rapid, simple and sensitive immunoagglutination assay with SiO2 particles and quartz crystal microbalance for quantifying *Schistosoma japonicum* antibodies. Clin. Chem., 52: 2065-2071.

Whitty, C.J., D.C. Mabey, M. Armstrong, S.G. Wright and P.L. Chiodini, 2000. Presentation and outcome of 1107 cases of *Schistosomiasis* from Africa diagnosed in a non-endemic country. Trans. R. Soc. Trop. Med. Hyg., 94: 531-534.

WHO, 2005. Deworming for health and development. Report of the Third Global Meeting of the Partners for Parasite Control, November 29-30, 2004. World Health Organization, Geneva.

Xue, C.G., M.G. Taylor, Q.D. Bickle, L. Savioli and E.A. Renganathan, 1993. Diagnosis of *Schistosoma haematobium* infection: Evaluation of ELISA using key hole limpet haemocyanin or soluble egg antigen in comparison with detection of eggs or haematuria. Trans. Roy. Soc. Trop. Med. Hyg., 87: 654-658.