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SCHISTOSOMA-SPECIFIC 26 KDA PROTEIN FOR A DIAGNOSIS OF ACUTE AND CHRONIC SCHISTOSOMIASIS

By

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Abstract

One of the world wide major public health problems is the schistosomiasis that is caused by *Schistosoma (S.) heamatobium*. It is also one of the main concerns for the public health community in Egypt. There are several immunodiagnostic methods used for that diagnosis of such disease, but some are more sensitive and specific than others. The purified 26 kDa *Schistosoma*-specific protein (PSPA-26) detection in serum samples is found out to be more valuable in diagnosis; it also helps in the early diagnosis which will lead to the early treatment before the irreversible damage takes place. PSPA-26 was purified from whole worms by DEAE-Sephadex G-75 ion exchange chromatography and then was injected into rabbits to produce specific polyclonal antibodies (anti-PSPA-26 pAb) which were then used as a primary capture in the indirect ELISA technique to reveal its reactivity using infected human sera. The anti-PSPA-26 was then labeled with horse-radish peroxidase (HRP) and used as a secondary capture. Sandwich ELISA was done for serum samples of human and hamsters infected with *S. heamatobium*. The results revealed a sensitivity of 85% for human and 80% for hamster's samples, and a specificity of 95% for human and 91.1% for hamsters samples by comparing them with those infected with other parasites and control samples. Data obtained concluded that PSPA-26 antigen can be used as a diagnostic marker for *S. haematobium* infection using the sandwich ELISA which is cost effective and applicable technique.

Keywords: *S. haematobium*, PSPA-26, diagnosis, ELISA.

Introduction

Schistosomiasis, which is also known as bilharziasis, is one of the most world wide spread disease causing the highest rates of morbidity and mortality after the malaria. Schistosomiasis infects about 200 million people worldwide. The contamination takes place by getting in contact with fresh water contaminated with cercaria which penetrates the skin causing infection (Liu *et al*, 2016).

There are 3 main types of *Schistosoma* (S.) that cause human infections; *S. mansoni, S. haematobium*, or *S. japonicum* (CDC, 2012).

In Egypt, the urinary schistosomiasis is representing a serious health problem to deal with. Its complications add more burden on the national control programs and on the national economy (El-Khoby *et al,* 2000). Due to controlling programs over the last decade, a decline in the prevalence of human schistosomiasis in Egypt has been reported, however the disease is still endemic in many foci (Bergquist, 2009; Kandeel *et al*, 2016).

Schematically, there are three different known approaches for the diagnosis of parasitic infections, direct (detecting parasitic ova in stool and urine), indirect (relaying on biochemical assays) and immunological methods which are the most sensitive and specific methods in measure the immune response (antibodies) to certain parasitic antigens and/or detecting circulating parasitic antigens (Bruschi and Castagna, 2004).

Proteases and peptidases are proteolytic enzymes that operate in virtually every biological phenomenon. They function not only as individual enzymes but often in cascades or networks. They also provide essential functions in all life forms (Turk *et al*, 2012)

Proteases operate at the host-parasite interface facilitating migration, digestion of host proteins and probably immune evasion (McKerrow *et al,* 2006; Dalton *et al,* 2006).

The cysteine proteases (CPs) comprise a large family that includes cathepsin. The role of the CP-B as hemoglobinases in blood feeding parasites has been well established in helminthes (Turk *et al,* 2012). In medically important *S. mansoni* CP-B (cathepsin B1) the predominant cysteine peptidase one of a large number of gut-associated digestive enzymes (Dvoraka *et al,* 2005), was considered an attractive target for anti-parasite chemotherapy (Abdulla *et al,* 2007). More over addition, this enzyme is able to elicit an antibody response in the hosts and its immunogenicity has been used as one of the main serodiagnostic markers for the schistosomiasis (Doleckova *et al,* 2009).

This study aimed to evaluate PSPA-26 purified antigen as detecting target in the serum of infected patients and hamsters to evaluate its efficiency to be used as an immunodiagnostic target of *S. haematobium* in both the early and late stages (acute and chronic stages).

Materials and methods

Animals: New Zealand white male rabbits were purchased from Agriculture Faculty, Cairo University (Giza, Egypt), weighing approximately 1.5 Kg and 2 months old. They were examined and confirmed to be free from parasitic infection. The rabbits were housed in the animal house at Theodore Bilharz Research Institute (TBRI) (Giza, Egypt), under standard laboratory care at 21°C, 16% moisture, the animals were supplied with filtered water with salts 1cm/5 liter and vitamins 1cm/10 liter, also there diet hold a minimum of 15% protein, 3% fat and 22% fiber. Internationally valid guidelines were applied on animal experiments. A number of 78 hamsters were included, 60 hamsters infected with 300 *S. haematobium* cercariae, 40 of them were scarificed after 8 week post infection (acute group) and 20 were sacrificed after 16 week post infection (chronic group), and 18 hamsters infected with other helminthic parasites (infected with *H. nana* and 8 with *F. gigantica*). Also, 20 hamsters free parasite infected were included as healthy control group.

Parasites: *S. haematobium* adult worms were brought from the Schistosome Biological Supply Program Unit (SBSP) at TBRI, Giza, Egypt. The worms were recovered by the perfusion of the portal mesenteric vasculatures of the laboratory infected hamsters using heparinized saline (Smithers and Terry, 1965).

A total of 140 individuals were enrolled in the present study. Sixty individuals are free from any parasitic infections served as normal control. Sixty patients infected with *S. haematobium,* 38 were acute and 22 were chronic. Chronic patients were selected on the basis of presence of hepatic fibrosis (degree I or II), determined by abdominal ultrasonography according to the World Health Organization (WHO) criteria. Other helminthic parasites infected groups (n=20) were included (10 patients infected with *H. nana* and 10 with *F. gigantica*).

Preparation and Purification of PSPA 26 kDa from *S. haematobium* worms: *S. haematobium* fresh adult worms were suspended in 10 ml of phosphate buffer saline (PBS) followed by dialysis against the lysis buffer [8M Urea, 2M Thiourea, 4% 3-3-Cholamidopropyl-dimethyl-amonio-propane sulfonate (CHAPS), 50mM dithiothreitol (DTT), 20 mM Tris and Complete Mini Protease Inhibitor Cocktail Tablets (Roche)].

After the dialysis step, the adult worms homogenization was done under the continuous agitation for 2 hours (hr) at room temperature, 10 repeated passages were performed through a 30-gauge hypodermic needle, centrifugation of the homogenate at 20,000×g for 30 min at 25°C and finally collection of the supernatant and store it at -70°C.

Fractionation of prepared supernatant was done by ammonium sulfate at 80% saturation. The precipitate was dissolved in PBSC (20mM sodium phosphate buffer containing 5mM L-cysteine, pH 6.0) and dialyzed against PBSC for 24 hr. The prepared crude

worm antigen solution was then stored at -80ºC as crude extract.

The crude antigen was purified by DEAE Sepharose CL-6B, where the antigen was further purified on the basis of molecular size by DEAE SephadexG-75 gel filtration chromatography. Absorbance at 280 nm of each fraction was measured and the purity of the produced protein was assayed by sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions (Laemmli, 1995). The protein content was measured based on Bradford dye-binding procedure which was dependent on the color change of Coomassie brilliant blue G-250 dye in response to various concentrations of proteins by using protein assay kit (Bio-Rad, Richmond, CA, USA, Bradford, 1976).

Characterization of PSPA 26: SDS-PAGE was applied to the purified PSPA 26 antigen under reducing condition, the antigen bands were shown in polyacrylamide gel by staining using Coomassie brilliant blue R-250 (Sigma).

Preparation of polyclonal antibody (pAb): Two rabbit, each received priming dose of antigen (1 mg) intramuscularly (I.M.) in complete Freund's adjuvant (CFA), (Sigma), followed by 3 booster doses, each was 0.5 mg antigen emulsified in equal vol. of incomplete Freund's adjuvant (IFA, Sigma). The first boosting was 2 wk. after priming dose, and the following boosting doses were given at weekly intervals (Fagbemi, 1995). The rabbits were bled for collection of serum 1 week later after a preliminary testing of titer by indirect ELISA. Rabbit serum that contains anti-purified PSPA 26 IgG pAbs was fractionated and kept at-20°C. Test blood samples were withdrawn before the injection of each immunization dose to detect the titer of antibodies.

Purification of rabbit anti-purified PSPA 26 pAbs was based on two different methods, ammonium sulfate precipitation (Nowotny, 1979), and caprylic acid treatment (Mckinney and Parkinson, 1987). Protein content was measured after each purification step using Barford method (Barford *et al,* 1976). The efficiency of the purification steps was measured by 12% SDS-PAGE (1mm) under reduced conditions (Laemmli, 1970). Labeling of pAb with horseradish peroxidase (HRP) (Periodate method) was done (Tijssen and Kurstak, 1984).

Reactivity of anti-PSPA 26 pAbs in patients' sera and infected hamsters by sandwich ELISA: Sandwich ELISA was done with some modifications (Engvall and Perlmann, 1971), Wells of polystyrene microtitre plates (96-flat bottomed wells, M129A Dynatech) were coated with 100 µl/well of anti-purified PSPA 26 pAb at a concentration of 5 µg/ml in 0.6 M carbonate buffer pH 7.4, and incubated overnight at room temperature. The plates were then washed 3 times with washing buffer 0.1 M PBS, pH 7.4, then blocked with 200 µl/well of 0.1% fetal calf serum (FCS, Sigma) in 0.1 M PBS, pH 7.4 for 1 hr. at 37°C. 100µl of human and hamster sera infected with *S. hematobium, H. nana and Fasciola (F.) gigantic*a diluted (1/250) in washing buffer were added to each well after washing then incubated for 1 hr. at 37°C. The plates were washed 3 times with washing buffer. 100 µl of polyvalent- peroxidase conjugate (Sigma) diluted in washing buffer (1/1000) were dispensed into each well and the plates were incubated for 1 hr. at 37°C. The plates were washed 5 times with washing buffer. 100µl of substrate solution one tablet of O-phenylene diamine dihydrochloride (OPD) (Sigma) dissolved in 25ml of 0.05M.

Phosphate citrate buffer, pH 5 with peroxidase H_20_2 (Sigma) was added to each well and the plates were incubated in the dark at room temperature for 30 min. 50µl/well of 8 N H_2SO_4 was added to stop the enzyme's action in the substrate solution. The absorbance was measured at 492nm using ELISA reader.

Statistical analysis: Data are expressed as mean (M) ±standard deviation (SD). Statisti-

cal analysis was performed with the aid of the SPSS computer program (version windows 16.0).

Results

Purification by DEAE-Sephadex A-5o ion exchange chromatography to obtain the OD280 profile of the antigen fractions: The eluted antigen appeared as a single peak with a maximum OD value equal to 2.1 at fraction number 40 (the fraction with the highest protein content). The fractions from 23-56 were collected (Fig. 1).

Purification by gel filtration chromategraphy on Sephadex G-75 HR: The fractions from 23–56 were further purified by DEAE Sephadex G-75 gel filtration column chromatography and three peaks were obtained representing the column elution volume fractions which contain the highest protein content with OD value 1.6 at fraction number 15 (Fig. 2).

Antigen profile: The different purification methods results eluted protein fractions that were then analyzed by 12.5% SDS-PAGE under reducing condition and only one band appeared at 26 kDa which represent the PSPA 26 antigen (Fig. 3).

The antigenicity of the PSPA 26 antigen was tested by indirect ELISA technique. Serum samples from infected human with *S. haematobium* gave a strong reaction against *S. haematobium* PSPA 26 antigen with mean OD reading equal to 1.64 and no cross reactions were recorded with sera of patients infected with other parasites e.g., *Fasciola, Hymenolepis, H.nana* and *Ancylostoma*.

Table 1: Reactivity of purified target PSPA 26 antigen by indirect ELISA

Serum Samples	OD readings at 492 nm (m ± SD)
S. haematobium	2.63 ± 0.43
F. gigantic	0.26 ± 0.04
H. nana	0.13± 0.38
Ancylostoma	0.18 ± 0.09

OD= Optical density, SD= Standard deviation.

Production of against PSPA 26 IgG pAb: Anti-PSPA 26 sera were obtained by immunizing New Zealand white rabbits with PSPA 26 antigen. Blood samples were withdrawn from rabbits before and after each immunizing dose, tested for specific anti-*S. haematobium* antibodies by indirect ELISA. An increasing antibody level started 1 week after 1st booster dose and 3 days after 2nd booster dose immune sera gave a high titre against *S. haematobium* PSPA 26 (Fig. 4).

Purification of rabbit's anti-*S. haematobium* PSPA 26 IgG pAbs: IgG fraction of rabbit's anti-*S. haematobium* pAb was purified using different purification steps including ammonium sulfate precipitation method followed by 7% the caprylic acid precipitation method and finally by ion exchange chromatography method.

Total protein content of rabbit's crude serum containing anti- *S. haematobium* pAb was 12.5 mg/ml. using the 50% ammonium sulfate precipitation method, the protein content became 5.9 mg/ml, while following 7% caprylic acid precipitation method, the content dropped to 3.1 mg/ml. Protein content of highly the purified anti-*S. haematobium* PSPA 26 pAb subjected to ion exchange chromatography was 2.3mg/ml (Fig. 5). The purity of anti-*S. haematobium* PSPA 26 pAb after each step was assayed by 12% SDS-PAGE under the reduced conditions.

The analysis of 50% ammonium sulfate-precipitated proteins by 12% SDS-PAGE under reducing conditions showed that precipitated proteins appeared as several bands. The purified anti- *S. haematobium* PSPA 26 pAb after ion exchange chromategraphy was represented by 2 bands, L & H-chain bands at 31 & 53kDa, respectively. Anti-body was free from other proteins (Fig. 6).

The specificity of purified anti-*S. haematobium* PSPA 26 pAb by indirect ELISA diluted 1/250 in PBS/T buffer gave strong reactivity to *S. haematobium* PSPA 26. The OD

means reading at 492 nm for *S. haematobium* PSPA for *Fasciolia gigantica, Hymenolepis nana* and *Ancylostoma duodenal* infected sera, respectively (Tab. 2).

Table 2: Specificity of rabbit anti-*S.haematobium* PSPA 26 pAb against parasitic antigens by indirect ELISA

Parasitic antigen	OD readings at 492 nm (m ± SD)
S. haematobium	1.84 ± 0.47
F. gigantic	0.20 ± 0.08
H. nana	0.14 ± 0.04
Ancylostoma	0.18 ± 0.03

Anti- *S. haematobium* PSPA 26 IgG pAb was used for *S. haematobium* PSPA 26 in human and hamster sera, sandwich ELISA was used for preliminary standardization and optimization of different materials concentrations and dilutions before application. Using anti-*S. haematobium* PSPA 26 pAb as antigen capture antibody was evaluated by coating an ELISA plate with different concentrations of purified pAb (2.5, 5, 10, 20 and 40µg/ml) against one concentration of *S. haematobium* PSPA 26 (10µg/ml) to specify optimum concentration of coating antibodies to be used. By OD readings at 492nm, optimum concentration of coating antibodies was 10µg/ml (Fig. 7).

Concentrations of HRP conjugated rabbit anti-*S. haematobium* PSPA 26 pAb were evaluated to specify optimum concentration of the conjugate antibodies to be used. An ELISA plate was coated with one concentration of *S. haematobium* PSPA 26 antigen (20ug/ml) and different concen-trations of the conjugate antibodies (5, 10, 15, 25 & 50µg/ml) were added. By OD read-ings at 492nm, optimum concentration of HRP conjugated antibodies was 15µg/ml (Fig. 8).

Optimal conditions were reached after standardization, an ELISA plate was coated with one concentration of anti-*S. haematobium* PSPA 26 pAb (10ug/ml) and different concentrations of *S. haematobium* PSPA 26 antigen started from 0.005 to 10µg/ml by serial dilution. Standard antigen binding curve extended by plotting of various *S. haematobium* PSPA 26 concentrations against corresponding OD readings (Fig. 9). The assay lower limit was 0.001 µg/ml

In *S. haematobium* antigen, cut off values were calculated as mean OD reading of negative controls +2 standard deviation (SD) of mean, tested samples showing OD values > cut off value were considered positive for *S. haematobium.* Cut off value was 0.428. In *S. haematobium* PSPA 26 antigen in human sera, 32 cases (84.2%) were positive in acute patients, 6 cases were negative (15.7%). But, 19 chronic cases were positive (86.3%) and 3 cases were negative (13.6%).

In other helminthic parasites, 4 cases were positive (3 with *F. gigantica*, 1 with *H. nana* infection), other 14 cases were negative. All the control patients were negative. There was a statistical significant (*P<0.001* & *P<0.01*) difference compared to positive in the *S. haematobium* group and the other two tested (Tab. 3).

Table 3: *S. haematobium* PSPA 26 antigen detection in human sera by sandwich ELISA.

Groups	Positive cases		Negative cases	
	(n.)	OD (M± SD)	*(n.)*	OD (M± SD)
Healthy control (n= 60)	-	-	60	0.37±0.12
S. haemtobium (Acute) n = 38	32	1.85±0.63 ***	6	0.28±0.22
(Chronic) n= 22	19	0.98±0.39**	3	0.17±0.09
F. gigantica (n= 10)	3	0.78±0.13	5	0.24±0.06
H. nana (n=10)	1	0.90±0.21	9	0.27±0.15

Detection of *S. haematobium* PSPA 26 antigen in hamster sera showed positive in 30 cases (75%) of acute infected group, 10 animals were negative (25%). The 18 chronic infection were positive (90%), two were negative (10%). All control hamsters were negative. In group infected with other parasites, seven animals were positive (five

with *F. gigantica*, two with *H. nana*), the other eleven experimented with animals were negative (Tab. 4).

Table 4: *S. haematobium* PSPA 26 antigen detection in hamster' sera by sandwich ELISA.

Groups	Positive cases		Negative cases	
	(n)	OD (M± SD)	*(n)*	OD (M± SD)
Healthy control (n= 60)	-	-	60	0.34±0.13
S. haemtobium (Acute) n = 40	30	1.49±0.38 ***	10	0.23±0.19
(Chronic) n= 20	18	0.86±0.26**	2	0.21±0.07
Fasciola (n= 8)	5	0.50±0.19	3	0.23±0.27
H. nana (n=10)	2	0.54±0.20	8	0.21±0.15

n= Number; M= Mean; SD= Standard deviation. *** $P<0.001$ ** $P<0.01$.

Sensitivity of pAb against PSPA 26 was 84.2% for acute and 86.4 for chronic cases with an overall sensitivity was 85%. Specificity was 95% compared to control and 88.3% compared to patients with other parasites. PPV & NPV were 89.6% and 85%, respectively. In hamsters, sensitivity of acute and chronic animals was 83.3% and 90.0%, respectively, with an overall sensitivity of 80%. Specificity of pAb against PSPA 26 was 91.1% compared to control and 85% compared to animals with other parasites. The PPV and NPV were 88.3% and 82.4%, respectively (Tab. 5).

Table 5: Overall sensitivity, specificity, positive predictive value and negative predictive value of sandwich ELISA to detect PSPA 26 antigen in serum samples of *S. haematobium* infected animals and patients.

Samples	Sensitivity	Specificity	PPV	NPV
Human	85 %	95%	89.6%	85%
Hamster	80 %	91.1%	88.3%	82.4%

Infected humans and hamsters: The OD values of *S. haematobium* infected patients were obviously (P<0.001) higher than negative controls and parasitic infected patients, 51/60 cases were positive, other 9 were negative. There was slight cross reactivity as fasciolosis showed highest, followed by *H. nana*. In hamsters' sera, 48/60 were positive, other 12 were negative. There was slight observation of cross reactivity as well, as fasciolosis showed the highest followed by *H. nana*. Detection of PSPA 26 antigens in sera by sandwich ELISA gave sensitivity of 85% and specificity of 95% in humans and sensitivity of 80% and specificity of 91.1% in hamsters. Humans gave 89.6% & 88.3%, while hamsters gave 85% & 82.4% for PPV & NPV, respectively (Tab. 5).

Discussion

Although *S. haematobium* is the most frequent human schistosome, publications regarding it are particularly scarce (Brindley and Hotez, 2013). Schistosomiasis diagnosis using immunological method was more sensitive and specific than ordinary parasitologic ones that appeared clearly in diagnosis of early or low-intensity infections (Hamilton *et al,* 1998; Corachan *et al,* 2002). So, the current study used antigen detection in sera of patients and hamsters to evaluate its sensitivity and specificity in both early and late diagnosis of *S. haematobium*.

In this study, chromatography proved to be very effective in t isolation of candidate diagnostic antigens in others (Fagbemi *et al,* 1995, Guobadia *et al,* 1997; Kumar *et al,* 2008). The purified PSPA-26 showed only one band at 26 kDa which represent our target antigen. Due to PSPA-26 has clear technical advantages where it is not expensive to produce, present in large quantities (~10 mg/ml), can be produced from the serum of an immunized animal and can be isolated merely 2-3 months after the initial immunization, so facilitates their rapid study. In addition, PSPA-26 contains the entire antigen-specific antibody population that gave a statistically relevant idea overall picture of the immune response (Lipman *et al,* 2005).

In the current purified PSPA26 fraction

purified from adults of *Schistosoma* diagnosed schistosomiasis in acute and chronic stages of experimentally infected hamsters and humans using ELISA proved as a sensitive and specific protein for diagnosis.

In the present study, a large control numbers were used to avoid cross reactivity with other helminthes (Rietveld *et al,* 1987; Gha-ffar *et al,* 1991; Aronstein *et al,* 1986; Correa-Oliveira *et al,* 1988, Abbas *et al,* 1993). ELISA proved highly sensitive and highly specific (Anderson *et al,* 1999) in a very early phase of infection and might constitute a potentially useful method for diagnosis of schistosomiasis antibodies, antigens or even peptides. ELISA proved a useful for epidemiology in low endemic areas, with a high sensitivity and specificity for antigen and antibody detection in schistosomiasis (Kanamura *et al,* 2002; Oliveira *et al,* 2008). So, ELISA was utilized here.

On detection of PSPA 26 antigen in human sera by traditional sandwich ELISA showed positivity of acute cases of 84.2% & 86.3% in the chronic cases. The increase in the positivity percentage in the chronic cases was due to lower antigen concentration in sera during the early stages of infection. The study showed a very low cross reactivity compared to others (Gryseels *et al,* 2006). Antigen detection in infected hamsters' sera showed 75% positivity of acute infected group, and 90% of chronic one. High sensitivity (85% & 80%), specificity (95% & 91.1%), PPV (89.6% & 88.3%) and NPV (85% & 82.4%) of human and animal serum antibody, respectively, against PSPA-26 showed that PSPA-26 can be used in the diagnosis of schistosomiasis. Salah *et al*. (2006) detected SEA in serum samples of *S. haematobium* infected patients. They found that the procedure gave a sensitivity of 89% and a specificity of 100%. Also, the data agreed with Elkawaz and Ghaffarifar (2009) by ELISA reported 82% sensitivity and 95% specificity in *S. haematobium* patients sera. Stothard *et al.* (2009) performed SEA-ELISA using sera showed sensitivity of 89% and specificity of 70%. Sandwich ELISA using anti-*S. haematobium* PSPA-26 pAb as a detector significantly increased sensitivity, specificity and incidence of positivity towards higher the antigen detection in both acute and chronic stage patients. Definitive diagnosis of schistosomiasis in patients is a clinically hard challenge. Chronic patients pass few eggs in urine, often missed when other diagnostic methods (Weerakoon *et al,* 2015). Detection of PSPA 26 antigen in sera gave high sensitivity & specificity. Sandwich ELISA sensitivity was 85% in human sera & 80% in hamsters' sera. Specificity was 95% in patients & 91.1% in hamsters.

Conclusion

Sandwich ELISA proved sensitive for detection of *S. haematobium* in humans and hamsters. Also, detection of PSPA 26 antigen by ELISA assays might be particularly in diagnosis infection in early and late stages and valuable in areas with low prevalence of schistosomiasis and for the assessment of cure after chemotherapy.

Explanation of figures

Fig. 1: Eluted profile for purified PSPA 26 antigen by DEAE Sepharose CL-6B ion exchange chromatography.

Fig. 2: Eluted profile for the purified PSPA 26 antigen by Sephadex G-75 column.

Fig. 3: SDS-PAGE of target antigens eluted from affinity chromatography columns. Lane 1: Low molecular weight standard, Lane 2: Homogenate product, Lane 3: Target antigen eluted from DEAE Sepharose CL-6B, Lane 4: Target antigen eluted from Sephadex G-75. Protein content of the purified *S. haematobium* PSPA 26 was measured by Biorad assay giving a measurement of 3.8mg/ml.

Fig. 4: Reactivity of anti-*S. haematobium* PSPA 26 antisera (1/250) against *S. haematobium* cathepsin proteinase by indirect ELISA. a- Before b- Priming c- 1st booster d- 2nd booster e- Immunization

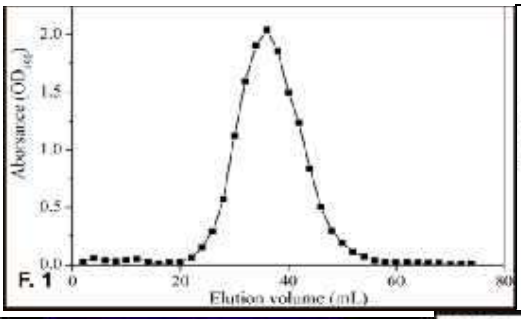
Fig. 5: OD280 profile of fraction obtained following rabbit's anti-*S. haematobium* PSPA 26 pAb purification by chromatography. eCluted IgG represented by a single peak with maximum OD value 2.6 at fraction number 9.

Fig. 6: 12% SDS-PAGE of anti-*S. haematobium* PSPA 26 pAb before and after purification. Lane 1: Molecular weight of standard protein, Lane 2: Crude anti-S. haematobium PSPA 26 pAb (before purification), Lane 3: Precipitated proteins after 50% ammonium sulfate treatment, Lane 4: Purified IgG-pAb after 7% caprylic acid treatment.

Fig. 7: Optimum concentration of the coating antibodies.

Fig. 8: Optimum concentration of the conjugate antibodies.

Fig. 9: Standard curve for *S. haematobium* PSPA 26 antigen detection



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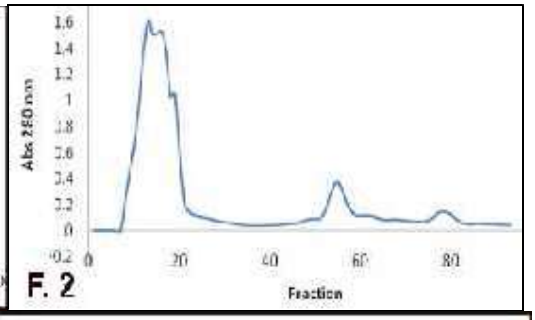

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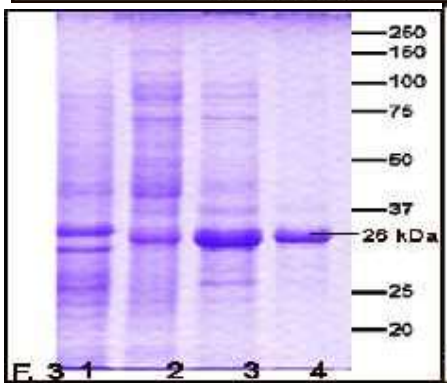

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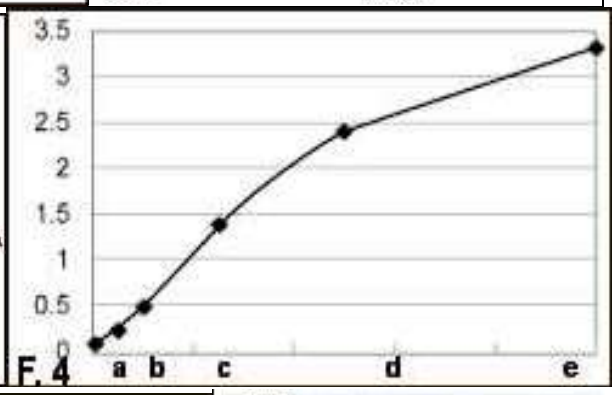

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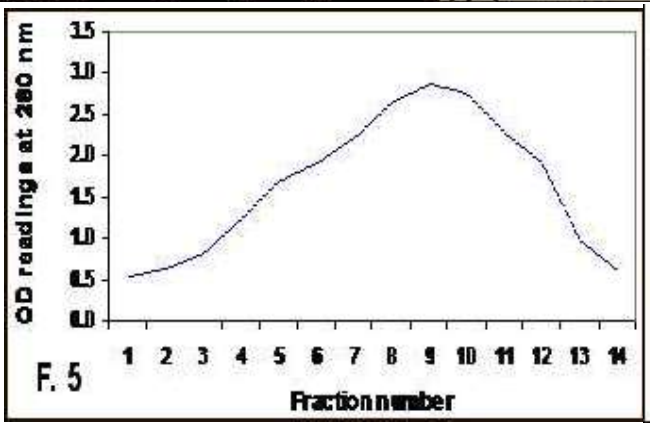
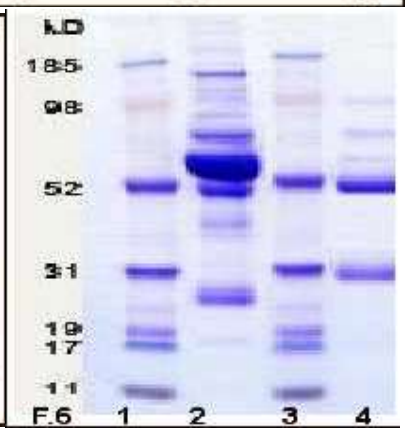


F. 5



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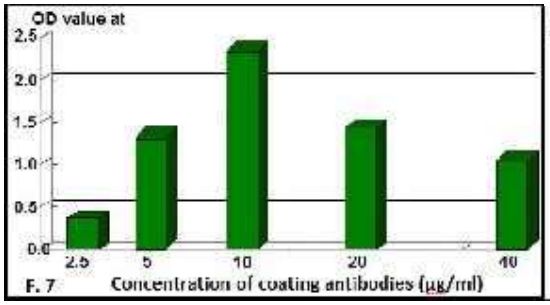

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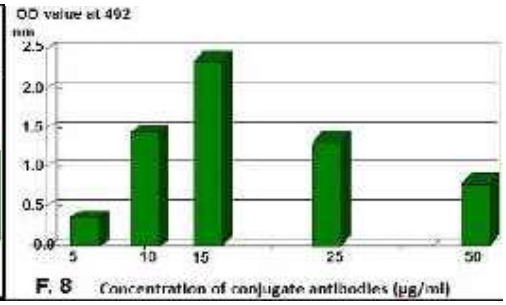

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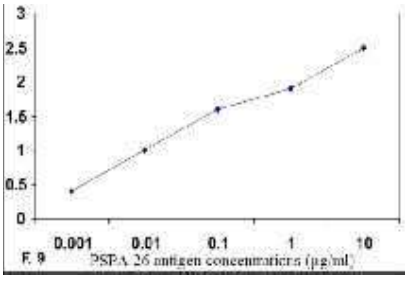

F. 9

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