# Home-made monoclonal antibody –based sandwich ELISA versus commercial fast dot- ELISA technique in the diagnosis of human schistosomiasis and fascioliasis

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**Abstract:** Two monoclonal antibodies (12D/10F) and (5F/6H) were prepared at Immunology Department, TBRI. The first was an IgM monoclonal antibody prepared against *S. mansoni* adult worm tegumental antigen and the second was of IgG subclass prepared against *F. gigantica* excretory / secretory products. Both monoclonals were evaluated by comparing the detection of specific *Schistosoma* circulating antigen (SCA) in serum and urine, and *Fasciola* circulating antigen in serum (FCA) and coproantigen by using MAb sandwich ELISA versus commercially available antigen capture fast dot-ELISA. Studied subjects comprised 42 *S. mansoni* infected patients, 35 *F. gigantica* infected patients, 30 patients harbouring parasites other than the parasite of infected group, and 20 healthy controls. The sensitivity and specificity of SCA assay in serum and urine by MAb sandwich ELISA was 92.9% and 96% for serum and 90.5% and 94% for urine respectively, compared to 71.4% and 76% for serum and 76.2% and 64% for urine respectively using fast dot-ELISA test. Accordingly, the diagnostic accuracy for MAb sandwich ELISA in both serum and urine was higher 94.6% and 92.4% respectively compared to 74% and 70% by fast dot-ELISA test. As well, the sensitivity and specificity of FCA assay in serum and stool by MAb sandwich ELISA was 97.1% and 96% for serum and 94.3% and 98% for stool respectively compared to 74.3% and 70% respectively for serum samples only using fast dot-ELISA test. Accordingly, the diagnostic accuracy by MAb sandwich ELISA was higher 96.5% and 96.5% for serum and stool respectively compared to 71.8% for serum only by fast dot-ELISA test. It is concluded that the home-made monoclonal antibodies prepared at Immunology Department, TBRI against *S. mansoni* and *F. gigantica* antigens showed high sensitivity and specificity and accordingly high diagnostic accuracy using sandwich ELISA compared to available fast dot-ELISA kits. This indicates the importance of using MAb sandwich ELISA as a confirmatory test for false negative results in field screening by fast dot-ELISA test.
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**Key words:** schistosomiasis; fascioliasis; monoclonal antibody; ELISA technique

## 1. Introduction

Diagnosis of schistosomiasis is usually based on the microcopic detection of eggs in stool and urine samples. This method has poor diagnostic efficiency when applied to individuals with low worm burden Wen et al., 2005). On the other hand, diagnosis of fascioliasis is achieved by finding the fluke eggs in feces. However, the flukes start releasing eggs in feces after 8 weeks of infection. So, the use of coprodiagnosis of human fascioliasis is often unreliable because the eggs are not found during the prepatent period (Berhe et al., 2004 and Gajewska et al., 2005). Immunodiagnostic assays for diagnosis of these two parasites have been developed and used for the detection of specific antibodies in serum. However these assays cannot differentiate between recent and past infection and has the problem of cross reactivity among different helminthic parasitic infections (Mott and Dixon, 1982). Antigen detection assays by monoclonal antibody (MAb) based-sandwich ELISA proved to be the most accurate technique for estimation of antigen concentration in serum samples of both schistosomal and fascioliasis patients and determination of disease activity (Silvana et al., 2001). In the case of *Schistosoma,* the use of monoclonal antibodies prepared against *S. mansoni* worm tegumental antigen has greatly increased the sensitivity and specificity of assays for detection of circulating schistosomal antigen (CSA) (Demerdash et al., 1995). Tegumental antigens develop within 3 hours of host penetration by cercariae and thus their detection would diagnose active *S. mansoni* infection very early, reflect worm burden and proved

to be an efficient immunodiagnostic tool for schistosomiasis (Davis, 1986; Hanallah et al., 2003). In fascioliasis, the use of monoclonal antibody (MAb) prepared against *Fasciola* excretory-secretory antigens (E/S Ags) as antigen detection assay can identify occult infections and provide a serological measure of parasite burden (Anuracpreeda et al., 2006). Several studies reported use of dot-ELISA for screening of anti-schistosomal antibodies ( e.g. Boctor et al., 1987) and for screening of anti-*Fasciola* antibodies (Intapan et al., 2003). Fast dot –ELISA (FD-ELISA) assays were described by Attallah et al., (1995, 1997 and 1999) for diagnosis of *S. mansoni* and *F. gigantica* infections using serum and urine. The aim of this work was to evaluate some home-made Mabs prepared at the Immunology Department, TBRI against schistosomal and *Fasciola* antigens, using sandwich ELISA, versus commercially available fast dot ELISA kits utilizing monoclonal antibodies against the same parasites.

## 2. Materials and methods

### 1. Studied Subjects:

This study was conducted on patients admitted to Tropical Medicine Department, Menoufiya University Hospitals and outpatients of Theodor Bilharz Research Institute (TBRI), Guiza. They were divided into two main groups. All patients and healthy volunteers were subjected to clinical and repeated parasitological stool examination using Kato-Katz technique (Martin and Beaver, 1968), merthiolate iodine formaldehyde-concentration (MIFC) method and formol ether concentration techniques (Erdman, 1981). Urine analysis was performed for all studied groups using sedimentation method (Peters et al., 1976). Ethical issues were strictly handled according to the International Ethical Guideling for Biomedical Research. Prior to stool and blood collection the purpose of the study was explained to all individually participate in this study.

**Group (1):** (a) 42 S. mansoni infected patients were selected for this study, based on the presence of Schistosoma eggs in their stool. (b) 30 patients harboring other parasites than Schistosoma [Fasciola (n= 10), hydatid cyst (n=10) and Wuchereria bancrofti (n=10)]. (c) 20 healthy volunteers.

**Group (2):** (a) 35 F. gigantica infected patients having the characteristic large operculated Fasciola eggs in stool. (b) 30 patients harboring other parasites than Fasciola [S. mansoni (n=10), hydatid cyst (n=10) and Wuchereria bancrofti (n=10)]. (c) 20 healthy volunteers. Both groups underwent detection of serum and urine circulating schistosomal antigen as well as serum circulating and coproantigen of Fasciola using MAbs raised against S. mansoni and F. gigantica by sandwich ELISA. All samples except coproantigen of Fasciola were detected by FD-ELISA.

**Processing of samples:**

Urine samples: 1 ml of freshly voided urine was boiled in water bath for 5 minutes and allowed to cool at room temperature before use.

Stool eluates: were prepared according to Espino et al. (1998) by adding approximately 3 parts of 0.01 M PBS, pH 7.2, containing 0.05% Tween 20 (PBST) to 1 part of stool in a centrifuge tube. The mixture was homogenized and then centrifuged at 900 x g for 3 minutes, after which the supernatant was aspirated and stored at -20 °C until used.

Blood samples: sera were separated and stored at -70 °C till required.

### 2. Preparation of Antigens:

#### 2.1. Adult worm tegumental antigen:

S. mansoni adult worm tegumental antigen (AWTA) was prepared from living worm according to Oaks et al. (1981).

#### 2.2. Fasciola gigantica excretory-secretory (E/S) products:

The E/S Ag was prepared from livers of cattle infected with F. gigantica according to Dalton and Hoffernen (1998).

### 3. Monoclonal Antibody Production:

#### 3.1. Preparation and Characterization of anti-S. mansoni MAbs:

BALB/c mice were immunized with S. mansoni (Sm) AWTA according to Gianfrigilia et al. (1983). Spleen cells were fused with non-secreting murine myeloma cells (P3X63 Ag.8) in the presence of 43% polyethylene glycol (Sigma) and cultured in 96 wells culture plates (Costar) to get hybridomas as modified from Galfre and Milstein (1981).

Hybdridomas were screened for anti-Sm AWTA antibodies by ELISA. Hybrids that were highly reactive to Sm AWTA and not reactive to Fasciola or Echinococous granulosus were cloned by limiting dilution method, using splenocyte feeder layer according to Galfre and Milstein (1981). Isotype analysis of MAb 12D/10F was done and proved to be of IgM class using a mouse hybridoma subtyping kit (Boehringer). Hybridoma cells were injected intraperitoneally into BALB/c mice for ascites production. Monoclonal antibody 12D/10F (IgM) was purified from ascitic fluid by euglobulin precipitation in distilled water according to Garcia-Gonzalez et al. (1988). Labeling of MAbs with

horseradish peroxidase conjugate (Sigma) was performecd by periodate method according to Nakane and Kawaoi (1974).

**3.2. Preparation and characterization of anti-Fasciola MAbs:**

Spleen cells from BALB/c mice, immunized with Fasciola gigantica E/S products according to Gianfriglia et al. (1983) were fused as mentioned above according to Galfre and Milstein (1981), screened for anti-Fasciola antibodies by ELISA, and highly reactive hybrids were cloned using a splenocyte feeder layer. Specificity determination was done by indirect ELISA as described by Demerdash et al. (1995) using Schistosoma, Wucherriria bancrofti and hydatid fluid antigens. Only those MAbs showing no cross reactivity with other parasites were selected. Hybridoma cells were injected intraperitoneally into BALB/c mice for ascites production. The produced ascites was characterized by detection of isotypes and the chemical nature of monoclonal-defined epitopes (De Jonge et al. 1988). Selected MAbs (5F/6H) of IgG1 subclass were purified by ammonium sulfate treatment followed by ion exchange chromatography according to Goding (1986). The MAb (5F/6H) was labeled by horse-radish peroxidase (Sigma) according to Tijssen and Kurstak (1984).

**4. Evaluation of Circulating Antigens by Sandwich-ELISA:**

This method was performed, with some modification from the original method of Engvall and Perlmann (1971). Quantification of the target antigen was achieved by sandwich ELISA using the two selected MAbs IgM (12D/10F) raised against S. mansoni antigen and IgG1 (5F/6H) raised against F.gigantica excretory/secretory (E/S) products. The technique was performed according to Demerdash et al. (1995). Briefly, microplates (Thomas scientific, Swedesboro, NJ, USA) were coated with 100 µl/ well of purified MAb (10µg/ml) in 0.1M carbonate buffer, pH 9.6 and left overnight at room temperature. Plates were blocked by addition of 200 µl of 5% fetal calf serum (FCS, Sera Med.) in 0.02 M phosphate buffer saline containing 0.05% tween 20 (PBS/T) pH 7.2 for 2 hours at 37°C. The undiluted patients' sera, urine or stool elutes were added 100 µl/ well and incubated for 2 hour at 37°C. Plates were washed with PBS/T; 100 µl/ well of 1:1000 dilution of peroxidase conjugated MAb (5 µg/ml) in PBS/T containing 1% of FCS were added; plates were incubated for 2 hours at room temperature, then thoroughly washed with PBS/T. The reaction was visualized by addition of 100 µl/ well of ortho-phenylene diamine (Sigma) for 30 minutes in the dark at room temperature; the reaction was stopped by adding 50 µl/ well of 8N $H_2SO_4$, then plates were read at 492 nm using an ELISA reader (Microplate Reader, Bio-Rad, Richmond CA, USA). Optimum concentrations/dilutions were achieved by block titration.

**5. Fast-Dot ELISA Technique:**

This technique was performed according to Attallah et al. (1999) using commercial kits obtained from ABC Diagnostics (Industrial Zone, New Damietta, Egypt). Fast detection of S. mansoni and F. gigantica circulating antigens by specific monoclonal antibodies in serum and urine of all groups were performed (no available kits for coprological detection of Fasciola CAg in stool). Briefly, the included device was wetted by three drops of washing solution (A); after complete absorption, 200 µl of the serum or urine sample dilution were added (20 µl serum or urine optimum sample with 200 µl sample diluent with mixing). Two drops of antibody solution (B) were added and allowed to be completely absorbed; then the device was washed by three drops of solution (A). Two drops of conjugated solution (C) were added and left for complete absorbance; each device was washed by three drops of washing buffer (A) and allowed until completely absorbed. Then 100 µl of substrate solution (D) were added, and the device was left for 2 minutes; then the reaction was stopped by adding two drops of stopping solution. After complete absorbance, the violet color intensity around control dot was recorded visually .Appearance of violet color reaction whatever its intensity was considered to be positive (+) and no violet color around the control ring was considered as negative (−); absence of control dot means invalid test.

**6. Data analysis:**

The mean values for each parasite (mean ± SD) were calculated from the mean values of the healthy individuals. The cut off value was calculated as the mean OD reading of negative controls ± 2 standard deviation and OD levels equal to or less than cut off value were considered negative. The diagnostic sensitivity, specificity, accuracy and predictive values were calculated using the method of Galen (1980). These values were calculated and expressed as follows:

Sensitivity: [number of true positives/(number of true positives + number of false negatives) × 100]

Specificity**:** [number of true negatives/(number of false positives + number of true negatives) × 100]

Accuracy: [number of true positives + number of true negatives/(number of true positives + number of

false negatives + number of false positives + number of true negatives) × 100]
Positive predictive value: [number of true positives/(number of false positives + number of true positives) × 100]
Negative predictive value: [number of true negatives/(number of false negatives + number of true negatives) × 100].

## 3. Result Analysis

Levels of circulating schistosomal or fasciola antigens in serum, urine and stool of different studied groups were measured by MAb sandwich ELISA at O.D. readings equal to 492 nm. The cut off value was calculated as the mean O.D. reading of negative controls +2 standard deviation of the mean. The O.D. readings equal to or less than cut off value were considered negative while those readings greater than the cut off value were considered positive. On the other hand, the levels of these circulating antigens measured by fast dot ELISA were calculated from the color intensity of the test. The schistosomal antigen levels in sera and urine of schistosomiasis patients, normal group and other parasites group detected by the two methods are illustrated in Fig 1 & Table 1. The Fasciola antigen levels in sera and stool of all studied groups detected by the two methods are illustrated in (Fig 2 & Table 2. The specificity, sensitivity, diagnostic accuracy, positive predictive and negative predictive values of these results were calculated and illustrated in Table 3 & Table 4.

**Table (1): Detection of SCA using MAb sandwich ELISA and fast dot-ELISA in different groups.**

| Patients | Total no. | Schistosomiasis | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | MAb sandwich ELISA | | | | Fast dot-ELISA | | | |
| | | Serum | | Urine | | Serum | | Urine | |
| | | + ve | -ve | +ve | -ve | +ve | -ve | +ve | -ve |
| Schistosomiasis | 42 | 39 | 3 | 40 | 2 | 30 | 12 | 32 | 10 |
| Other Parasites | 30 | 2 | 28 | 3 | 27 | 8 | 22 | 10 | 20 |
| Healthy Control | 20 | - | 20 | - | 20 | 4 | 16 | 8 | 12 |

SCA: *Schistosome* circulating antigen. MAb: monoclonal antibody

**Table (2): Detection of FCA using MAb sandwich ELISA and fast dot- ELISA in different groups.**

| Patients | Total No. | Fascioliasis | | | | | |
|---|---|---|---|---|---|---|---|
| | | MAb sandwich ELISA | | | | Fast dot-ELISA | |
| | | Serum | | stool | | Serum | |
| | | + ve | -ve | +ve | -ve | +ve | -ve |
| Fascioliasis | 35 | 34 | 1 | 32 | 3 | 26 | 9 |
| Other Parasites | 30 | 1 | 29 | 1 | 29 | 10 | 20 |
| Healthy Control | 20 | - | 20 | - | 20 | 5 | 15 |

FCA: *Fasciola* circulating antigen. MAb: monoclonal antibody.

Table (3): Percent sensitivity, specificity, diagnostic accuracy, positive and negative predictive values of anti-schistosomal MAb using sandwich ELISA and fast dot-ELISA for detection of CSAg in serum and urine.

| Item | Schistosomiasis Sandwich ELISA | | Schistosomiasis Fast dot-ELISA | |
|---|---|---|---|---|
| | Serum | Urine | Serum | Urine |
| Sensitivity | 92.9 | 90.5 | 71.4 | 76.2 |
| Specificity | 96 | 94 | 76 | 64 |
| Accuracy | 94.6 | 92.4 | 74 | 70 |
| Positive predictive | 95.1 | 92.7 | 71.4 | 64 |
| Negative predictive | 94.1 | 92.2 | 76 | 76.2 |

Table (4): Percent sensitivity, specificity, accuracy, positive and negative predictive values of anti-*Fasciola* MAb using sandwich ELISA and fast dot-ELISA for detection of FCA in serum and stool.

| Item | Fascioliasis Sandwich ELISA | | Fascioliasis Fast dot-ELISA |
|---|---|---|---|
| | Serum | Stool | Serum |
| Sensitivity | 97.1 | 94.3 | 74.3 |
| Specificity | 96 | 98 | 70 |
| Accuracy | 96.5 | 96.5 | 71.8 |
| Positive predictive | 94.4 | 97 | 63.4 |
| Negative predictive | 98 | 96 | 79.5 |

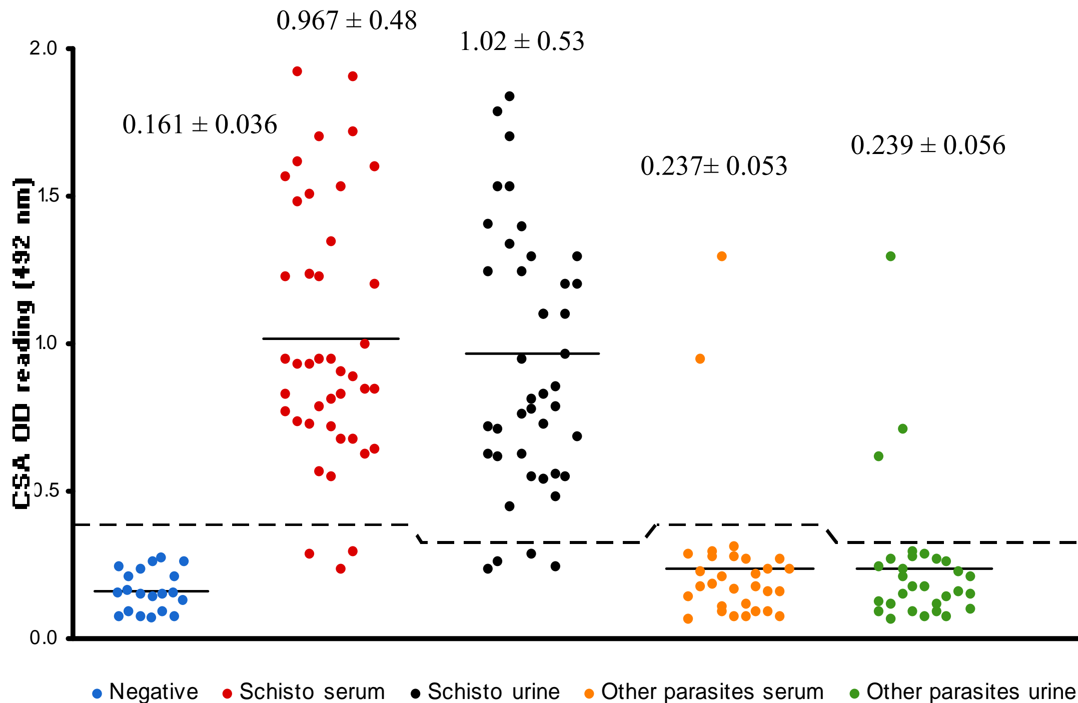


Fig. (1): circulating antigen level (OD at 492 nm) in serum and urine samples of studied groups using MAbs sandwich ELISA. Each point represents SCAg level of a single patient.

------- represents the cut-off value (lower detection limit) of the assay (0.384 in serum & 0.32 in urine).

____ represent mean of O.D. value.

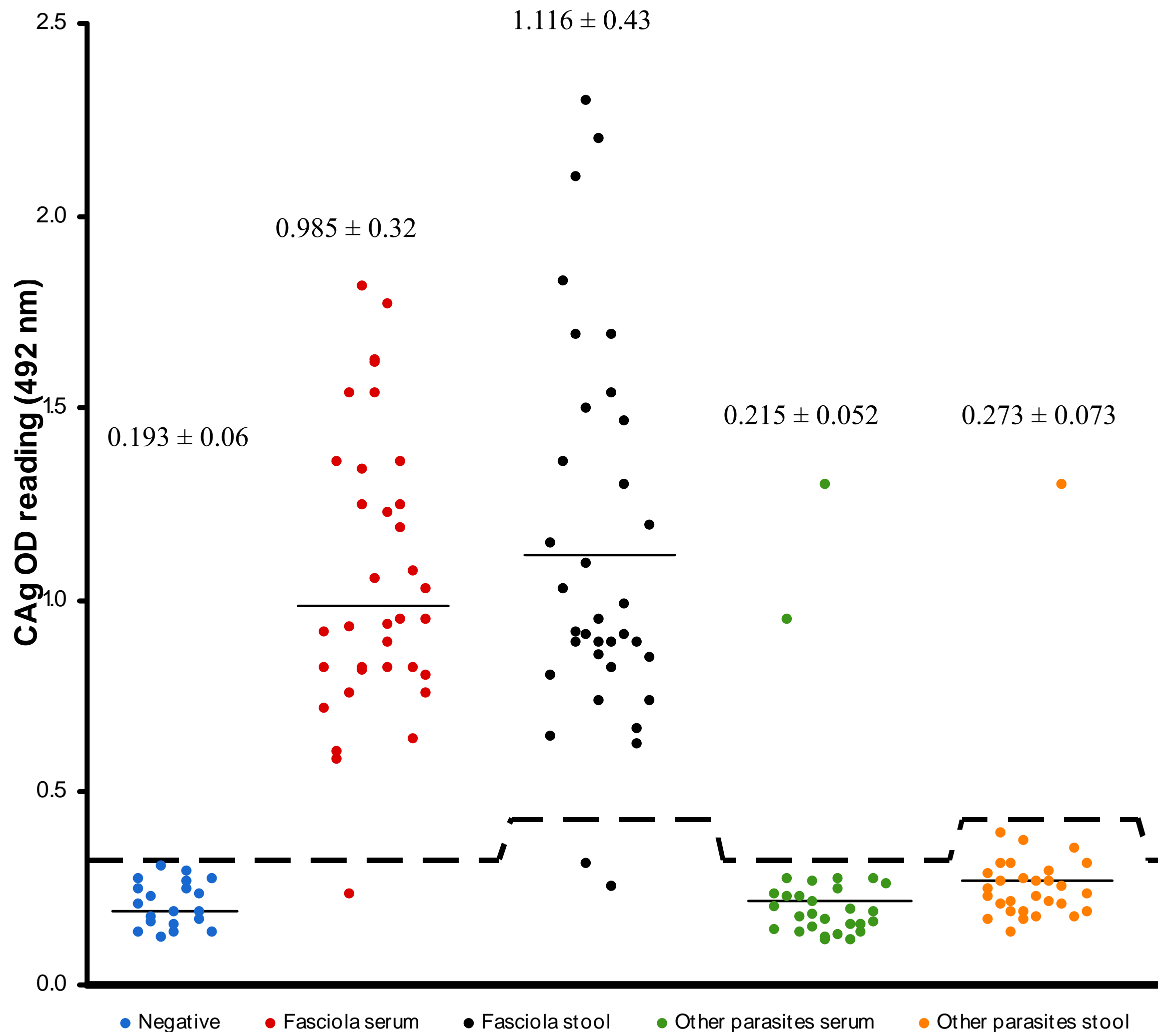


Fig. (2): *Fasciola* sero and copro circulating antigne level (OD at 492 nm) in serum and stool samples of studied groups using MAb sandwich ELISA. Each point represents FCAg level of a single patient.
------- represents the cut-off value (lower detection limit) of the assay (0.324 in serum & 0.428 in stool).
____ represent mean of O.D. values.

**4. Discussion**

Parasitological screening has many drawbacks; it leaves many infected subjects undiagnosed (Berhe et al., 2004). So, sensitive and specific diagnostic tools are necessary in order to treat patients early and to avoid the major clinical complications caused by the parasite (Estuningsih et al., 2004). Although higher sensitivity was reported for antibody detection using partially purified fractions of *Schistosoma* and *Fasciola* antigens, avoiding cross-reactions to antibodies induced by other helminthic infections is quite impossible (Van Lieshout et al., 2000). *Schistosoma* circulating antigens were used to indicate the infection intensity and to assess cure (Salah et al., 2006). The use of MAbs has greatly increased the sensitivity and specificity of the assays

for detection of circulating schistosome antigen (Van Lieshout et al., 2000). Mahmoud et al. (2002) demonstrated the effectiveness of CSA detection by sandwich ELISA using anti- *S. mansoni* SEA MAb in diagnosis of recent *S. mansoni* infection before the appearance of parasitological parameters. Mansour et al. (1998) demonstrated the value of the use of anti-*Fasciola* monoclonal antibodies for detection of circulating Fasciola *antigen* as an indicator of the activity of fascioloiasis infection in serum. Immunodiagnosis needs a sophisticated laboratory work, hence cost and training facilities. To overcome the clinical and direct parasitological diagnostic problems, there is a need for a reliable, simple, cost-effective diagnostic tool to determine parasitic infections. Attallah et al. (1999) developed a test on the basis of an IgG2a MAb for the detection of *Schistosoma* circulating antigen excreted in urine; they concluded that, fast dot-ELISA (FDA) test has a number of advantages that makes it a preferable technique over the other diagnostic assays. It is a simple, rapid, noninvasive and sensitive assay for the detection of circulating antigens among humans with all clinical stages. This assay is applicable in the field and for mass screening and control programs. Mansour et al. (2009) demonstrated a new technique dot-immunogold filtration assay (DIGFA) for rapid detection of antigenaemia in the sera of schistosomiasis patients.

It is rapid and simple procedure with clear visual interpretation of results that makes it particularly suitable for field testing and epidemiological surveys. This technique exhibits a similar sensitivity and specificity as sandwich ELISA. Mohamed et al. (2007) used latex agglutination (ALT) for CSA assay as a valuable applicable screening diagnostic technique in field survey especially for urine samples. The main shortcoming of these techniques is that the results are only qualitative and all authors recommended a confirmatory sandwich ELISA for CSA assessment in sera or urine for query false negative results.

In this study, a MAb prepared against *S. mansoni* worm tegumental antigen (12D/10F) was evaluated for its efficacy in immunodiagnosis of active *S. mansoni* infection using patients' serum and urine samples. At the same time a Mab prepared against *F.gigantica* E/S products antigen (5F/6H) of IgG1 subclass was also evaluated for its efficacy in immunodiagnosis of active fasciliasis infection in patients' serum and stool samples. The results of CSA assay in seum and urine by MAb sandwich ELISA were compared with fast dot-ELISA as a reasonable commercial diagnostic test for CSA detection in serum and urine. Similarly, the results of CFA assay in serum and stool by Mab sandwich ELISA were compared with fast dot-ELISA test in serum (no available test for CFA in stool samples).

Our results showed that the sensitivity and specificity of both CSA and CFA assays by MAb sandwich ELISA were higher than those by fast dot-ELISA. Therefore, the diagnostic accuracy of MAb-sandwich ELISA for both CSA and CFA was higher compared to fast-dot ELISA test.

The false positivity among three cases of other parasites group may be attributed to the fact that they came from endemic eareas and were not subjected to rectal endoscopy.

These results agree with those of Demerdash et al. (1995), Hanallah et al (2003) and El-Bassiouny et al. (2005); they used different MAbs-based sandwich ELISA for detection of CSA in both serum and urine samples of *S. mansoni* infected patients with high sensitivity and specificity.

In addition, Van Etten et al. (1994) and Russell et al. (2006) concluded that detection of CSA in urine has a potential for development of non-invasive rapid diagnostic screening test, while serum antigen detection may provide a more direct measure of worm burden for epidemiological research (Polman et al., 2000). Also the level of CSA in serum and urine was positively correlated with the number of eggs excreted in stool of schistosomiasis patients, denoting the reliability of CSA detection as an indicator for intensity of infection and monitor of cure instead of using rectal snip invasive technique (Hendawy et al., 2006).

Our results of CFA coproantigen are in agreement with those detected by Estunngsih et al. (2004). They used MAbs to *Fasciola gigantica* E/S antigens for detection of coprologic antigens of infected sheep. They concluded that applying MAb-based sandwich ELISA to stool samples was able to detect the immature fluke in patent period. In addition, following antihelminthic treatment and removal of parasites, the anti-*Fasciola* antibody levels still remained high for at least 6 weeks when the levels of coproantigens were no longer detected in the faeces within 2 weeks of treatment. Hassan et al. (2008) reported that 26-28 kDa coproantigen proved of high sensitivity and specificity in diagnosis of fascioliasis, with positive correlation between numbers of *F. gigantica* egg per gm faeces and mean sandwich ELISA O.D.

## 5. Conclusion

The home-made monoclonal antibodies prepared at Immunology Department, TBRI against *S. mansoni* and *F. gigantica* antigens showed high

sensitivity and specificity and accordingly high diagnostic accuracy using sandwich ELISA compared to available fast dot-ELISA kits. This indicates the importance of using MAb sandwich ELISA as a confirmatory test for false negative results in field screening by fast dot-ELISA technique.

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**References**

1. Attallah, A. M.; El-Masry, S. A.; Rizk, H.;Ismail, H.; El-Bendary, M.; Handoussa, A. E.; El Shazly, A.M. and Arafa, M. A. (1997): Fast- Dot ELISA using urine, a rapid and dependable field assay for diagnosis of schistosomiasis. J. Egypt. Soc. Parasitol., 27(1): 279-289.
2. Anuracpreeda, P.; Wanichanon, C.; Chaithirayanon, K.; Preyavichyapugdee, N. and Sobbon, P. (2006): Distribution of 28.5 kDa antigen in the tegument of adult *Fasciola gigantica.* Acta Trop., 100 (1-2): 31-40.
3. Attallah, A. M.; El-Masry, S.; Rizk, H.; Ismail, H.; El-Bendary, M.; Handoussa, A. E. and Ezzat, F. (1995): Fast- Dot ELISA: A rapid, simple, sensitive, specific and field applicable assay for diagnosis of human schistosomiasis Trans. Roy. Sec. Trop. Med. Hyg., 84:554-555.
4. Attallah, A. M.; Younes, E.; Ismail, H.; El Masry, S. A. Tabll, A.; Elenein, A. A. and El Ghawalby, N. A. (1999): Immunochemical characterization and diagnostic potential of a 63 KDa *Schistosoma* antigen. Am. J. Trop. Med. Hyg., 60(3):493-497.
5. Berhe, N.; Medhin, G.; Erko, B.; Smith, T.; Gedamu, S.; Bereded, D.; Moore, R.; Habte, E.; Redda, A.; Gebre-Michael, T. and Gundersen, S.G. (2004): Variations in helminth faecal egg counts in Kato-Katz thick smears and their implications in assessing infection status with *Schistosoma mansoni*. Acta Trop., 92: 205–212.
6. Boctor, F. N.; Stek, M. S.; Peter, J. B. and Kamal, R. (1987): Simplification and standardization of Dot-ELISA for human schistosomiasis *mansoni*. J. Parasitol., 73: 589-592.
7. Dalton, J.P. and Heffernan, M. (1989): Thio l protease released in vitro by *Fasciola hepatica*. Mol. Biochem. Parasitol., 35: 161-166.
8. Davis, A. (1986): Recent advances in schistosomiasis: Q. J. Medicine, 58: 95-110.
9. De Jonge, N.; Gryseels, B.; Hilbrath, G. W.; Polederman, A.M. and Deelder, A. M. (1988): Detection of circulating anodic antigen by ELISA for seroepidemiology of schistosomiasis *mansoni*. Trans. R. Soc. Trop. Med. Hyg., 82: 591-594
10. Demerdash, Z.; Mohamed, S. H.; Shaker, Z. A.; Hassan, S. I., El Attar, G. M.; Saad-Eldin, H. A.; Abadeer, N. I. and Mansour, M. (1995): Detection of circulating schistosome antigens in serum and urine of schistosomiasis patients and assessment of cure by monoclonal antibody. J. Egypt Soc. Parasitol., 25(2): 471-484.
11. El-Bassiouny, A.; Salah, F.; Kamel, M. et al. (2005): Effective diagnosis of active schistosomiasis by a pair of IgG monoclonal antibodies against *Schistosoma mansoni* soluble egg antigen. Egypt J. Med. Sci., 26(2): 369-382.
12. Engevall, E. and Perlmann, P. (1971): ELISA, quantitative assay for IgG. J. Immunohistochem., 8: 871-874.
13. Erdman, D. (1981): Clinical comparison of ethyl acetate and diethyl ether sedimentation technique. J. Clin. Microbiol., l4: 483-485.
14. Espino, A.; Diaz, A.; Perez, A. et al. (1998): Dynamics of antigenemia and coproantigens during a human *Fasciola hepatica* outbreak. J. Clin. Microbiol., 36: 2723–2726.
15. Estunngsih, E.; Widjayanti, S.; Adiwinata, G. and Piedrafita, D. (2004): Detection of coproantigens by sandwich ELISA in sheep experimentally infected with *Fasciola gigantica.* Trop. Biomed., 21(2):51-6.
16. Gajewska, A.; Smaga-Kozlowska, K. and Wisniewski, M. (2005): Pathological changes of liver in infection of *Fasciola hepatica*. Wiad Parazytol., 51:115-123.
17. Galen, R. S. (1980): Predictive value and efficiency of laboratory testing. Pediatr. Clin. North. Am., 27: 861–869.
18. Galfre, G. and Milstein, C. (1981): Properties of monoclonal antibodies: Strategies and procedures. Meth. Enzymol., 73: 3-7.
19. Garcia-Gonzalez, M.; Bettinger, S.; Ou, S.; Oliver, P.; Kadouch, J. and Pourletty, P. (1988): Purification of murine IgG3 and IgM monoclonal antibodies by euglobulin precipitation. J. Immunol. Meth, 111: 17-23.
20. Gianfrigilia, A.; Armellini, D. and Massone, A. et al. (1983): Simple immunization protocol for high frequency production of soluble antigen specific hybridomas. Hybridoma, 2: 451-457.
21. Goding, J. W. (1986): Monoclonal antibodies: Principles and Practice. Alden Press Oxford, London and Northampton. P: 112-116.
22. Hanallah, S. B.; Mohamed, S. H.; Maher, K. M.; Amer, N. M.; Sayed, H. A.; Demerdash, Z. A. and Shaker, Z. A. (2003): Amonoclonal antibody against tegument of adult *S. mansoni* worms detects active schistosomal infection. Kasr. El Aini Med. J., 9:209-223.
23. Hassan, A. A.; El-Bahy, M. M.; Abou-Zinadah, N. Y. and Shalaby, H. A. (2008): The diagnostic

efficacy of *Fasciola gigantica* coproantigen in naturally infected cattle and buffaloes. J. Egypt Soc. Parasitol., 38(1): 115-30.
24. Hendawy, M. A.; Mohamed, S. H. and Ibrahim, R.A. (2006): Nitrocellulose cassettes (NCC) as a field applicable technique for rapied and effective diagnosis of schistosomiasis and fascioliasis. New Egypt J. Med., 35(6): 86-92.
25. Intapan, P. M.; Maleewong, W.; Nateeworanart, S.; Wongkham, C.; Pipitgool, V.; Sukolapong, V. and Sangmaneedet, S. (2003): Immunodiagnosis of human fascioliasis using an antigen of *Fasciola gigantica* adult worm with the molecular mass of 27 KDa by a DOT-ELISA., Southeast Asian J. Trop. Med. Pub. Health, 34 (4): 713-717.
26. Mahmoud, F. S.; Hassanein, H. I. and Mohamed, S.H. et al. (2002): The dynamics of serum and hepatic schistosomal antigens in two different strains of mice. Egypt J. Schisto. Infect. Endem. Dis., 24: 19-36.
27. Mansour, W. A.; El-Assal, F.; El-Mahdy, M. M.; Hendawy, M. A.; Salem, R. and Demerdash, Z. A. (2009): A monoclonal antibody-based dot immunogold filteration assay: a rapid field applicable technique for immubodiagnosis of active schistosomiasis. Australian J. of Basic and Applied Science, 3: 784-789.
28. Mansour, W. A.; Kaddah, M. A.; Shaker, Z. A.; Al-Assal, F. M. and Derbala, M. F. (1998): A monoclonal antibody diagnosis active *Fasciola* infection in humans. J. Egypt. Soc. Parasitol., 28: 711-727.
29. Martin, L. and Beaver, P. (1968): Evaluation of Kato thick smear technique for quantitative diagnosis of helminth infection. Am. J. Trop. Med. Hyg., 17: 382-391.
30. Mohamed, S. H.; Rabia, I. B. and Diab, T. M (2007): A monoclonal antibody-based Latex agglutination test for detection of circulating schistosomal antigens in human *Schistosomiasis mansoni*. The new Egyptian J. of Med. 37: 168-174.
31. Mott. P. K. and Dixon, H. (1982): Collaborative study on antigens for immunodiagnosis of *Schistosomiasis mansoni*. Bule WHO, 63: 729-753.
32. Nakane PK and kawaoi A (1974). Peroxidase-labeled antibody: A new method of conjugtion. J Histochem Cytochem, 22: 1084-1091.
33. Oaks, J. A.; Cain, A.; Mower, D. A. et al. (1981): Distribution and removal of the tegument from *Schistosoma mansoni* withtriton X-100. J. Parasitol., 67: 761-774.
34. Peters, P.A.; Warren, K. S. and Mahmoud, A. F. (1976): Rapid, accurate quantification of schistosome eggs via nuclopore filters. J. Parasitol., 62: 154-155.
35. Polman, K.; Diakhate, M.M.; Engels, D.; Nahimana, S.; Van Dam, G.J.; Ferreira, S.F.; Deelder, A.M. and Gryseels, B. (2000): Specificity of circulating antigen detection for schistosomiasis *mansoni* in Senegal and Burundi specific serum CAA assay remains the method of choice. Trop. Med. Int. Health., 5: 534–537.
36. Russell S. J.; Narcis, B.; Kabatereine; Edridah, M.; Tukahebwa; Francis K.; David R.; William M.; Joanne, P.; Webster and Alan F. (2006): Use of circulating cathodic antigen (CCA) dipsticks for detection of intestinal and urinary schistosomiasis. Acta Tropica., 97: 219–228.
37. Salah, F.; ElBassiouuny, A.; Rabia, I; Demerdash, Z.; Roshdy, M. and Shaker, Z. (2006): Human schistosomiasis *haematobium*: effective diagnosis of active infection using a pair of monoclonal antibodies against soluble egg antigen. Parasitol. Res.,99 (5):528-33.
38. Silvana, C.; Mo´nica, I.; Rodrı´gueza, E. A.; Guarneraa, Carlos, C.; Tamara, T.; Sergio, O. and Angela (2001): Immunodiagnosis of fasciolosis using recombinant procathepsin Lcystein proteinase. Diagnostic Microbiology and Infectious Disease, 41: 43–49.
39. Tijssen, P. and Kurstak, P. (1984): Highly efficient and simple methods for the preparation of peroxidase-antibody conjugate for enzyme immunoassays. Anal. Biochem., 136: 451.
40. Van Etten, l.; Folman, C.; Eggelte, T.; Kremenser, P. and Deelder, A. M. (1994): Rapid diagnosis of schistosomiasis by antigen detection in urine with a reagent strip. J. Cli. Microbiol., 32: 2404-2406.
41. Van Lieshout, L.; Polderman, A. M. and Deelder, A. M. (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.
42. Wen, L. Y.; Chen, J. H.; Ding, J. Z.; Zhang, J. F.; and Zhang, J. (2005): Evaluation on the applied value of the dot immunogold filtration assay (DIGFA) for rapid detection of anti-*Schistosoma japonicum* antibody. Acta Tropica, 96: 142-147.

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