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# IMMUNO-MAGNETIC BEADS ELISA FOR DIAGNOSIS OF *SCHISTOSOMA MANSONI* INFECTION

**By**

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**Abstract**

Diagnosis of *Schistosoma mansoni* infection is a challenge in low transmission regions. Serological diagnosis proved more sensitive and extensively used to detect anti- *Schistosoma* antibodies. This study evaluated the immunomagnetic beads-enzyme-linked immunosorbent assay (IMB-ELISA) for diagnosing *S. mansoni* infection in comparison with the commercial IHA, indirect ELISA and microscopy stool examination.

A total of 100 subjects were divided into four groups (25 each). G1: cases actively passing *S. mansoni* eggs, G2: chronic cases of *S. mansoni*, G3: cases with other parasitosis and G4: normal healthy subjects.

*S. mansoni* diagnostic rate was 25%, 46%, 48%, & 50% by parasitological, IHA, Indirect ELISA & IMB-ELISA, respectively. In G1, the four tests gave positivity rates of 100%, 88%, 96%, & 100%, respectively. In G2, the four tests gave 0%, 84%, 88% & 96%, respectively. Sensitivity of various tests were estimated in relation to total true positive *S. mansoni* cases (G1 & G2), and gave 50%, 86%, 92% & 98% for the applied tests, respectively. Cross-reactivity rates were estimated in relation to *S. mansoni* negative cases (G3 & G4). Specificity rates were 100%, 94%, 96% & 98%, respectively.

**Key words:** Fayoum University Hospitals, *Schistosoma mansoni*, Magnetic beads, ELISA, IHA

## Introduction

Schistosomiasis endemicity in both tropical and subtropical countries forms great health troubles, with around 200 million people infected and 700 million people at infection risk worldwide (WHO, 2012). WHO (2016) estimated that treatment must be provided for at least 206.4 million people, and only 89 million people of them were reported as treated. The WHO control plan depends on large-scale periodic population treatment to decrease the disease transmission, which necessitates prior diagnostic screening (WHO, 2018). In addition, sensitive and specific diagnostic tests are needed for early detection of cases prior development of any complications (WHO, 2018).

Microscopic egg detection, especially by Kato-Katz (KK) is the standard method for quantitative detection of *S. mansoni* eggs in fecal samples due to its high specificity (WHO, 2018**)**. The test, however, is labour-intensive, time-consuming, and to some extent messy due to low worm burden and/or high day to day fluctuation in egg counts. In addition, the test should be repeated to increase its sensitivity which raises its cost, and the laboratory hazards of handling stool samples.

For the regions where there is low disease transmission, follow up of the treated subjects necessitates using highly sensitive diagnostic techniques to replace copro-scopic searching for eggs which is a waste of time and efforts. False-negative subjects will act to maintain the disease endemicity, despite the application of control measures (Espirito-Santo *et al*, 2014; WHO, 2018).

PCR was utilized for schistosomiasis detection using various test forms. However, the method is not standardized and its sensitivity needs to be confirmed, in addition to the high cost needed for the equipment, chemicals and training the personnel exceeding the budget of primary health-care settings (Xu *et al*, 2011).

Immunological techniques for antibody detection have been widely used since they attracted the scientists' attention by their advantages over stool examination as being highly sensitive and easy for use. These included forms of indirect immunofluorescent-

antibody tests (IFATs), IHAs, and ELISAs. Different antigenic preparations were applied such as the cercarial antigen (CA), the soluble egg antigen (SEA), and the purified or the crude adult worm antigen (AWA) (Kinkel *et al*, 2012). Several types of ELISA techniques have been successfully employed for the large-scale laboratory diagnosis of *S. mansoni* infection by using the antibodies against different types of antigens in automated and quantitative assays (Espirito-Santo *et al*, 2014).

The magnetic bead-based immunoassay was launched in 1980. It is commonly used to isolate nucleic acids, proteins, organelles, cells and other molecules. It was applied for hormones, cytokines and tumour markers detection (Liu *et al*, 2010). The magnetic separation was used in many forms in both indirect ELISA and Sandwich ELISA for detection of many parasitic infections as *S. mansoni* (Teixeira *et al*, 2007), *S. japonicum* (Liu *et al*, 2010; Lei *et al*, 2011; Xu *et al*, 2011; Yu *et al*, 2012a, b; 2014), toxoplasmosis (Hegazy *et al*, 2015), neurocysticercosis (Hernández-González *et al*, 2017).

This study aimed to evaluate the IMB-ELISA diagnosing *S. mansoni* infection in comparison with the IgG antibody-based detection by both IHA, and indirect ELISA and with the microscopy stool examination.

## Material and Methods

This case-control study was conducted in the period from October 2015 to June 2017. The study received approval by the Local Health Authorities and the Ethics Committee of Faculty of Medicine, Fayoum University. Subjects were informed about the study aim and gave written consents.

Tested subjects: Subjects were 100: 72 males and 28 females, their age ranged from 12- 45 years. They were divided into four groups. G1: Active *S. mansoni* group: 25 cases with positive *S. mansoni* eggs in stool. Egg count by KK ranged from 50-100 egg/gram (EPG). They were negative for other parasites including *S. haematobium*. They were collected from rural health care units and the Center for Diagnosis of Schistosomiasis El-Hadkka (Fayoum Governorate). G2: Chronic *S. mansoni* group: 25 cases with a history of past infection and previous Praziquantel treatment and presented with symptoms and signs of chronic *S. mansoni* infection, and parasitologically negative for both types of schistosomiasis and other parasitic infections. G3: Cross-reactivity group: 25 cases positive for other parasitic infections; fascioliasis (n=4), ancylostomiasis (n=9), and giardiasis (n=12), and parasito-logically negative for both types of schistosomiasis. Cases of G2 & G3 were chosen from the attendee of the Department of Tropical Medicine, Faculty of Medici- ne, Fayoum University. G4: negative con- trol group: 25 subjects with no history of previous exposure to canal water, and parasitologically negative for both types of schistosomiasis and other helminthes. They were selected from hospital attendee of matched age and sex as cases.

Sampling, Parasitological examination, and IHA: From each participant, two stool samples were taken on 2 consecutive days also a sample of 3mL venous blood was withdrawn. Samples were delivered to the Department of Parasitology, Faculty of Medicine, Fayoum University, and sera were separated then stored at -20°C until needed. Direct wet mount smear, and iodine-stained smear, Formol-éther concentration technique (Cheesbrough, 1987), and KK stool technique were performed to diagnose parasitic infections as 3 slides/stool sample (Katz *et al*, 1972).

The IHA was applied to detect anti-*Schistosoma* IgG antibodies (Fumouze Diagnostics, SOFIBEL, S.A.S., France) according to the manufacturer's directions. The red blood cells (RBCs) sensitized with *Schistosoma* antigens were added to serially diluted serum samples, and each sample was tested twice. In each test, non-sensitized RBCs, positive and negative control sera were included as controls. After 2hr incubation at room temperature, the dilution before that

gave a clear sharp dark spot was recorded as the titer at a cutoff 1:160, and was presented as a reciprocal value.

*Schistosoma mansoni* egg antigen preparation: *S. mansoni* eggs were purchased from Theodor Bilharz Research Institute, Egypt. Further immunological tests were developed and adjusted in Parasitology Department, Faculty of Veterinary Medicine, Cairo University. *S. mansoni* egg antigen (SEA) was prepared by placing eggs in 0.9% NaCl, homogenization for 1 hr on ice and 20,000 rpm centrifugation for 3 hr at 4°C (van Gool *et al*, 2002). Supernatant was collected, aliquoted, kept at -20°C, and the antigenic protein content was determined (Lowry *et al*, 1951).

Preparation of hyper-immune sera (RHIS) in rabbits: SEA was used to raise specific polyclonal antibodies in rabbits to be used as reference control sera in ELISA tests (Dunne *et al*, 1986). Rabbits were injected subcutaneously with 2.4µg of SEA emulsified in mineral oil. Three following injections were done using 1.2µg SEA divided into 3 doses at 2 weeks interval. Sera were collected of the vaccinated animals one week after the last injection. The collected sera were stored at -20°C until used.

Coupling of *S. mansoni* SEA to magnetic beads: The magnetic beads-carboxy-terminated with diameter around 2mm were brought (Beijing Bio Biology Company, Beijing, China) used according to Yu *et al*. (2012). At first, the beads were activated by adding them to 0.1 M 2-morpholine-ethane- sulfonic acid hydrate (MES hydrate**)** buffer of pH 6.0, N-hydroxysuccinimide and 1-ethyl-3-(dimethylaminopropyl)-carbodiimide hydrochloride (Sigma-Aldrich, St. Louis, Mo, USA).The suspension was mixed and incubated for 20 minutes at 25°C. This was followed by three times washing of the beads using 0.01 M phosphate-buffered saline (PBS), pH 7.4. For antigenic coating, the *S. mansoni* SEA was added, mixed and incubated with the active beads at 37°C for 2 hours followed by two times washing steps. Bovine serum albumin (BSA-Sigma-Aldrich St. Louis, Mo, USA), 1.5% (w/v) was added after the 2nd beads-antigen incubation, for blocking of any free binding sites on the beads. The washing buffer (Tris-buffer solution with 0.05% Tween 20, pH 7.4-TBST) was used to wash the beads (three times). The coated beads were added to TBST containing 0.01% sodium azide, BSA and kept at 4°C until used.

Immunomagnetic beads-ELISA Technique: Specific IgG antibodies to *S. mansoni* SEA antigens present in sera were measured using IMB-ELISA (Yu *et al*, 2012), after checkerboard titration. The method was performed in small flat-bottom tubes fitted to a magnet rack (Lei *et al*, 2011). A 60µL of antigen-conjugated magnetic beads (0.2mg) were used per tube, and 30µL of serum sample diluted 1:200 in 0.01 M PBS (pH 7.4) was added to each tube. RHIS used as a reference positive control, negative control sera and PBS (blank control) were included in each test set. The entire mixture was incubated with continuous rotation for 20 minutes at 37°C to avoid IMB precipitation.

For washing, the first step was IMB separation. The tubes were placed for 2 minutes on the magnetic rack to remove the supernatant. The second step was adding the TBST to IMB with gentle vortexing to get rid of any unbound antibodies (repeated thrice). Then, 100µL of Horse-radish peroxidase-conjugated (HRPC) goat anti-human IgG and anti-rabbit IgG (Sigma, St. Louis, Mo, USA) diluted 1:1000 was added to the IMB, mixed for one hour at 37°C, and then IMB were separated, the liquid was removed, and three times washing were performed as described before. After washing, 100µL of substrate buffer Ortho-phenylenediamidine (OPD) was added at a concentration of 340 µg/mL and incubated with gentle shaking for 20 minutes at 37°C. The reaction was terminated by addition of 300µL of stopping reagent 0.2 M $H_2SO_4$. IMBs were separated; the reaction fluid was transmitted to the polystyrene high binding flat-bottom 96-well ELISA plates (Libro, USA). Optical density

(OD) was estimated using an automated Titerteckmultiscan plate reader (Titertek-Berthold, Berthold Detection Systems GmbH, Pforzheim, Germany), at 450nm (Lei *et al*, 2011). Serum samples were tested in triplicates and the mean OD was used as the final result. Cut-off values for assays were at least two times higher than mean OD for negative control group.

Indirect ELISA was performed (Doenhoff *et al*, 1993) after checkerboard titration. Overnight coating of the flat-bottom 96-well ELISA plates was performed using 200µ/well of 20µg/mL of *S. mansoni* ESA in carbonate buffer (pH 9.6) at 4°C.

The plates were washed with PBS (50mM, pH 7.2), and blocked with 0.2% BSA in PBS Tween-20 (PBS-T, pH 7.2), 100µL/well. After three times of washing, sera (1:100) were added 100µL/well, (three replicate/sample), incubated for 2hrs at 37°C. RHIS was used as a reference positive control. HRPC-goat anti-human and anti-rabbit IgG (1:2000) were pipetted, incubated at 37°C for 1hr, followed by washing. Then, the substrate buffer, OPD, 340µg/mL was added, kept for 15 minutes at room temperature, and terminated using 0.2M $H_2SO_4$. The absorbance was read using the same ELISA plate reader at 450nm as before.

Statistical analysis: Data were computerized and analyzed by SPSS 17 program (SPSS Inc, Chicago, Ill). Sensitivity (SN) and specificity (SP) rates of various tests were estimated. The degree of agreement (DA) between results of various tests was determined by Cohen's Kappa (K) index at 95% confidence intervals. The K index was reported as a poor DA if value < 0.20, a low DA if ranged between 0.20 &0.40, a moderate DA between 0.41 & 0.60, a good DA between 0.61 & 0.80, with an excellent DA between 0.8 & 1.00 (Altman, 1990). Bivariate or Pearson's correlation estimated the correlation between every two tests, and degree of significance. A $P$ value $\leq 0.01$ was considered significant. The receiver-operating characteristic (ROC) curve graphically plotted the test SN against (1- SP) of the reference standard technique. The cut-off value gave the best balance of SN & SP, and area under the curve (AUC) determined the test degree of accuracy.

## Results

For the egg passers group (G1), parasitological examination, IHA, Indirect ELISA and IMB-ELISA tests detected 100%, 88%, 96%, & 100% of cases, respectively. For *S. mansoni* chronic cases (G2) the four tests detected 0%, 84%, 88% & 96%, respectively. The tests SN rates were estimated in relation to total number of true positive *S. mansoni* cases (G1 & G2), and gave 50%, 86%, 92% & 98% for the four tests, respectively.

For cross-reactivity group (G3) the four tests detected 0, 3, 2 & 1 fascioliasis cases, respectively. No cross-reactivity was detected with sera of cases infected with other parasites or normal control sera. Cross-reactivity or SP rate was estimated in relation to *S. mansoni* negative sera (G3 &G4). The SP rates were 100%, 94%, 96% &98%, respectively.

Out of 100 subjects, 25 cases were positive by parasitological tests, while IHA, Indirect ELISA and IMB-ELISA tests detected 46%, 48%, & 50% of cases, respectively. Mean ±standard deviation (SD) of optical density (OD) values (OD) of positive and negative samples detected by the indirect ELISA & IMB-ELISA tests given (Tab.1).

Application of tests on *S. mansoni* infected groups (G1, G2) and the estimated SN rates of different tests in comparison with IMB-ELISA were presented (Table 2). For the group actively passing eggs (G1), SN of parasitological examination, indirect ELISA and IHA tests were 100%, 96% & 88%, respectively. SN rates for chronic cases (G2) were 0%, 91.7% and 87.5 % respectively. Total SN rates in both *S. mansoni* groups (G1, G2) were 51%, 93.9% and 87.8%, respectively.

Table 1. Sensitivity and specificity rates detected by different techniques at different groups

| Group (number) | | Parasitology No. +ve (%) | IHA No. +ve(%) | Indirect ELISA No.+ve(%) | IMB-ELISA No. +ve(%) |
|---|---|---|---|---|---|
| G1 (25) | | 25 (100) | 22 (88) | 24 (96) | 25 (100) |
| G2 (25) | | 0 (0.0) | 21 (84) | 22 (88) | 24 (96) |
| Sensitivity % | | 50 | 86 | 92 | 98 |
| G3 | Fascioliasis (4) | 0 (0.0) | 3 (75) | 2 (50) | 1 (25) |
| | Ancylostomiasis (9) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| | Giardiasis (12) | 0 (0.0) | 0 (0.0) | 0(0.0) | 0(0.0) |
| | Total G3 (25) | 0 (0.0) | 3 (12) | 2 (8) | 1 (4) |
| G4 (25) | | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Specificity % | | 100 | 94 | 96 | 98 |
| Total detection rate | | 25 (25) | 46 (46) | 48 (48) | 50 (50) |
| OD range | Positive | - | - | 0.32 - 0.763 | 0.5- 1.9 |
| | Negative | - | - | 0.05 – 0.015 | 0.1-0.2. |
| M±SD positive OD values | | - | - | 0.65 ± 0.25 | 1.8 ± 0.25 |
| Cut off value | | - | 160 | 0.222 | 0.5 |

Table 2: Sensitivity rates of various tests in relation to IMB-ELISA for diagnosis of *S. mansoni* groups.

| Tested group | IMB-ELISA | Parasitology | | | ELISA | | | IHA | | | SN % | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | P | N | T | P | N | T | P | N | T | Parasitology | ELISA | IHA |
| G1: active S. *mansoni* | Positive (P) | 25 | 0 | 25 | 24 | 1 | 25 | 22 | 3 | 25 | 100 | 96% | 88 |
| | Negative(N) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | |
| | Total (T) | 25 | 0 | 25 | 24 | 1 | 25 | 22 | 3 | 25 | | | |
| G2: chronic *S. mansoni* | P | 0 | 24 | 24 | 22 | 2 | 24 | 21 | 3 | 24 | 0 | 91.7 | 87.5 |
| | N | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | | | |
| | T | 0 | 25 | 25 | 22 | 3 | 25 | 21 | 4 | 25 | | | |
| Total | P | 25 | 24 | 49 | 46 | 3 | 49 | 43 | 6 | 49 | 51 | 93.9 | 87.8 |
| | N | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | | | |
| | T | 25 | 25 | 50 | 46 | 4 | 50 | 43 | 7 | 50 | | | |

The 100 subjects were compared regarding SN and SP rates (Tab. 3). In comparison with microscopy examination, estima-ted SN rates increased gradually from IHA (88%) to Indirect ELISA (96%) then IMB-ELISA tests (100 %), while SP rates were 68.0%, 68.0% & 66.7%, for the three tests, respectively. ROC curve analysis for comparison of various tests with parasitological examination was built and presented with its data (Fig. 1, Tab. 4).

In comparison with IMB-ELISA test, the performance of other tests was presented (Tab. 3). Parasitological examination, IHA and indirect ELISA test SN rates were 50.0%, 88.0% & 94.0%, respectively, while SP rates were 100%, 96% and 98 %, respectively.

In comparison with indirect ELISA, sensitivity rates of microscopy examination, IHA & IMB-ELISA tests were 50%, 93.8% & 97.9%, respectively, but specificity were 98.1%, 98.1% & 94.2 %, resp-

ectively.

In comparison with IHA test, sensitivity rates of microscopy examination, IMB-ELISA & indirect ELISA test were 47.8%, 95.7%, &97.8%, respectively, but specificity rates were 94.4%, 88.9% & 94.4%, respectively.

Kappa index for degrees of agreement (DA) and *P* value estimated form the bivariate correlation between results of each two tests given (Tab. 3). DA was excellent between every two serological tests (K index > 0.8), with a significant correlation between them ($P \leq 0.001$). There were moderate DA between microscopy examination and each of three serological assays (K index ranged from 0.437- 0.5).

Table 3: Comparison of sensitivity (SN) and specificity (SP) rates, degree of concordance (Kappa index), and *P* value from bivariate correlation between results of every two tests used to diagnose *S. mansoni* infection among total examined subjects (n 100)

| Test | | Parasitological examination | | | [d]SN & SP compared to parasitology | IHA | | | [c]SN & SP compared to IHA | Indirect ELISA | | | [b] SN & SP compared to indirect ELISA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | P | N | T | | P | N | T | | P | N | T | |
| [a] IMB-ELISA data and SN and SP rates compared to IMB-ELISA | P | 25 | 25 | 50 | [d] SN = 100 % SP = 66.7 % | 44 | 6 | 50 | [c] SN = 95.7 % SP = 88.9 % | 47 | 3 | 50 | [b] SN = 97.9 % SP = 94.2 % |
| | N | 0 | 50 | 50 | | 2 | 48 | 50 | | 1 | 49 | 50 | |
| | T | 25 | 75 | 100 | | 46 | 54 | 100 | | 48 | 52 | 100 | |
| | | [a] SN = 50.0 % SP = 100 % K = 0.5 | | | | [a] SN = 88.0 % SP = 96.0 % K = 0.84 P ≤ 0.001 | | | | [a] SN = 94.0 % SP = 98.0 % K = 0.92 P ≤ 0.001 | | | |
| [b]Indirect ELISA data and SN and SP rates compared to indirect ELISA | P | 24 | 24 | 48 | [d] SN = 96 % SP = 68.0 % | 45 | 3 | 48 | [c] SN = 97.8 % SP = 94.4 % | | | | |
| | N | 1 | 51 | 52 | | 1 | 51 | 52 | | | | | |
| | T | 25 | 75 | 100 | | 46 | 54 | 100 | | | | | |
| | | [b] SN = 50.0 % SP = 98.1% K = 0.49 | | | | [b] SN = 93.8 % SP = 98.1 % K = 0.92 P ≤ 0.001\ | | | | | | | |
| [c] IHA, SN &SP rates of Parasitology compared to IHA | P | 22 | 24 | 46 | [d] SN = 88 % SP = 68.0 % | | | | | | | | |
| | N | 3 | 51 | 54 | | | | | | | | | |
| | T | 25 | 75 | 100 | | | | | | | | | |
| | | [c] SN = 47.8 % SP = 94.4 % K = 0.437 | | | | | | | | | | | |

[a] Performance of various tests in relation to IMB-ELISA, [b] Performance of various tests in relation to indirect ELISA. [c] Performance of various tests in relation to IHA, [d] Performance of various tests in relation to parasitological examination

Table 4: ROC Curve data comparing the performance of various tests to standard microscopy

| Test used | Area | Std. Error | Asymptotic Sig. | Asymptotic 95% Confidence Interval | |
|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound |
| Indirect-ELISA_ | 0.914 | 0.029 | 0.000 | 0.858 | 0.970 |
| IMB_ ELISA | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 |
| IHA | 0.780 | 0.051 | 0.000 | 0.680 | 0.880 |

## Discussion

Application of governmental control programs including snail control and provision of Praziquantel therapy has decreased schistosomiasis transmission in Egypt. This created a low level of transmission and infection intensity, where microscopy failed to detect cases. This required highly sensitive and specific serological tests to detect infected cases before spreading infection and development of complications (Kinkel *et al*, 2012; Hinz *et al*, 2017). These tests were needed for accurate follow up of cases after treatment to indicate antibody titres, in egg negative cases with high antibody positivity indicating re-infection or treatment failure (Yu *et al*, 2012).

In low transmission regions of schistosomiasis, antigen-based detection assays using monoclonal antibodies including circulating antigens neither showed high SN & SP for detection of chronic cases nor better than antibody-based detection to assess the chemotherapy outcome (Gryseels *et al*, 2006; Legesse and Erko; Zhu *et al*, 2012). The IMB-ELISA for antibody detection was effective with the same sensitivity as antigen detection and evading costs to prepare monoclonal antibodies. The test was indicated in areas with low transmission levels (Liu *et al*, 2010).

The present study compared three antibody-based tests for diagnosis of schistosomiasis *mansoni* including IMB-ELISA.

The results showed that the IMB-ELISA had high SN and SP, with minimal cross-reactivity rates with other parasites, while indirect-ELISA and IHA showed different levels of cross-reactivity with cases of fascioliasis. Although ELISA showed high SN in detecting egg-passers, the SN to confirm chronic cases was lower than IMB-ELISA. The IMB-ELISA detection rate for chronic schistosomiasis was 96%, with significant differences from the results by using the indirect-ELISA (88%) and IHA (84%).

The present results of indirect ELISA ranged with previous studies. Van Gool *et al*

(2002) reported SN & SP rates of 93.3% & 98.8% for indirect SEA-ELISA to detect *S. mansoni* egg passers. Kinkle *et al* (2012) compared three commercial indirect ELISA and three home-made indirect ELISA tests using three different antigens (AWA, SEA, & cercarial antigens) together with IHA to diagnose *S. mansoni* egg-passers, the SN range of applied ELISA tests was (47.6-90.5%) and the SP was (88.4-100%). The indirect SEA-ELISA test achieved SN & SP rates of 90.5% & 97.1%, respectively (Kinkle*et al*, 2012). As regard *S. mansoni* infection, the indirect SEA-ELISA test had a SN rate ranged from 56%-100% and SP ranged from 6%-99%, respectively (Hinz *et al*, 2017).

The present results were compared with Yu *et al* (2012) using IMB-SEA ELISA versus indirect ELISA for diagnosing *S. japonicum* infection in persons with low-intensity infection. The IMB-SEA ELISA had a higher SN of 96.55% than indirect ELISA 91.38% with significant difference ($P < 0.01$). In follow up of treated cases, SN of IMB-SEA ELISA was 73.3%, but ELISA was 66.7% ($P < 0.01$). The two tests performed similarly when tested for cross-reactivity gave a specificity of 50%. Also, the magnetic micro-bead separation to detect *S. japonicum* antibodies showed that magnetic ELISA method was more precise and sensitive than traditional SEA-ELISA in the epidemiological survey (Liu *et al*, 2010).

The present results of IHA test were in range with previous studies that evaluated its performance for serodiagnosis of schistosomiasis. It gave SN of 88% & SP of 98.9% for *S. mansoni* egg-passers (van Gool *et al*, 2002). Kinkle *et al*. (2012) estimated SN & SP rates of 76.2% &99% for IHA tests for *S. mansoni* egg-passers. In comparison with the microscopy, the SN & SP of magnetic ELISA were 99 % & 80.9%, while the SN & SP of IHA were 62.9% and 84.5% detecting *S. japonicum* antibodies (Liu *et al*, 2010).
By comparing the 4 types of IHA tests with indirect ELISA and magnetic beads FITC ELISA with the standard KK technique, SN of IHAs ranged from 92%-98%, indirect ELISA was 95%, & FITC ELISA was 98%. The SP of IHAs ranged from 70%-93%, indirect ELISA was 93.6%, and FITC ELISA was 94.3% (Xu *et al*, 2011).

In the present study, the high SN & SP for IMB-ELISA was explained by using the magnetic beads as the solid phase in the immunoassay. The magnetic beads increased the surface area for antigen or antibody binding helped by gravity. So, they were easy with quick and complete separation of antigen or antibody from supernatant solution without loss of reactants in the washing buffer, and reduced time required for coating or incubation step from 12-14hr to 2hr (Liu *et al*, 2010). The technique rendered completely automated, and it's safe, decreasing the manual labour and provision of rapid, precise results. It requires simple methods and could replace the indirect ELISA & IHA (Liu *et al*, 2010; Yu *et al*, 2012). Also, the test can be used for antigen detection with very high SN & SP in Sandwich ELISA technique after the availability of the specific monoclonal antibodies.

## Conclusion

IMB-ELISA proved to be a sensitive and specific method for diagnosis of *S. mansoni* in patients with low-intensity infections.

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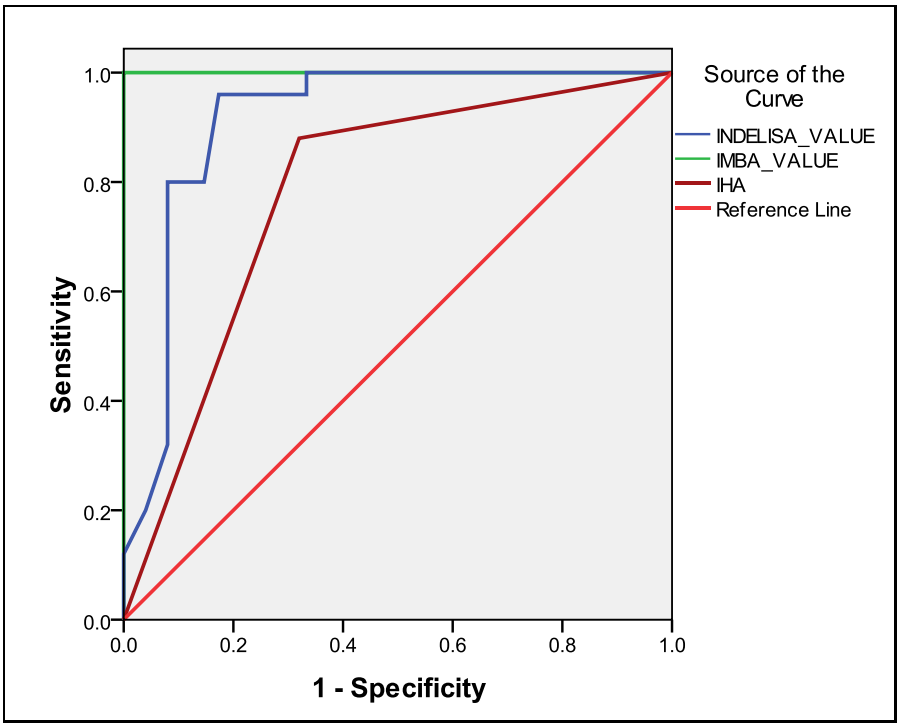
**Explanation of figure**

Fig. 1: ROC Curve to compare performance of various tests to standard parasitological examination


Source of the Curve
INDELISA_VALUE
IMBA_VALUE
IHA
Reference Line
Sensitivity
1 - Specificity
0.0
0.2
0.4
0.6
0.8
1.0