Nano-Immunoassay for Diagnosis of Active Schistosomal Infection

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Abstract: Sandwich ELISA using Gold nanoparticles (AuNPs) conjugated with anti-schistosomal monoclonal antibody (MAb) was evaluated for its sensitivity and specificity in diagnosing human Schistosoma mansoni infection. The study was conducted on serum samples of 116 subjects: 71 S. mansoni-infected patients, (gp I), 25 patients infected with parasites other than schistosomiasis, (gp II) and 20 uninfected healthy individuals (gp III). Patients in gp I were further subdivided according to egg count in their stool samples into light, moderate and severe infection. Results were compared with those after using MAb sandwich ELISA system. It was found that AuNPs-MAb/ELISA reached a lower detection limit of 10 ng/ml compared to 85 ng/ml on using MAb/ELISA and the optimal concentration of AuNPs-MAb used was 12 folds less than that of MAb. The sensitivity and specificity of ELISA using AuNPs-MAb for detecting circulating schistosomal antigen (CSA) were 100% & 97.8% compared to 87.3% & 93.38 % respectively on using MAb/ELISA system. A more significant positive correlation was detected between ova count and CSA levels on using AuNPs-MAb/ELISA(r= 0.882) than with MAb/ELISA system(r= 0.770 ). In conclusion, loading AuNPs with MAb (6D/6F) increased the sensitivity and specificity of sandwich ELISA for detection of CSA, thus active and light infections could be easily detected. Moreover this binding will decrease the amount of MAb consumed in the assay and lower the cost. The significant positive correlation that was detected between ova count and OD reading in ELISA using AuNPs-MAb enables its use to detect the severity of infections and follow up of patients after treatment for monitoring of cure.

Key words: Nanoparticles • Monoclonal antibodies • Gold • Schistosomiasis • Circulating Schistosomal Antigen

INTRODUCTION

Schistosomiasis is one of the most prevalent human parasitic infections. It is a major source of morbidity affecting more than 207 million people in 76 countries. It was estimated that 97% of the infected cases are in the African continent [1, 2]. 120 million have symptoms, 20 million have severe illness [3] and a further 779 million people are at risk [4]. Diagnosis of schistosomiasis is, however, beset with several problems. The infections are generally chronic and characterized by the presence of parasite eggs in stool or urine. Hence routine diagnosis is microscopically, although it is of limited value because of inadequate sensitivity especially in light infections, <50 eggs/g feces for S. mansoni and <50 eggs/10 ml urine for S. haematobium [5, 6]. By time, antibody and antigen detection assays have been developed that led to an increase in sensitivity and specificity of these assays, especially the latter [7]. Consequently, many tests have been developed to detect different circulating schistosome antigens (CSA) in urine or serum samples of infected hosts, e.g sandwich ELISA [8-10] dot-ELISA [11] latex agglutination test [12] and dipstick assay [13-17] using either polyclonal or monoclonal antibodies (MABs) [18-21]. So far, no immunodiagnostic assays have provided a “gold standard” for determining prevalence, identifying infected individuals for selective population chemotherapy, or assessing the effectiveness of

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intervention, including chemotherapy follow-up. However, the available antigen-based serological assays that detect schistosome-specific circulating molecules are also not sufficiently sensitive for early cases, especially those with a low intensity of infection [22, 23]. The use of nanoparticles promises to help promote in vitro diagnostics to the next level of performance [24, 25]. Loading of MAbs on gold nanoparticles combines the properties of the nanoparticles themselves (Larger surface area to volume ratio, which implies that they may render higher binding efficiencies) with the specific and selective recognition ability of the antibodies to antigens [26]. This study aimed at increasing the diagnostic efficiency of monoclonal antibody (MAb)-based antigen detection assays through gold nanoparticles conjugated with anti-schistosomal MAb (6D/6F), hence early and light Schistosoma infections could be easily detected. In addition, a decrease in the cost per test would be an important goal to be achieved.

MATERIALS AND METHODS

Preparation of MAbs
Revival of Cells and Propagation of Hybridoma Cell Lines: Three hybridoma cell lines of MAbs against tegumental Schistosoma antigens (AWTA) were prepared at the Immunology Department of TBRI and preserved in liquid nitrogen till further use [9]. Revival of cells was carried out under laminar flow according to Goding [26]. The frozen cells were thawed rapidly by immersing cryotubes in water bath at 37°C. Thawed cells were centrifuged at 250 xg for 5 min. at room temperature and were then resuspended in 1 ml serum-free medium (SFM). Cells were counted and diluted to a concentration of 20 x 10^5 cells/ml in growth media (SFM supplemented with 20% fetal calf serum (Hyclone), heat inactivated at 56°C for 30 min and seeded at 24 well culture plate (Becton Dickinson Labware, Bedford, MA). The cells were split every 48h and maintained in culture plates for about one week before transferring them to 25 cm² tissue culture flasks. Hybridomas were screened for anti-S mansoni tegumental antibodies by ELISA. Hybrids that were highly reactive were cloned by the limitation dilution, and 0.01% (w/v) sodium azide was added. The obtained solution was stored at 4°C in a dark-colored glass bottle for several months.

Characterization of Monoclonal Antibodies
Assessment of Reactivity and Specificity of MAbs to Parasitic Antigens: The reactivity of MAbs against various parasitic antigens such as S. mansoni and S. haematobium SEA, extracts of Fasciolag natricula and Echinococcus granulosus at concentrations of 30 µg (protein)/ml was determined by indirect ELISA as described by Mohamed et al. [19].

Isotypic Analysis of MAb: Determination of isotype of MAbs was done by indirect ELISA [28] using ELISA plates coated with 30 µg/ml of S. mansoni soluble egg antigen and a panel of anti-mouse immunoglobulin peroxidase conjugates (Goat anti- mouse IgM, IgG, IgG1, IgG2a, IgG2b, IgG3 and IgA) (Sigma Aldrich, Germany).

Identification of Target Antigens Recognized by MAbs: Using enzyme- linked immunoelectrotransfer blotting, Mab recognized 50 -65 kDa band of electrophoresed S. mansoni AWTA [9].

Purification and Conjugation of MAb: MAb was purified according to Garcia et al. [29]. The ascitic fluid containing IgM MAb was centrifuged at 1500 rpm 37°C for 15 minutes. The clear supernatant was collected in dialysis bags and soaked in 2% boric acid pH 6.0 at 4°C. The boric acid was changed every other day for 3 days. At the 4th day the supernatant was collected, ultracentrifuged at 100,000 xg at 4°C for 1 h. Supernatant was discarded and final precipitate was dissolved in a suitable amount of 0.2 M PBS, pH 7.2. MAbwas conjugated to horseradish peroxidase (Sigma Aldrich, Germany) following the periodate method according to Tijssen and Kurstak [30].

Gold Nanoparticles (AuNPs): Characterization and Functionalization: AuNPs were purchased from Nanotech Egypt Company for Photo-electronics-Dream Land with average diameter of 20 nm checked with a transmission electron microscope (JEOL, JEM-2100). AuNPs were functionalized according to Omidfar et al. [31]. Briefly, 100ml tetrachlorauricacids (0.01% w/v HAuCl4) were brought to boiling and then a solution of 1% trisodium citrate was added with constant stirring. When the solution’s color changed from light yellow to wine red, after about 8 min, the solution was cooled down. The pH was adjusted to 8.5 using 0.01 M potassium carbonate and 0.01% (w/v) sodium azide was added. The obtained solution was stored at 4°C in a dark-colored glass bottle for several months.

Loading of AuNPs to MAb: To prepare AuNPs-MAb according to Kumar et al. [32] 600ug of tegumental Mab; 30µg/ml, in phosphate-buffer (pH 7.5) was added to 20ml pH-adjusted AuNPs solution. The mixture was gently mixed for 3 h and subsequently 4ml of 10% BSA solution.
was added to block the residual surface of the AuNPs. The mixture was then incubated for 20 min at room temperature before being centrifuged at 13,000 rpm for 45 min at 4°C for three times. After the last centrifugation, the pellets were re-suspended in 2 ml phosphate buffer (pH 7.2, 0.01 M) containing 1% BSA and 0.05% sodium azide. AuNPs-MAb was stored at 4°C before being used.

**Optimization of Working Dilutions of Coating AuNPs-MAb and Determination of the Lower Detection Limit by Indirect ELISA [33, 34]:** The prepared AuNPs-MAb was tested in different concentrations to obtain optimum dilutions by checker-board titration against known positive and negative sera. A standard curve was set up using serial dilutions of *S. mansoni* SEA from 3 ug/ml to 0.6 ng/ml. Optical density (OD) readings at 492 nm were plotted against the concentrations of the antigen preparations to determine the lower detection limit.

**Application of Anti-*Schistosoma* MAb-Based Sandwich ELISA for Antigen Detection in Serum Samples of Schistosomiasis Patients**

**Study Population:** This study was conducted on serum samples of 116 subjects. Seventy one patients (Male and female with age ranging from 20 to 50 years) infected with *S. mansoni* (Eggs were quantified in stool samples on 3 consecutive days by modified Kato's thick smear technique) [35] representing active infection with no other parasites including *S. haematobium* (gp I), 25 patients harboring parasites other than *Schistosoma* such as *Fasciola gigantica* and *Echinococcus granulosus* (gp II) and 20 age and sex matched healthy individuals were collected to serve as negative controls (gp I). Patients infected with *S. mansoni* (gp I), were further subdivided according to egg count in their stool samples into light (≤ 50 egg per gram (epg) (n = 17)), moderate (51-100 epg (n = 33)) and severe infection (>100 epg (n = 21)).

**Ethical Agreement and Consent:** This study was approved by the internal review board of Theodor Bilharz Research Institute, Cairo- Egypt for the use of the serum samples and data. Written informed consents were obtained from patients for the use of their data and publish the results of the study.

**Detection of CSA in Serum Samples of Schistosomiasis Patients by Sandwich ELISA Using AuNPs-MAb:** After several optimization trials, the following sandwich ELISA according to Engvall and Perlmann [28] and modified by Demerdash *et al.* [34] was performed for circulating antigen detection. Microtitration plates (Thomas Scientific) were coated with 100 ul/well of AuNPs-MAb 1/12000 (250 ng protein/well) in 0.1 M carbonate buffer pH 9.6 and left overnight at room temperature. Plates were blocked by the addition of 200 ul/well of 2.5% fetal calf serum (FCS; Seromed) in 0.02 M phosphate buffered saline with 0.05% Tween 20 (PBS/T) pH 7.2 for 2 h at 37°C. 100 ul of serum samples diluted 1/4 in diluent buffer were pipetted into the wells of the blocked plate in duplicate and incubated for 2 h at 37°C. Plates were washed 3 times with PBS/T. 100 ul of 1/1000 HR-MAb in diluent buffer were added to each well and the plate was incubated for 1 h at 37°C. The wells were then washed 5 times, 3 min each. The reaction was visualized by the addition of 100 ul/well of O-phenylenediamine (Sigma) for 20 minutes in the dark at room temperature. The reaction was stopped by adding 50 ul/well of 8N H2SO4 and plates were read at 492 nm using an ELISA reader (Microplate Reader, Bio-Rad, Richmond, CA, USA).

**Statistical Analysis:** Results are expressed as mean ± standard deviation (X ± SD) or number (%). Comparison between the mean values of variables was performed using One Way Analysis of Variance (ANOVA) and if significant results were obtained, Least Significant Difference (LSD) test as a post-hoc test was used. Pearson correlation coefficient was used to determine significant correlations between the egg count/g stool and ELISA OD readings. Receiver Operating Curve (ROC) was used to determine the sensitivity, specificity and accuracy of sandwich ELISA using MAb or AuNPs-MAb. The data were considered significant if p value was ≤ 0.05 and highly significant if p < 0.01. Statistical analysis was performed with the aid of the SPSS computer program (Version 16 windows).

**RESULTS**

**Reactivity of Monoclonal Antibodies to Schistosoma Antigen by Indirect ELISA:** After screening of different prepared MAbs, one MAb (6D/6F) raised against adult worm tegumental *Schistosoma* antigen (Anti- AWTA MAb) was chosen for this study, due to its high reactivity to *Schistosoma* antigen as it gave highest optical density (1.843 ±0.23) value.
Table 1: Reactivity of anti-S.mansoni adult worm tegumental antigen (anti-Sm AWTA) monoclonal antibody to S. mansoni adult worm tegumental antigen (AWTA), S.mansoni soluble egg antigen (SEA), S.haematobium SEA, Fasciola and Hydatid antigens compared to hybridoma growth medium

<table>
<thead>
<tr>
<th>Coating antigen</th>
<th>Hybridoma growth medium</th>
<th>6D/6F Mab</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. mansoni AWTA</td>
<td>0.056</td>
<td>2.216</td>
</tr>
<tr>
<td>S. haematobium SEA</td>
<td>0.130</td>
<td>1.940</td>
</tr>
<tr>
<td>S. mansoni SEA</td>
<td>0.095</td>
<td>2.203</td>
</tr>
<tr>
<td>Fasciola antigen</td>
<td>0.071</td>
<td>0.254</td>
</tr>
<tr>
<td>Hydatid antigen</td>
<td>0.049</td>
<td>0.203</td>
</tr>
</tbody>
</table>

Production of Ascitic Fluid Containing MAB: Ascitic fluid was induced in 60 BALB/c female mice (8 weeks old) for MAB production by intraperitonial injection of 2x10^6 hybridoma cells. Ten to 20 ml of ascitic fluid were obtained from each mouse containing 3 mg/ml of total protein as measured by Bio-Rad protein assay kit and were tested for the presence of specific anti-Schistosoma Ag antibodies by indirect ELISA. Ascitic fluid was highly reactive to S. mansoni SEA.

Characterization of (6D/6F) MAB: Isotyping of anti-AWTA MABs was performed using Boehringer sub-isotyping kit. The produced MAB gave positive results with anti-IgM and with κ-type light chain. Target antigen of the prepared MAB was found to be glycoprotein in nature following periodate treatment. MAB reacted with repetitive epitopes on SEA when tested by immunoelectrophoresis.

Determination of 6D/6F Monoclonal Antibody Specificity: Specificity of MABs was determined by ELISA against various parasitic antigens including F. gigantica and Echinococcus granulosus 6D/6F MAB was found to be strongly reactive with S. mansoni AWTA, S. mansoni SEA and S. haematobium SEA and did not show cross-reactivity with extracts of F. gigantica or Echinococcus granulosus Table (1).

Optimization of Working Dilutions of AuNPs-MAB: After chequer-board titration against known positive and negative sera, the optimal dilution of AuNPs-MAB was found to be 1:12000 (250ng/well) compared to 1:1000 (3ug/well) using MAB.

Detection of CSA in Serum Samples of Different Studied Groups by Sandwich ELISA Using Either AuNPs-MAB or MAB

Subject Groups: This study was conducted on the following subjects:

Group I: Patients infected with S. mansoni (Eggs were quantified in stool samples on 3 consecutive days by modified Kato’ thick smear technique. Siongok et al. [35] representing active infection with no other parasites including S. haematobium (71 cases).

Group II: Patients infected with parasites other than Schistosoma (25 cases).

Group III: Uninfected age and sex matched healthy controls (20 individuals).

Sandwich ELISA: CSA levels were detected in sera of different studied groups by sandwich ELISA at OD readings equal to 492 nm using AuNPs-MAB for antigen capture and detection. The results were compared to those obtained by using MAB. The results were expressed as the mean (X) OD of each group ± standard deviation (SD) of the mean of the studied group. In order to measure the incidence of positivity for circulating Schistosoma antigen in the studied groups, it was necessary at first to determine the cut off point for positivity or the line of demarcation between positive and negative results. The cut off value was calculated as the mean OD readings of negative controls + 2 SD of the mean. The OD readings equal to or less than the cut off value were considered negative, while those readings greater than the cut off value were considered positive. It was found that serum levels of CSA using either AuNPs-MAB or MAB were highly significant (P<0.01) in Schistosoma infected group (gp III) compared to their corresponding levels in the other two groups gp II and gp I as shown in Table 2. No significant difference was detected in CSA level between other parasites group II and control group I.

Comparison of Immunological and Parasitological Results of Subject Groups

Results of Subject Groups Using AuNPs-MAB in Sandwich ELISA: The cutoff point used to determine a positive or negative serum sample was equal to 0.23. This value corresponded to 10 ng/ml of S.mansoni SEA in the standard curve. Positive CSA levels were detected in serum samples of all 71 S. mansoni infected patients (With positive S.mansoni eggs in their stool samples). Thus the sensitivity of antigen assay by sandwich ELISA using AuNPs-MAB was 100%. On the other hand, level of CSA in serum samples of all 20 healthy negative controls plus 24 out of 25 of other parasites group was found to be below cut off value, giving an overall specificity of 97.8 % (Table 3, Fig. 1).
Table 2: Mean serum levels of circulating Schistosoma antigen (CSA) in the studied groups using either AuNPs-MAb or Mab. Data are presented as mean ± SD.

<table>
<thead>
<tr>
<th>Study population</th>
<th>Schistosoma I (n=71) Mean CSA</th>
<th>Other parasites II (n=25) Mean CSA</th>
<th>Control III (n=20) Mean CSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mab (6D/6F) (Cut off point=0.29)</td>
<td>1.12± 0.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.18 ± 0.05</td>
<td>0.21 ± 0.04</td>
</tr>
<tr>
<td>AuNPs-MAb (Cut off point=0.23)</td>
<td>1.02± 0.49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.17 ± 0.05</td>
<td>0.17 ± 0.03</td>
</tr>
</tbody>
</table>

<sup>a</sup> p< 0.01 relative to control group.
<sup>b</sup> p< 0.01 relative to other parasites group.
<sup>p</sup>< 0.01 highly significant.

Table 3: Comparative evaluation of sandwich ELISA OD readings using AuNPs-MAb and parasitological examination for diagnosis of S. mansoni infection

<table>
<thead>
<tr>
<th>Parastology examination</th>
<th>Control + Other parasites (n= 45)</th>
<th>Schistosoma (n= 71)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative (n= 44)</td>
<td>44 (97.78%) TN (True negative)</td>
<td>0 (0%) FN (False negative)</td>
</tr>
<tr>
<td>Positive (n= 72)</td>
<td>1 (2.22%) FP (False positive)</td>
<td>71 (100%) TP (True positive)</td>
</tr>
</tbody>
</table>

Data are expressed as number (percent).

TN: True negative, TP: True positive, FN: False negative, FP: False positive.

Fig. 1: Distribution curve showing (CSA) levels detected in sera of different studied groups measured as optical density (OD) values at 492nm using AuNPs-MAb in sandwich ELISA. Each small circle represents CSA level of a single patient. The horizontal line represents the cut-off value. CSA values below cut off were considered negative and values above were considered positive.

Results of Subject Groups Using MAb in Sandwich ELISA: The cutoff point used to determine a positive or negative serum sample on using MAb for detection of CSA levels of different studied groups was equal to 0.29. In this study, positive CSA levels were detected in sera of 62 out of 71 Schistosoma infected patients. Thus the sensitivity of antigen assay using MAb was determined 87.3%. On the other hand serum samples of all 20 healthy negative controls plus 22 out of 25 other parasites group (A total of 42 subjects out of 45) were negative for Schistosoma Ag and gave CSA levels below cut-off value, giving an overall specificity of 93.38% Table 4 and Fig. 2. It was observed that all nine schistosomal patients who were negative on using MAb /ELISA and positive with AuNPs-MAb/ELISA had an egg count below 50 epg feces (Light infection).
Table 4: Comparative evaluation of sandwich ELISA optical density (OD) readings using MAb and parasitological examination for diagnosis of *S. mansoni* infection

<table>
<thead>
<tr>
<th>Parasitology examination</th>
<th>Mab Control + Other parasites (n= 45)</th>
<th>Schistosoma (n= 71)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative (n= 51)</td>
<td>42 (93.33%) TN (True negative)</td>
<td>9 (12.68%) FN (False negative)</td>
</tr>
<tr>
<td>Positive (n= 65)</td>
<td>3 (6.67%) FP (False positive)</td>
<td>62 (87.32%) TP (True positive)</td>
</tr>
</tbody>
</table>

Data are expressed as number (Percent).

Table 5: Mean optical density (OD) readings of circulating *Schistosoma* antigen (CSA) levels in sandwich (ELISA) using either MAb or AuNPs-MAb classified according to intensity of infection in group (I)

<table>
<thead>
<tr>
<th>ELISA OD readings</th>
<th>Ova count (X ± SD)</th>
<th>Mab Cut off point=0.29</th>
<th>AuNPs-MAb Cut off point=0.23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light infection ≤ 50epg (n= 17)</td>
<td>24.12 ±14.17</td>
<td>0.39 ± 0.19 (n=8) 0.25±0.027 (n=9)</td>
<td>0.54 ± 0.25 (n=17)</td>
</tr>
<tr>
<td>Moderate infection 51-100 epg (n= 33)</td>
<td>73.94 ±10.59</td>
<td>1.09 ± 0.29 (n=33)</td>
<td>0.95 ± 0.33 (n=33)*</td>
</tr>
<tr>
<td>Severe infection &gt;100 epg(n= 21)</td>
<td>141.90 ±31.8</td>
<td>1.76 ± 0.31 (n=21)</td>
<td>1.53 ± 0.4 (n=21)*</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD.

* p< 0.01 relative to moderate and severe infection groups.

**p< 0.01** = highly significant

![Distribution curve showing CSA levels detected in sera of different studied groups measured as optical density (OD) values at 492 nm using MAb in sandwich ELISA. Each small circle represents CSA level of a single patient.](image)

Relation between Intensity of Infection and CSA Levels in *S. mansoni* Patients: Patients infected with *S. mansoni* were classified according to egg count in their stool samples into light (Egg count ≤ 50 egg per gram (Epg)), moderate (Egg count 51-100epg) and severe infections (Egg count > 100 epg). Mean OD readings of CSA levels using either MAb or AuNPs-MAb in patients with light infection (0.39 ± 0.19 and 0.54 ± 0.25, respectively) was found to be significantly lower (p< 0.01) than their corresponding levels in both moderate (1.09 ± 0.29 and 0.95 ± 0.33) and severe (1.76 ± 0.31 and 1.53 ± 0.4) infections as shown in Table 5.

Correlation between Intensity of Infection and CSA Levels in *S. mansoni* Patients: A significant positive correlation was detected between ova count and OD readings using AuNPs-MAb (r= 0.882 and p=0.001) more than using MAb(r= 0.770 and p=0.001) in the *Schistosoma* infected group as shown in Fig. 3 & 4.
DISCUSSION

In Egypt, a national control program against schistosomiasis was started from 1993 to 2006 leading to a gradual decrease in the prevalence of the 2 schistosome species (1.9% for *S. mansoni* and 1.1% for *S. hematobium* [2]. Since then, schistosomiasis is considered as a neglected disease [1, 2]. By time, attention paid to schistosomiasis has been reduced leading to an increase of its prevalence again specially in rural areas reaching to 10% [36-38]. The use of ELISA for detection of schistosomal soluble egg antigen is recommended by
many authors as a promising complementary field-applicable method for monitoring treatment and infection dynamics in endemic areas [17, 39]. This is an alternative to traditional parasitological approaches which became more fallible as both the infection prevalence and intensity diminished through control. CSA detection using anti-ATWA/ sandwich ELISA systems is now routinely used for diagnosis of active infection and monitoring of cure in our laboratory with a sensitivity 87.3% and specificity 93.38%, for all in and out-patients at TBRI [9, 35, 40]. However, further efforts are still needed to increase the sensitivity of CSA assay especially for light and early infections.

This study aimed at increasing the diagnostic efficiency of monoclonal antibody (MAb)-based antigen detection assays through gold nanoparticles conjugated with MAb (6D/6F) which was chosen due to its high reactivity to Schistosoma antigens.

The prepared MAb was raised in ascites fluid, purified, HRP conjugated and applied in sandwich ELISA as both antigen capturing and HRP conjugated detecting MAb. Modified nanoparticles gold labeled MAb sandwich ELISA was performed as well, comparing its results with MAb-based sandwich ELISA. It was found that nanoparticles labeled-MAb/sandwich ELISA system reached a lower detection limit of 10 ng/ml compared to 85 ng/ml on using MAb – based sandwich ELISA and the optimal concentrations of nanolabeled MAb were found to be twelve folds less than the optimal concentrations of unlabeled MAb. Selection of gold nanoparticles in this study is due to the fact that they allow increased sensitivity by several orders of magnitude, extremely stable, have a long shelf-life. [41]. Modulation of the physicochemical properties of gold can be easily achieved by adequate synthetic strategies and give gold nanoparticles advantages over conventional detection methods currently used in clinical diagnostics [26].

In agreement with our results, Azzazy et al. [41] stated that conjugation of MAbs with gold decreases the amount of MAbs consumed and thus reduce cost for many diagnostic applications. Same result was found by Nagatani et al. [42] who showed the advantage of decreasing the antibody concentration by using gold nanoparticles for detection of prostate-specific antigen in serum samples. These results are in accordance with that obtained by Tanaka et al. [43] who employed antibodies conjugated with AuNPs for detection of human chorionic gonadotropin hormone (HCG) antigen with a limit of detection reached 10pg/ml compared to 1ng/ml by conventional sandwich ELISA. In this study, the sensitivity and specificity of sandwich ELISA using AuNPs–MAb and MAb were 100% & 97.8% compared to 87.3% &93.38%, respectively, on using MAb. The higher sensitivity and specificity detected in our work are in agreement with Ding et al. [44]. They stated that tests employing gold nanoparticles functionalized with antibodies enable high specificity and sensitivity by reducing background noise due to minimal non-specific binding of the gold nanoparticle, which in turn, creates an assay with 2-3 orders of magnitude more sensitive than conventional ELISA-based methods. Ahmed et al. [45] reported that using anti-S. haematobium antibodies coupled with nanoparticle for antigen detection by sandwich ELISA gave a sensitivity of 95% and 93.7% specificity compared to 87% and 87.3%, respectively, on using conventional microplate-based ELISA technique. It was observed that all nine patients with negative CSA levels byMAb and positive levels with MAb/AuNPs had an egg count below 50 epg feces (Patients with light infections). Conventional ELISA lacks sensitivity when testing light infections [9, 22] while gold nanoparticles conjugated to MAb are incredibly sensitive to detect nanomolar concentration of CSA. This finding supports the use of AuNPs- MAb as alternative to MAb in sandwich ELISA for detection of active and light infections.

**CONCLUSION**

Our data demonstrated that loading AuNPs with MAb (6D/6F) increased the sensitivity and specificity of sandwich ELISA for detection of CSA, thus active (Early) and light infections could be easily detected. Moreover this binding will decrease the amount of MAb consumed in the assay and lower the cost. The significant positive correlation that was detected between ova count and OD reading in sandwich ELISA using AuNPs- MAb enables its use to detect the severity of infections and follow up patients after treatment for monitoring of cure.

**REFERENCES**


