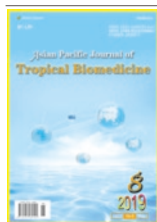

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Development of new lateral-flow immunochromatographic strip using colloidal gold and mesoporous silica nanoparticles for rapid diagnosis of active schistosomiasis

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ABSTRACT

Objective: To develop a new sandwich based lateral flow immunochromatographic strip for rapid detection of circulating *Schistosoma mansoni* antigen in serum and urine samples of patients with active schistosomiasis.

Methods: This lateral flow immunochromatographic strip was prepared by using anti-*Schistosoma mansoni* soluble egg antigen monoclonal antibody conjugated gold nanoparticles (MAb-AuNPs) as antigen-detecting antibody, while crystalline material (MCM)-41-MAb bioconjugate was immobilized at the test line as antigen-capturing antibody. Both antigen capturing and detecting antibodies formed sandwich complexes with circulating *Schistosoma mansoni* antigen in the positive samples. Sandwich complexes immobilized at the test line gave distinct red color. The assay reliability was examined by using urine and serum samples of 60 *Schistosoma mansoni* infected patients, 20 patients infected with parasites other than *Schistosoma*, and 20 healthy individuals as negative controls. Results were compared with those obtained *via* sandwich enzyme linked immunosorbent assay (ELISA).

Results: The detection limit of circulating *Schistosoma mansoni* antigen by lateral flow immunochromatographic strip was lower (3 ng/mL) than the detection limit by ELISA (30 ng/mL). The sensitivity and specificity of lateral flow immunochromatographic strip in urine samples were 98.3% and 97.5%, respectively compared to 93.5% and 90.0% by ELISA. In serum samples, they were 100.0% and 97.5%, respectively compared to 97.0% and 95.0% by ELISA. The strip test took approximately 10 min to complete.

Conclusions: This new lateral flow immunochromatographic strip offers a sensitive, rapid, and field applicable technique for diagnosis of active schistosomiasis.

1. Introduction

Schistosomiasis is one of the most neglected tropical diseases causing significant morbidity and mortality in low and middle-income countries, where the prevention and control programs are facing many challenges[1].

Diagnosis of schistosomiasis, is usually performed by parasitological examination (microscopic detection of eggs), and/or immunological

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methods (antibody and antigen detection)[2]. Demonstration of parasite eggs in urine or feces directly indicates the presence of the worms. This approach has many disadvantages including high fluctuation in egg count especially in light infection. In addition, it is relatively time consuming technique that needs experienced staff[3]. On the other hand, immunological methods such as enzyme linked immunosorbent assay (ELISA) offer the advantages of high sensitivity and specificity, however, they are time-consuming and require well-equipped labs with skilled personnel[4]. In recent years, lateral flow immune assays (LFIAs) have gained a great interest in diagnostic applications for rapid detection of analytes because of its convenient use and visual endpoint[5]. Because the sensitivity of conventional LFIAs is considerably lower than ELISA, many efforts have been made to increase the sensitivity of these tests by the employment of colloidal gold nanoparticles (AuNPs), or the use of liposome[6]. The unique properties of mesoporous silica nanoparticles (MSNs) such as controlled particle size, vast surface area, porosity and high chemical stability, make them more effective in protein immobilization when compared with conventional materials[7,8]. Mobile crystalline material (MCM-41) type silica binds proteins, mainly by electrostatic forces to their porous surface, guaranteeing the stability and immunological reactivity of this immobilized protein[9].

In this study, we developed a novel, rapid and accurate lateral flow immunochromatographic strip (LFIS) using gold nanoparticles and MCM-41 type silica for the detection of circulating *Schistosoma mansoni* (*S. mansoni*) antigen (CSA) in serum and urine of patients with active schistosomiasis.

2. Materials and methods

2.1. Ethical statement

This study was reviewed and approved by the ethics committee of Theodor Bilhariz Research Institute (TBRI, No. 05/09/16) and methods including sample collection were carried out in accordance with relevant guidelines and regulations. Samples were collected from endemic hot spots in the Nile Delta (Elkhamseeny and Sandala villages in Kafr Elsheikh Governorate). Informed consent and full medical histories were taken from the patients (all patients were above 18 years old).

All the animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH) (publication No 86-23, revised, 1985)[10]. and were approved by the Institutional Review Board of TBRI (592016). All methods involving animals [immunization, fusion procedure and large scale production of monoclonal antibody (MAb)] were carried out in accordance with relevant guidelines and regulations.

2.2. Materials

RPMI 1640, fetal calf serum, streptomycin, penicillin, bovine serum albumin (BSA), anti-mouse polyvalent, horse raddish peroxidase (HRP), goat anti-mouse immunoglobulin (IgG) antibody, MCM-41, polyethylene glycol (1 300-1 600 Mw), and O-phenylene diamine dihdrochloride 10 mg (OPD) were obtained from Sigma Aldrich, USA. *S. mansoni* soluble egg antigen (SEA) was prepared in our lab. Non-secreting murine myeloma cell line (P3X36Ag8) was kindly provided by the department of Medicine, Western Reserve School of Medicine, Cleveland, Ohio, USA. The cell line was propagated in the laboratory and stored in liquid nitrogen at -197 ℃. High-flow nitrocellulose membranes (cat no HF09002XSS), cellulose fiber sample pads and absorption pads (cat no CFSP173000) were purchased from Merch Millipore (Darmstadt, Germany). Gold nanoparticles (20 nm) were purchased from Dream nanotech, Egypt. All other chemicals used in the study were of the highest quality and all buffer solutions were prepared with ultrapure water (Milli-Q purification system, Millipore Co., Bedford, MA, USA). The dispenser used was Hamilton Bonaduz dispenser (Switzerland). The intensity of the test strip color was detected by gel documentation system (Gel Doc XR+) (BIO Rad Laboratories, USA) as a "volume" and analyzed using "Image lab" software.

2.3. Production, characterization and purification of MAb

Balb/c mice (8-10 weeks of age) were injected with *S. mansoni* SEA (100 µL of 2.3 mg/mL). After several immunization doses, their sera were tested for the presence of anti-SEA antibodies by indirect ELISA. The mouse with the highest serum antibody titer was selected for cell fusion. Splenocytes of the chosen mouse were fused with Balb/c myeloma cell line (P3x63Ag.8) according to Galfre and Milstein[11]. After the propagation of hybridoma cells, they underwent three rounds of sub-cloning to ensure their monoclonality. MAbs produced by hybridoma cell lines were tested for their reactivity against *S. mansoni*, and other parasite antigens, by indirect ELISA. Those showing strong reactivity against *Schistosoma* antigen and having no cross-reactivity with other parasite antigens were the target for large-scale production by intraperitoneal injection of hybridoma cells into Balb/c mice for ascitis production. Determination of isotype of MAb was done by indirect ELISA using a panel of anti-mouse immunoglobulin peroxidase conjugates (goat-anti-mouse IgM, IgG, IgG_1, IgG_{2a}, IgG_{2b}, IgG_3, and IgA), Kappa and lambda light chain. MAb (4D/1D) was purified from ascitic fluid using the ammonium sulfate precipitation method according to Nowotny[12], followed by treatment with caprylic acid[13]. A fraction of purified MAb was conjugated to HRP using the periodate method according to Tijssen and Kurstak[14].

2.4. Preparation of MAb–AuNPs conjugate

2.4.1. Optimized condition for MAb–AuNPs conjugation

A half mL of 10% NaCl was added to 1 mL of AuNPs containing different concentrations of MAb (30, 15, 10, 5, 2.5 µg/mL) and was

shaken for 10 min. The minimum amount of MAb for conjugation was detected by the color changes from reddish to blue. The optimum concentration for conjugation was 15 μg/mL, which is the lowest concentration of MAb solution with no change in the color[15].

2.4.2. Conjugation procedure

AuNPs with an average diameter of 20 nm were conjugated with MAb (4D/1D) according to Tanaka *et al*[16] with some modifications. Briefly, 11 μL (30 μg) of MAb solution (2.7 mg/mL) was diluted with 5 mM KH_2PO_4 solution at pH 7.5 in ultra-pure water to final volume of 200 μL which were then added to 1.8 μL of AuNPs (20 nm) and mixed immediately. After resting at room temperature for 20 min, the mixture was blocked by 200 μL of 10% BSA (w/v) (in 50 mM KH_2PO_4 solution, pH 9.0). Following centrifugation at 8 000 *g* for 10 min at 4 ℃ and pulse sonication for a few seconds, conjugated MAb-AuNPs was added to 2 mL of preserving solution (1% (w/v) BSA, 0.05% and 150 mM NaCl in 20 mM Tris-HCl buffer, pH 8.2) and then stored at 4 ℃ for further use.

2.5. Preparation of MCM-41-MAb conjugate

Briefly, 1 mg of MCM-41 was dispersed in 1 mL of phosphate buffer solution (PBS) (0.1 M, pH 7.2). Then, 18.5 μL (50 μg) of MAb (4D/1D) (2.7 mg/mL) were added to 181.5 μL of MCM-41 solution to reach a final volume of 200 μL (1:4 ratio). The mixture was stirred overnight at 4 ℃. Blocking for non-specific binding was performed by using 200 μL of BSA 10% (w/v) (in PBS 0.1 M, PH 7.2). Finally, the mixture was centrifuged for 4 min at 4 ℃, the supernatant was discarded and the white sediment was dispersed in PBS (0.1 M, pH 7.2). The MCM-41-MAb bioconjugate was stored at 4 ℃ before use.

2.6. Preparation of sensitive LFIS

The lateral flow test strip was composed of a sample pad, absorption pad and a nitrocellulose membrane with detection zone which consisted of a test line and a control line.

2.6.1. Preparation of test line solution

Five hundred μL of the MCM-41-MAb solution was mixed with 20% sucrose solution [previously diluted 50 mM potassium dihydrogen phosphate buffer (pH 7.5) and 20 μL of 2-propanol].

2.6.2. Preparation of control line solution

Forty μL of goat anti-mouse IgG (2 mg/mL) was mixed with 60 μL of 2-propanol and 100 μL of 50 mM potassium dihydrogen phosphate buffer.

The mixture was dispensed and immobilized on a nitrocellulose membrane sheet using Hamilton dispenser. Several trials proceeded for identification of the optimum distance of test and control lines on the membrane that give the maximum reaction. Nitrocellulose membrane was dried at room temperature (RT) for 1 h. Blocking procedure was applied by using a 50 mM boric acid buffer (immersion for 3 min) containing 0.5% (w/v) skim milk (pH 8.5) to prevent nonspecific adsorption. The strip was then incubated for 45 min at RT. The blocked membrane was washed for 1 min by 5.0 mM PBS (pH 7.5) containing 0.01% (w/v) sodium dodecyl sulfate and then dried overnight at RT. The sheet was cut to adequate sizes (3 cm long, 0.5 cm wide) and stored in a plastic bag at 37 ℃ till used (Figure 1).

2.6.3. Visual detection limit

Serial dilutions of *S. mansoni* SEA (as spiked samples) starting from 3 ng/mL to 500 ng/mL were prepared. About 50 μL of each dilution was mixed with 5 μL of MAb-AuNPs in a test well. The sample pad of the LFIS was immersed into the well and the solution was absorbed by capillary force. The intensity of the red color in the test line region is proportional to the concentration of SEA.

2.7. Sample collection

Stool samples were collected from patients and healthy individuals and examined by Kato-Katz technique to identify *S. mansoni* eggs. According to stool examination results, tested individuals were classified into 3 groups: (1) Active *S. mansoni* group, including 60 *S. mansoni* infected patients, (2) Other parasite group, including 20 patients harboring parasites other than *Schistosoma* (*Fasciola gigantica* and *Echinococcus granulosus*), (3) Negative control group, including 20 gender and age matched healthy individuals. Blood and urine samples were collected from all groups. Urine samples were used as such without pretreatment, while blood samples were incubated 1 h at RT, centrifuged for 10 min at 3 000 rpm, and sera were collected. Both serum and urine samples were stored at -80 ℃ until being used.

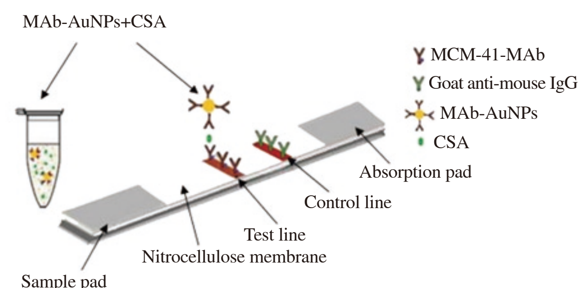

Figure 1. A diagram of the principle of sandwich based lateral flow immunochromatographic strip for detection of circulating *Schistosoma mansoni* antigen (CSA) in urine or serum samples of patients with active schistosomiasis. Serum or urine sample containing target antigen was mixed in a vial with MAb-AuNPs before migrating along the nitrocellulose membrane where they are captured at the test line by MCM-41-MAb forming a distinct red color at the test line.

2.8. Application of sandwich ELISA using MAb-AuNPs

All serum and urine samples were analyzed by sandwich ELISA according to Kamel *et al*[17], where the MAb-AuNPs was employed as antigen capturing for coating the plate, however, MAb-HRP conjugate was used as antigen detection. Briefly, the 96-well microtiter plate was coated with 100 μL/well of MAb-AuNPs in 0.1 M carbonate buffer (pH 9.6). After incubation for 24 h at RT, plates were washed and blocked by 0.1% BSA-PBS for 2 h at 37 ℃. After washing 3 times, either 100 μL of serum (1:2 in diluent buffer) or urine samples were pipetted into the wells of the blocked plate in duplicate and incubated for 2 h at 37 ℃. Plates were washed 3 times and 100 μL of MAb-HRP in diluent buffer was added to each well and the plate was incubated for 1 h at 37 ℃. The wells were then washed 5 times, 100 μL/well of substrate solution was added and the plate was incubated at RT in the dark for 30 min. The reaction was stopped by addition of 50 μL/well 8 N H_2SO_4. The absorbance of the content of each well was read by the microplate reader, Bio Rad at wavelength 492 nm against the reagent blank.

2.9. Application of sandwich based LFIS

96-well microtiter plates were used for paralleled assay of all samples. Briefly, 5 μL of MAb-AuNPs and 50 μL of serum (after dilution) or urine sample were mixed in the well. The sample pads of prepared test strips were immersed in the wells and the mixed solution then absorbed to the test strip by capillary force.

In positive cases, AuNPs-MAb-Ag complex was captured at the test line by (MCM-41-MAb) forming a sandwich immune complex with distinct red color due to colloidal plasmon resonance phenomena. The intensity of the color, which was assessed both visually and by gel documentation system (Gel Doc XR+) , is directly proportional to the concentration of the antigen in the tested sample.

In successful test, the control line should always appear, while the test line only appears when the sample is positive. If the control line doesn't appear or only the test line appears, this means that the testing procedure or the test strip was invalid and the test should be repeated (Figure 2).

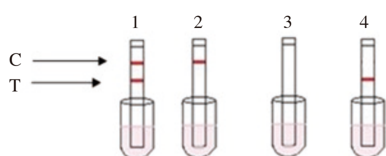

Figure 2. Schematic diagrams to show valid and invalid test strip. 1- Positive sample (with both bands) (C= control line, T= test line); 2- Negative sample (only control band); 3, 4 - Invalid test.

2.10. Statistical analysis

Data were analyzed using IBM SPSS advanced statistics, version 24 (SPSS Inc., Chicago, IL). Numerical data were described as mean and standard deviation while qualitative data were described as number and percentage. *Chi*-square (Fisher's exact) test was used to examine the relationship between qualitative variables as appropriate. Receiver operating characteristics (ROC) curve was done to calculate sensitivity, specificity, positive and negative predictive values. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Production and characterization of MAb to CSA

From a panel of anti-*S. mansoni* MAbs, 4D/1D MAb was chosen for detection of CSA due to its high reactivity against *S. mansoni* SEA. Using indirect ELISA, 4D/1D MAb was found to be strongly reactive to *S. mansoni* and not reactive to *Fasciola gigantica* and *Echinococcus granulosus*. Isotypic analysis of 4D/1D revealed that it was of the IgG_1 subclass with kappa light chain that recognized repetitive epitope on SEA when tested by immunoelectrophoresis.

3.2. Sandwich ELISA results

The lower detection limit of the SEA by MAb-AuNPs based sandwich ELISA was 30 ng/mL (detected after testing serial dilutions of *S. mansoni* SEA starting with 1 μg protein concentration/mL). The cut off value was calculated as the mean OD readings of negative controls +3SD of the mean. All subjected cases were tested by sandwich ELISA using MAb-AuNPs. In serum, positive CSA levels were detected in 58 out of 60 *S. mansoni* infected patients. On the other hand, levels of CSA in serum samples of all 20 healthy negative controls plus 18 out of 20 of other parasite group were negative (below cut off value). Using ROC curve, the area under the curve (AUC) was 0.98 (95% *CI* 0.96-1.00) with 95.0% specificity and 97.0% sensitivity. In urine samples, positive CSA levels were detected in 56 out of 60 *S. mansoni* infected patients, along with negative results in all healthy controls and in 16 out of 20 other parasite group. Using ROC curve, the AUC was 0.95 (95% *CI* 0.91-0.99) with 90.0% specificity and 93.5% sensitivity (Table 1).

A significant positive correlation was detected between intensity of infection (egg count) and OD readings in both serum and urine [(r= 0.974; P<0.001) and (r= 0.881; P<0.001), respectively] in the *S. mansoni* infected cases. The sensitivity in *Schistosoma* infected patients excreting ≤ 50 epg (light infection) was 86.0% in serum and 73.3% in the urine.

3.3. LFIS results

3.3.1. Optimal conditions of MAb-AuNPs and MCM-41-MAb for the sensitive sandwich based LFIS

AuNPs with an average diameter of 20 nm were used for conjugation. The diameter of AuNPs was checked with a transmission electron microscope (JEOLI, JEM-2100) (Figure 3). Transmission electron microscope images showed the close distribution of colloidal gold which provides a backbone for probe preparation and strong signal production in the strip.

MCM-41 was selected for immobilization of MAb as a capturing antibody at the test line. This type of silica nanoparticles with 2.1-2.7 and surface area of 1 000 m^2/g minimizes the non-specific adsorption and provides the desired biocompatibility. Its filament structure with a hollow position is a very suitable structure for protein immobilization. The scanning electron microscopic image (JEOL JSM-IT-100) of employed MCM-41 in the absence of MAb is demonstrated in Figure 4a. After conjugation with MAb, a thick structure was produced as an indicator of protein adsorption (Figure 4b).

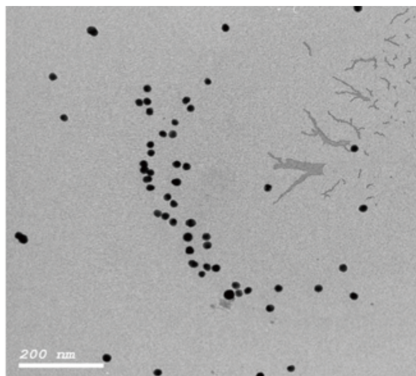

Figure 3. Analysis of gold nanoparticles by transmission electron microscope that identifies their size and shape. The diameter ranges between 15 and 20 nm.

Table 1. Percentage of sensitivity, specificity, positive predictive value, negative predictive value and total accuracy of sandwich ELISA using MAb-AuNPs for detection of circulating *Schistosoma mansoni* antigen in serum and urine samples of all groups.

ELISA	Sensitivity	Specificity	PPV	NPV	Total accuracy
Serum	97.0%	95.0%	96.7%	95.0%	96.0%
Urine	93.5%	90.0%	93.3%	90.6%	92.0%

Following several optimization trials, these test conditions were adopted: the ratio of MAb-AuNPs to test sample was 1:10 (5 μL of AuNPs were added to 50 μL of test sample), the optimum sample dilution was 1:2 for serum sample, while urine samples were used without pretreatment. The best concentration for a conjugated mixture of MCM-41-MAb was found to be 4 μL of MCM-41 (1 mg/mL) for each one μg of MAb.

3.3.2. Sensitivity and specificity of the LFIS

The visual detection limit of our LFIS (the concentration at which the faintest test line color developed) was 3 ng of *S. mansoni* SEA/mL (Figure 5). Serum and urine samples from all groups (60 *S. mansoni* infected patients, 20 patients infected with other parasites and 20 healthy individuals) were examined using the strips. Test validity was verified by detection of the control line in all tested strips. Positive samples should give colored test and control line. The intensity of red color of the test line was measured using gel documentation system. The cut-off value of the strip was determined *via* the ROC curve. For serum samples, the AUC was 1 (95% *CI* 1.000-1.000) and the cut-off value of 1.1×10^5 intensity (volume) was corresponded to 97.5% specificity and 100.0% sensitivity. For urine samples, the AUC was 0.99 (95% *CI* 0.972-1.000) and the cut-off value of 0.5×10^5 intensity was corresponded to 97.5% specificity and 98.3% sensitivity (Table 2). According to these cut-off values, positive CSA level was detected in the serum of all 60 *S. mansoni* infected patients, one case out of 20 of other parasite group and in 0 out of 20 healthy controls. On the other hand, in urine samples, positive CSA level was detected in 59 out of 60 *S. mansoni* infected

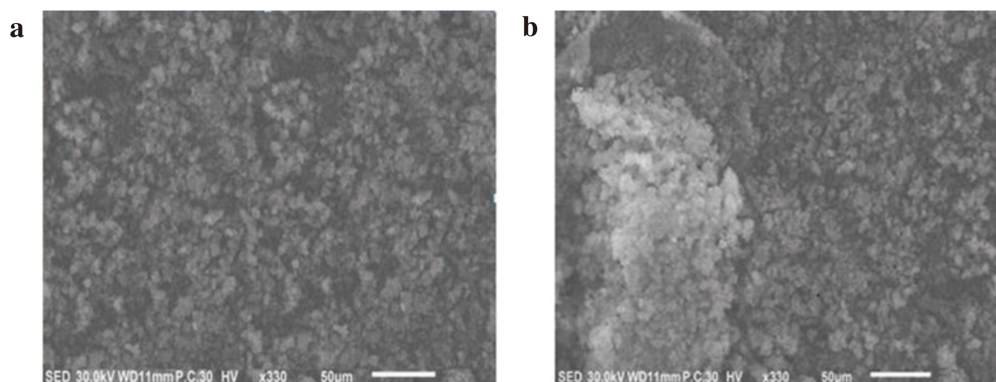

Figure 4. Scanning electron microscopic images of (a) MCM-41, (b) MCM-41-MAb (scale bar=50 μm).

patients, one case out of 20 of other parasite infected cases and in 0 out of 20 healthy controls.

A significant positive correlation was detected between ova count in *S. mansoni* infected cases and the intensity of our LFIS in serum (r = 0.950; P<0.001) and urine (r= 0.900; P<0.001) samples. Moreover, the sensitivity of LFIS in *Schistosoma* infected patients excreting ≤ 50 epg (light infection) was 100% in serum and 93.3% in the urine.

3.4. Correlation between LFIS and sandwich ELISA results

Both assays were significantly correlated in serum (r=0.943; P<0.001) and urine (r = 0.897; P<0.001) samples (Figure 6a &b).

Table 2. Percentage of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and total accuracy of lateral flow immunochromatographic strip (LFIS) for detection of circulating *Schistosoma mansoni* antigen in serum and urine samples of all studied groups.

LFIS	Sensitivity	Specificity	PPV	NPV	Total accuracy
Serum	100.0%	97.5%	100.0%	97.5%	99.0%
Urine	98.3%	97.5%	98.3%	97.5%	98.0%

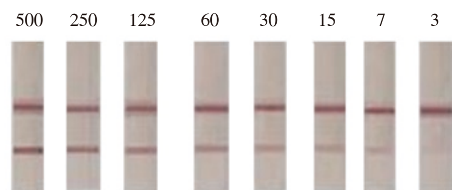

Figure 5. Determination of the visual detection limit of lateral flow immunochromatographic strip (LFIS) using different concentrations of soluble egg antigen (SEA) starting with 500 ng/mL till 3 ng/mL. Detection limit of SEA by LFIS test strip was determined at 3 ng/mL.

4. Discussion

Development of point-of-care testing (POCT) devices that are sensitive, specific, rapid, easy to interpret and field applicable, to monitor and detect infectious diseases, is crucial, especially in developing countries. Using these tests for diagnosis of schistosomiasis is an efficient and promising tool, especially in rural communities of disease-endemic countries. They could replace the conventional microscopy approach if they had simple and rapid tests with sufficient accuracy and field applicability. Generally, the presence of circulating antigens in urine or serum samples is directly correlated with parasite load and can differentiate between active and past infection[18]. Several studies discussed the employment of circulating antigens such as circulating anodic antigen (CAA) and circulating cathodic antigen (CCA) for POCT of active schistosomiasis detection[19,20]. The POC-CCA urine strip test is a commercially available lateral flow test applied for routine detection of *S. mansoni* infections, however, it has a low sensitivity and specificity for low endemic settings[21]. Moreover, limited sensitivity and false positive results have been reported when POC-CCA was applied in Brazil and in some parts of Africa[22]. Several studies worked for its improvement by concentrating urine samples through their lyophilization[23], or using larger sample volume[24]. Furthermore, CAA based assay has high complexity that is related to urine sample pre-treatment step using trichloroacetic acid followed by centrifugation step[25].

In the current study, we developed a novel sandwich based LFIS for rapid detection of *S. mansoni* CSA in urine and serum samples using both gold and mesoporous nanoparticles to guarantee more sensitivity and specificity of the assay. MSNs have unique and favorable features such as large pore size, ordered uniform pore structure, biocompatibility, chemical stability and ease of surface modification, making them suitable for the broad spectrum of

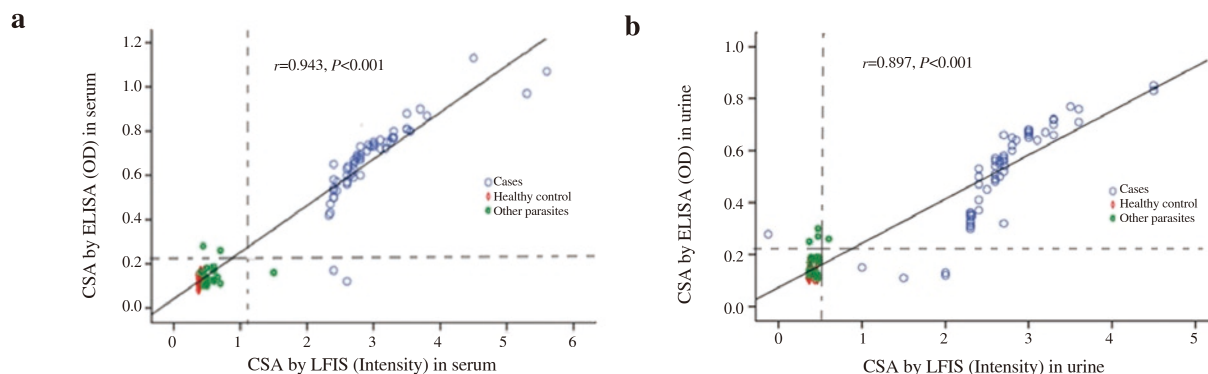

Figure 6. Correlation between circulating *Schistosoma mansoni* antigen (CSA) levels detected by lateral flow immunochromatographic strip (LFIS) (intensity) and sandwich ELISA (OD) readings. Serum (a) and urine (b) samples from 60 *Schistosoma mansoni* infected patients, 20 infected with other parasites and 20 control subjects are analyzed by both assays and the results of the two assays are correlated. Lines of dots indicate the cut-off values for each assay.

biomedical applications[26]. MCM-41 type silica was employed for immobilization of capturing Ab at test line of the strip. Its use offered stronger reaction at the test line when compared to the non-MCM-41 immobilized Ab. On the other hand, AuNPs with an average particle diameter of 20 nm, were employed for CSA capturing from the serum or urine samples. This combination of both gold and mesoporous nanoparticles is the effective key factor providing higher sensitivity and specificity for our CSA detection assay.

All serum and urine samples of all subjected groups including *S. mansoni* infected group, other parasite infected group and healthy control group were analyzed by sandwich ELISA using AuNPs-MAb as described in our previous work[17], and results were compared with those of LFIS. Both assays were also compared regarding intensity of infection in *S. mansoni* infected cases.

We have found that the results of both assays were relatively well correlated in serum (r= 0.943; P<0.001) and in urine (r=0.897; P<0.001). But the sensitivity and specificity of LFIS in urine (98.3% and 97.5%) and serum (100% and 97.5%) samples respectively were higher than those obtained by sandwich ELISA. The higher sensitivity and specificity of LFIS could be attributed to the employment of MCM-41 silica type nanoparticles for immobilization of MAb onto the membrane surface test line as a capture bioconjugate. These results were corroborated by results of Omidfar *et al* who reported that modified membrane using MCM-41 for protein immobilization showed more stability than bare membrane[27]. Moreover, Wang *et al*[28], also reported that MSNs are used to improve the sensitivity of immunosensors used for the analysis of biologically active proteins.

Furthermore, the correlation between LFIS obtained results and the intensity of the infection (egg load) was higher and showed more sensitivity in light infection than that obtained on correlation with sandwich ELISA.

In conclusion, LFIS, developed based on employment of gold and silica type nanoparticles, provides rapid and sensitive detection for CSA in urine and serum samples of patient with active schistosomiasis. Its key advantages are the simplicity and fast detection (10 min). Furthermore, its highly sensitivity and specificity, especially in urine samples, guarantee its application with more accuracy and rapid detection.

Conflict of interest statement

We declare that there is no conflict of interest.

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