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Original article

Evaluation of a novel homemade sandwich ELISA and lateral flow assays conjugated with gold-nanoparticles for detection of human fascioliasis antigens

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ABSTRACT

Background and rationale: Fascioliasis is a neglected parasitic infection that affects both humans and animals. It is caused by the trematode parasites known as *Fasciola hepatica* and *Fasciola gigantica*, which primarily infect the bile duct. Fascioliasis has a significant negative social and economic impact, with estimated infected rate in livestock (18%) and 2.6 million persons worldwide. The highly prevalence was recorded in least developed countries. The actual challenge is to obtain a cheap and efficient diagnosis technique to diagnose the early mild stage of fascioliasis, parasitological examination techniques are usually effective in the final stage of infection when a large number of fascioliasis eggs are execrated in stool**. Aim:** The aim of this study was conducted to develop and compare a novel diagnostic lateral flow assay and sandwich ELISA for detection of active fascioliasis infection. The conjugation of fascioliasis tegumental antibodies to AuNPs through covalent conjugation using EDC/Sulfo-NHS, to application in the setup of immuno-chromatography lateral flow assay (LFA) and sandwich ELISA. The novel LFA succeeded to diagnose (37 out of 38 true positive infected fascioliasis group), (2 out of 81 true negative other parasites infected group) and (0 out of 27 negative control group), the sensitivity, specificity, NPV and PPV of were 97.4%, 98.2%, 99.1%, and 94.8%, respectively. while the sensitivity, specificity, NPV and PPV of sandwich ELISA were 94.7%, 96.3%, 98.1%, and 90%, respectively. **Conclusion**: The higher sensitivity and specificity were recorded in LFA. Lateral flow assays (LFA) technique could be used as Ideal diagnostic techniques in monitoring and control of fasciolosis infection.

Introduction

Fascioliasis is a foodborne zoonotic neglected parasitic infection that affects both humans and animals especially in livestock include cattle, sheep, goat, pigs and horses. It is caused by the trematode parasites belonging to the genus *Fasciola* known as *Fasciola hepatica* and *Fasciola gigantica (F. gigantica)*, which primarily infect the bile duct [1-3]. Humans become occasional hosts by eating aquatic vegetable or by drinking contaminated water containing a live infective stage metacercariae of the parasite [4,5]. Human fascioliasis is one of the most widespread helminthes worldwide, causing significant morbidity and mortality rates and many socio-economic issues and medical importance are

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unimpeachable, it is estimated that worldwide average from 2.6 to 17 million people are infected with fascioliasis [5, 6]. Fascioliasis is mostly prevalent in more than 70 of less developed countries with temperate climates, while as is also widespread in Egypt, Iran, Bolivia, Ecuador, Peru and Asia [7].

In Egypt, human fascioliasis has a large prevalence rate especially in schoolchildren at Alexandria and Behera governorates in Nile delta and in adults at Assyout Governorate in Higher Egypt. There are high-risk infection rate Governorates which requires additional monitors and control measures, the most common signs and symptoms reported in patients were right upper quadrant pain, high temperature fever, eosinophilia, hepatic focal lesions [8-10].

Diagnosis of fascioliasis at early infective stage is very critical point, it is necessary to enhance early treatment to prevent reaching non-repairable infective stage. Due to challenging diagnosis of fascioliasis infection like difficulty of getting *Fasciola* antigens from human source and the searching for an alternative from animal source, intensity of infection varies from one endemic area to another, parasitological diagnosis methods are not useful in low density or chronic infection and needs a specialized skill, difficult in the routine diagnostic laboratory and in antibodies detection, not all patients infected with Fascioliasis have metacercariae in the blood; infection may not be patent because the parasites are immature. Point-of-care testing methods have been explored to improve the detection of this infection. Laboratory tests include the demonstration of antigens (Ags) and specific antibodies (Abs). Determine the ideal diagnosis methods or tools depend on several factors; diagnosis purposes, financial restraints, population number to be examined, the prevalence or widespread percent of disease, the availability of laboratory tools and qualification and experience of the technical personnel. Nanotechnologies-based immunoassays are powerful and potentially beneficial methods to maximize sensitivity, minimize sample volume requirements, and produce fast and accurate results. The main purposes for diagnosis of fascioliasis infection are evaluation of patient treatment and monitoring and control of mass treatment for groups of patients or epidemiological studies. Sandwich ELISA and lateral flow assays (LFA) conjugate with gold nanoparticles (AuNPs) are among the most commonly used assays in detection of fascioliasis Ag, these techniques proved to be highly accurate, sensitive and specific for antigen detection in serum and in stool. Such techniques depend on the use of specific well purified antibodies as a coating capture and as conjugate detectors. Polyclonal tegumental antibodies (pTAb) have clear technical advantages. pTAb are inexpensive to produce relative to the cost of monoclonal (mAb) technology [11].

The main purpose of this study is to report the isolation and covalent conjugation of *Fasciola* tegumental antibodies from live adult *Fasciola* worms with AuNPs to enhance and determine the accuracy and effectiveness of sandwich ELISA and rapid test in diagnosis of human fascioliasis infections. This study provides an improved immunoassay format for assessment of human fascioliasis antigen through homemade sandwich ELISA and lateral flow assays.

Material and methods

Human immune sera

In this study, after the parasitological examination by examining stool (fecal) specimens under the light microscope. Blood samples were collected under ethical condition from four different governorates in Egypt, Assiut, Cairo, Qalyubiyya and Gharbia Governorates. The study subjects 146 individuals' samples that were divided into three main groups: The first group was patients with propounded clinical demonstration of fascioliasis infection – 38/146 (26%), the second group was patients with other parasitic (OP) infections other than fascioliasis – 81/146 (55.5%), the third group was healthy controls people (HC) – 27/146 (18.5%). The use of human sera samples was approved by the medical ethics committee of the Theodor Bilharz Resarch Institute (TBRI) (Ethics No. CU-I-S-28-16).

Preparation of fascioliasis tegumental antigen

Live flukes *F. gigantica* adult worms have a richness amount of tegumental antigen rather than died worms. The live adult worms were collected from the liver of infected livestock animals from the slaughterhouses of Cairo districts in Egypt **(Figure 1A).** Adult worms washed in Tyrode medium. The *F. gigantica* tegumental antigen was isolated by using 7% of Triton X-I00 for tegumental cells lysing to extract the antigen. Then the washed worms were immersed in 7% Triton X-I00 in 0.01 mM phosphate buffer solution (PBS) and gently continuous shaking for 20 minutes (min) under incubation condition at

0–4°C **(Figure 1B).** The suspension was vortexed for 5 min, the adult worms removed by gently hand picking, then centrifuged at 500 rounds per minute (rpm) for 10min. The supernatant containing the tegumental antigen was dialyzed against PBS to eliminate Triton X-I00 from the protein sample, then against distilled water and stored at -20°C until use [12].

Production of fascioliasis polyclonal tegumental antibodies

Eight months old New Zealand white rabbits (~1.4 Kg) purchased from animal house, at agriculture research center, Cairo University, Egypt. Before start the experiments, the rabbit was tested to confirm that it was free from any parasitic infections and cross-reactivity with other parasites. The rabbit was immunized intramuscularly at four legs with a priming dose of 1mg of *F. gigantica* tegumental Ag mixed with 50 µl of alminimum hydroxide (13mg/ml). After two weeks rabbit was injected with first booster dose (0.5 mg antigen mixed with 50 µl of alminimum hydroxide) followed by 3 booster doses, one week between each dose. Taking in consideration, rabbit serum samples were collected before each intramuscularly fortification dose, whenever the priming dose or the booster doses to test with indirect ELISA to detect the cross-reaction *F. gigantica* tegumental antibodies titre [13]. When the titre was reached the higher peak, the rabbit bled and blood sample was collected and anti-sera were stored at -70 °C till used.

Purification of fascioliasis polyclonal tegumental antibodies

In the first, fully saturated ammonium sulfate (FSAS) solution was applied drop-wise with continuous stirring to the anti- *F. gigantica* tegumental serum containing IgG antibody to reach 50% saturation i.e. (1:1of SAS to rabbit anti-*F. gigantica* tegumental serum), thereafter the mixture was centrifuged for 15 minutes at 3500 rpm in Heraues refrigerated centrifuge at 4°C. Supernatant solution was removed. The precipitate was dissolved in 0.01 M PBS, pH 7.2. The ammonium sulfate was eliminated by dialysis in 0.01 M PBS, pH 7.2 for 72 hours at 4°C. Then followed by, Seven percent of caprylic acid was added drop wise to the serum containing partially purified anti- *F. gigantica* tegumental IgG antibody with gently continuous stirring for 20 min at 4ºC. The solution was separated by centrifuged at 3000 rpm for 30 minutes at 4ºC temperature.

Perpetration of AuNPs

AuNPs with an average diameter 20±5nm have been synthesizing via a chemical reduction method. Briefly, 1 ml of 1% HAuCl4 solution in 100 ml of distilled water was added to a clean 500 ml Erlenmeyer flask on a stirring hot plate and heated up to boiling under continuous stirring by a magnetic stirring heater, after boiling 1 ml of 1% tri-sodium citrate was added under constant stirring and boiling conditions. After while the color of the solution has changed from yellow to dark red after approximately 3 min **(Figure 2),** continue boiling the solution for 5 min more. Then the solution was cooled at RT, the gold nanoparticles solution was stored in a brown bottle to prevent the sample away from light.

Characterization of AuNPs

AuNPs were characterized by transmission electron microscopy (HR-TEM) JOEL JEM-2100 operating at 200 Kilovolt (kV) equipped with Gatan digital camera Erlangshen ES500, AuNP sample was characterized by placing one drop of a dilute suspension of AuNPs on a carbon-coated copper grid for 3 minutes at room temperature, preparation and characterization were carried out in Nanotech, 6th of October city, Giza, Egypt.

Antibodies optimum concentration (conc) and pH optimizations for the surface of AuNPs

Determine the optimum concentration of antibody and adjust the pH of the surface of colloidal gold nanoparticles for testing the stability of conjugation of AuNPs and Abs, we tested four different pH titration solutions [pH 7.5 solution of 4-(2-hydroxethyl)-1-piperazineethanesuulfonic acid, pH 8.5 solution of PBS, pH 9.5 and 10.5 solutions of potassium carbonate buffer (PCB)]. This step was repeated three times to determine the most stable concentration. Thereafter, 20µl of 100 mM from each pH solution was mixed with 20±4nm of colloidal AuNPs solutions, respectively, and then serial dilutions of antibodies (5µl, 10µl, 15µl and 20µl) were added, respectively. Next, vortex for 5 seconds and rotated the mixture for 15 minutes. To confirm the conjugation stability of antibodies with the gold nanoparticles, 50µl from each mixture was added and mixed with 50µl of 10% sodium chloride solution (1:1 V:V), each mixture was vortexed and rotated for 1 hour at room temperature (RT). The optimal conc of Abs and pH were detected by observing changes in the color and aspect of the mixtures

Covalent conjugation of the AuNPs with fascioliasis tegumental abs

After identification the ideal amount of antibodies and pH concentrations by sodium chloride solution, 8.5 ul 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (50uM) was added to 1 ml of AuNPs for 30 mins at 25ºC in checker incubator, after incubation 17ul of Sulfo-N-hydroxysuccinimide (NHS) (50uM) was added to the mixture(EDC/Sulfo NHS are a bio-conjugation that increase electrostatic attraction and enhance the conjugation rate between Abs and AuNPs), which left under checking condition for 30mins. Subsequently, the mixture was centrifuged at 3600 rpm for 5 mins at 25ºC. Appropriate amounts of anti-fascioliasis rabbit IgG were added to the colloidal gold solution with continuous and gentle rotation under incubation condition for 20 minutes. Thereafter, 3µg of 17β-estradiol was added to enhance the orientation attachment of antibodies on the AuNPs surface to utilize the minimum number of antibodies [14] and vortex to resuspend, under continuous and gentle shaking for 1h at 25ºC.

Fabrication of LFA

The principle of preparation of LFA is based on the Sandwich immunoassay as illustrated in the following diagram in (**Figure 3)** and disrobe below; the LFA is prepared from four pads (sample, adjustment, conjugate and absorbent pads), nitrocellulose membrane (NCM), self-adhesive (PE) protective film and semirigid polyethylene sheet (Plastic Backing film). All (membrane, pads, protective film) were assembled on semirigid polyethylene sheet. Briefly, the nitrocellulose membrane (NCM) is stamped in the middle of the Plastic Backing film, followed by the gold conjugated pad with 2mm overlap at NCM end, then, thereafter, the adjustment pad with 2mm overlap at conjugated pad end, subsequently the sample pad with 2mm overlap at adjustment pad end, then the absorption pad was added with overlap of 2mm at NCM end and finally the gold conjugated pad and adjustment pad were covered by the protective film with overlap of 2mm at sample pad and NCM. The assembled master card (Strip) was cut into 2.7mm strips using ECHO1 cutter.

Determine the optimum concentration and testing of human serum samples by LFA

One hundred and forty-six (146) serum samples were collected from individuals infected with (fascioliasis, some may have also been infected with other parasites, and others were healthy control samples). All participants have undergone ELISA and microscopy screening. Human serum samples of variable concentrations (5 µl, 10 µl, 15 µl, 20 µl, and 25 µl) were diluted in 100 µl PBS, added to sample pads, and allowed to migrate. After 10 minutes, the color development of the segments was examined. The presence of two-colored lines (test and control) signifies a positive diagnosis. A single-colored control line devoid of a control line indicates that the sample was negative.

Determine the optimum concentration and testing of human serum samples by using sandwich ELISA

One hundred (100) µl/well Au-F-pAb (5 ng/ml carbonate buffer, pH 9.6) was added to ELISA plate and incubated overnight at RT. ELISA plate was washed 3 times with 0.1 M PBS-tween20 (PBS/T), pH 7.4. The unattached spaces were covered by incubating 200 µl/well containing 2.5% bovine serum albumin and PBS/T at 37°C for one hour. PBS/T was used 3 times to wash the plates. After determining the optimal concentration by standardizing the sandwich ELISA against fascioliasis antigens and antibodies, 100 µl of serum diluted samples were pipetted at 37°C for 1 hour in duplicate wells. Following 3 washes, 100 µl/well of horseradish peroxidase-conjugated tegumental fascioliasis pAbs was added and incubated at RT for one hour. The plate was rinsed with a washing buffer 3 times. Subsequently, each well was filled with 100 µl of O-phenylenediamine dihydrochloride (OPD) solution, and the plate was incubated in the dark environment at RT for 30 minutes or until first color changes are observed. Lastly, to discontinue the reaction between enzyme and substrate, 50 µl of 8 N Conc sulphuric acid was placed in each well and the result was determined by using an ELISA reader at 492 nm.

Statistical analysis

Statistical analysis was performed with the aid of the Statistical Package for the Social Sciences (SPSS) computer program; data are expressed as mean ± standard deviation (SD). The cut off, sensitivity (Se), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) were calculated as the following:

Cut off value = $(The\ mean\ values\ of\ negative\ samples + (3 \times standard\ deviation\ SD))$

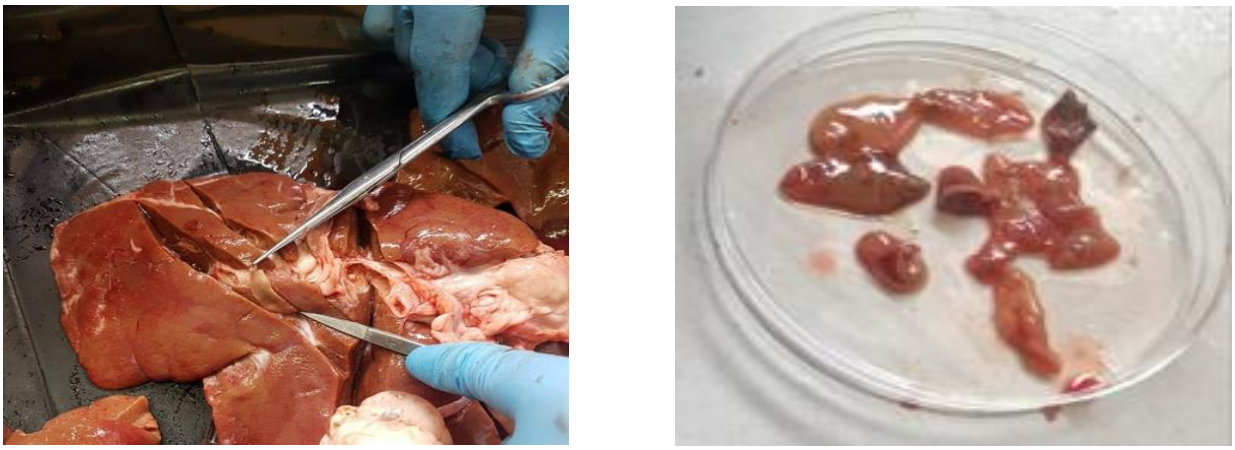
$$Se = \left(\frac{\text{No.of true positive results}}{\text{No.of +ve results + No.of false −ve results}} \times 100\right)$$

$$Sp = \left(\frac{\text{No.of true negative results}}{\text{No.of−ve results + No.of false+ve results}} \times 100\right)$$

$$PPV\ (\%) = \left(\frac{\text{No.of true positive results}}{\text{No.of true +ve results + No.of false +ve results}} \times 100\right)$$

$$NPV\ (\%) = \left(\frac{\text{No.of true negative results}}{\text{No.of true −ve results + No.of false −ve results}} \times 100\right)$$

Figure 1. **(A)** Collection of live adult *Fasciola* from bile duct of infected animal, **(B)** Production of tegumental Ag.



(A) (B)

Figure 2. Preparation of AuNPs by a chemical reduction method.

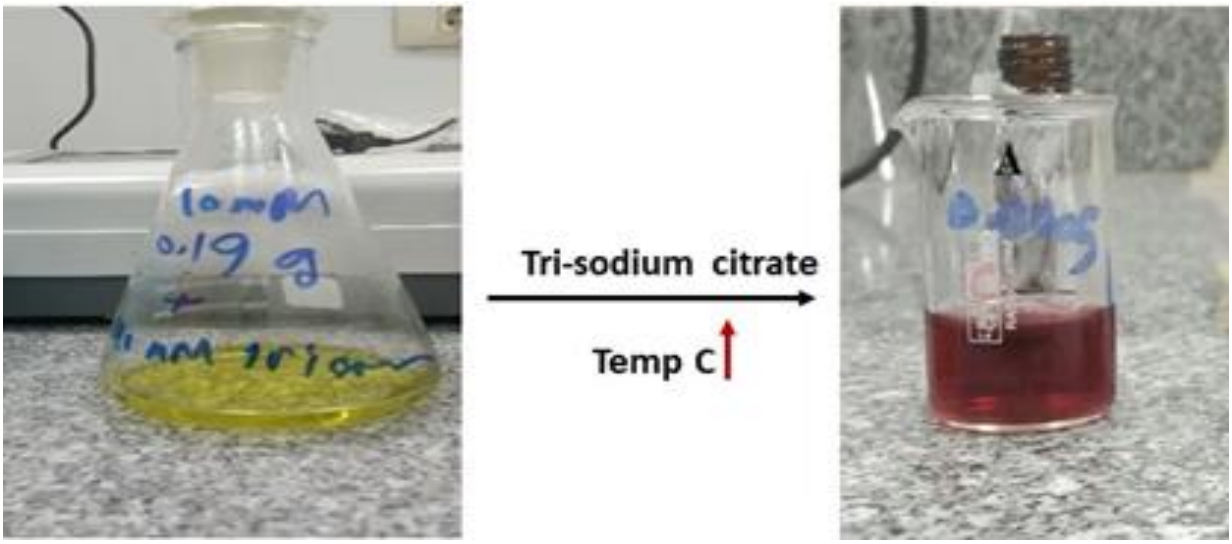
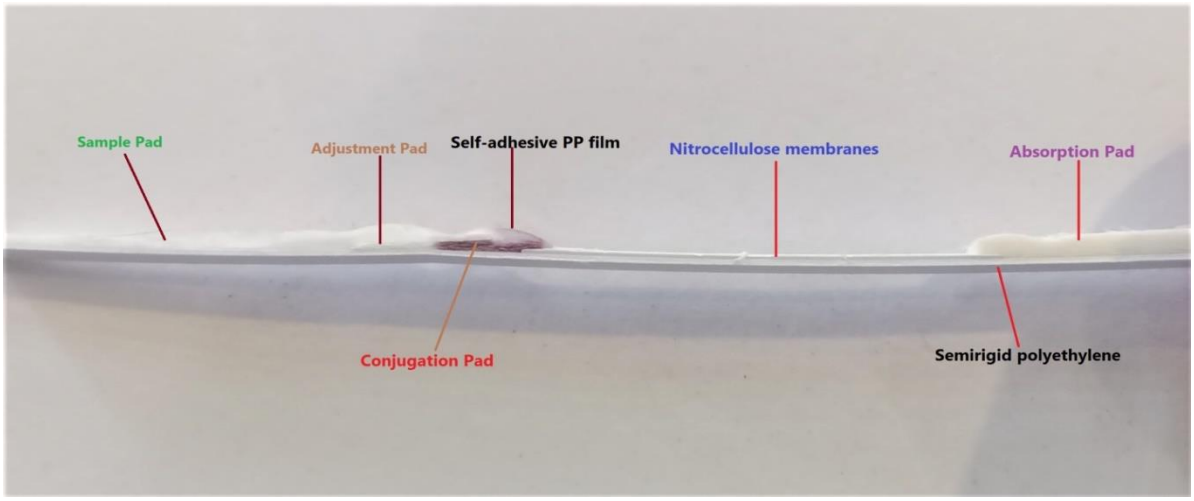


Figure 3. Illustration photo of lateral flow assay strips (LFA) for antigen detection.



Results

Production of fascioliasis polyclonal tegumenal antibodies

The rabbit's serum sample was tested by indirect ELISA to determine concentration of specific anti- *F. gigantica* Abs. the fascioliasis Abs titre began increase after 1 week from the primary dose. After four weeks from the primary dose the anti-sera titre was given the higher peak, and the protein content of anti-tegumental Abs was 2.44 mg/ml **(Figure 4).**

Characterization of AuNPs

AuNPs have been characterized via HR-TEM. AuNPs have a faint pink to reddish black color, with 200ppm concentration and spherical shape appearance; with an average size around 20 ±4 nm **(Figure 5).**

The optimum conc of sandwich ELISA and LFA used for assessment of anti- *F. gigantica* IgG Abs

The application of AuNPs-anti-tegumental IgG-pAb as antigen capture antibody was evaluated by coating an ELISA plate with different dilutions of AuNPs-anti-tegumental *F. gigantica* IgG pAb (1/50, 1/100, 1/200, 1/400, 1/800, 1/1600, /1/3200 µg/ml) against different dilutions of Fascioliasis sera antigen (as described above) and different dilutions of the conjugate antibodies (as described above). By optical density OD readings at 492 nm, the optimum dilutions of AuNPs-anti-tegumental IgG pAb conjugated, fascioliasis human sera antigens and peroxidase-conjugated anti-tegumental *F. gigantica* IgG pAb were 1/1600, 1/200 and 1/800 µg/ml, respectively. The optimum dilution of human fascioliasis sera for LFA was 10 µl diluted in 100 µl PBS.

Assessment of fascioliasis antigen in human blood samples by using sandwich ELISA conjugated with AuNPs.

The cut off values for positivity was calculated and equaled 0.254. The mean OD value of *Fasciola* human antigens was 1.302±0.339 using tegumental *F.gigantica* antibody conjugated with AuNPs. Two out of 38 fascioliasis infected group expressed false negative results. The sensitivity of sandwich ELISA using tegumental *F.gigantica* Abs conjugated with AuNPs was 94.7 %. All 27 negative HC group samples were below the cut off value **(Table 1),** while 4 out of 81 of OP group were positive giving 96.3% specificity **(Figure 7),** the percentage of positive results that are true positive (PPV) was 90% and the percentage of negative results that are true negative (NPV) was 98.1% **(Table 3) (Figure 8).**

Assessment of fascioliasis antigen in human serum samples by using homemade LFA

Using homemade LFA for detection fascioliasis positive infections. The red color of the stripes in the control and test lines was observed after an average time of 7 to 10 minutes. One out of 38 fascioliasis infected group samples showed false negative results, 2 out of 81 were gave weak false positive within OP infection group **(Figure 6.A).** All of the HC patients tested negative **(Figure 6.B). (Table 2).** The Se, Sp, NPV and PPV for antigen in serum were 97.4%, 98.2%, 99.1% and 94.8%, respectively **(Figure 8).**

Table 1. Accuracy of reactivity of human *Fasciola* antigens using anti-tegumental *F. gigantica* IgG by Nano-sandwich ELISA at 492nm.

Groups	No of samples	+ve samples	Mean of OD	SD	Cutoff
Negative control	27	0	0.226	0.014	0.254
Other parasites	81	4	0.247	0.113	
Fascioliasis	38	36	1.302	0.339	

Table 2. Accuracy of reactivity of human *Fasciola* antigens using anti-tegumental *F. gigantica* IgG by LFA.

Groups	No of samples	Positive cases	Negative cases	Invalid cases
Negative control	27	0	27	0
Other parasites	81	2	79	0
Fascioliasis	38	37	1	0

Table 3. Diagnostic indices (Se, Sp, NPV and PPV) of sandwich ELISA conjugated with AuNPs and LFA detecting *Fasciola* antigens in human sera.

	Se	Sp	NPV	PPV
Sandwich ELISA with AuNPs	94.7 %	96.3 %	98.1 %	90 %
LFA	97.4 %	98.2 %	99.1 %	94.8%

Figure 4. Rabbit immunized reactivity against tegumental *Fasciola* antigen.

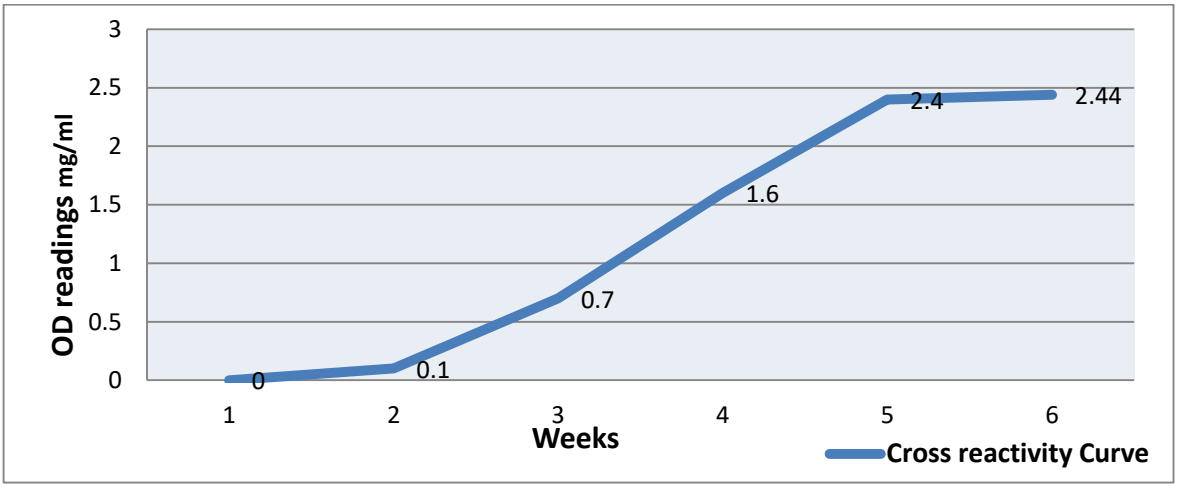


Figure 5. AuNPs with spherical shape appearance under transmission electron microscopy (TEM).

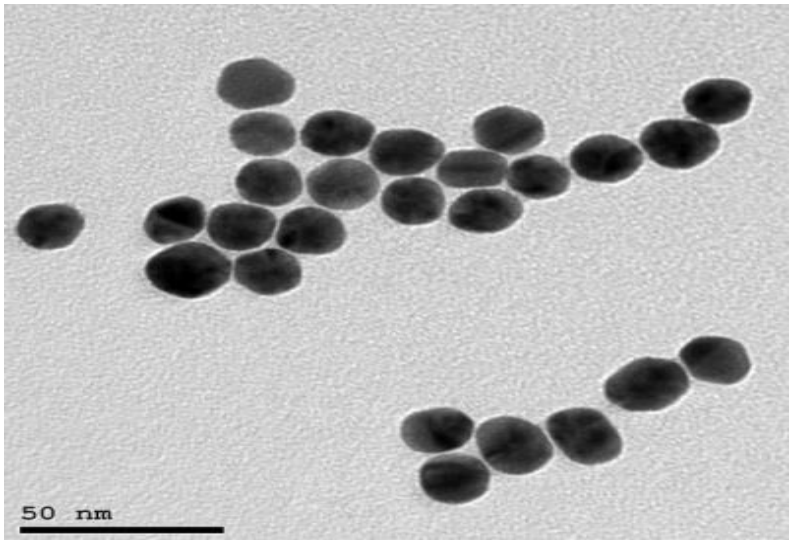
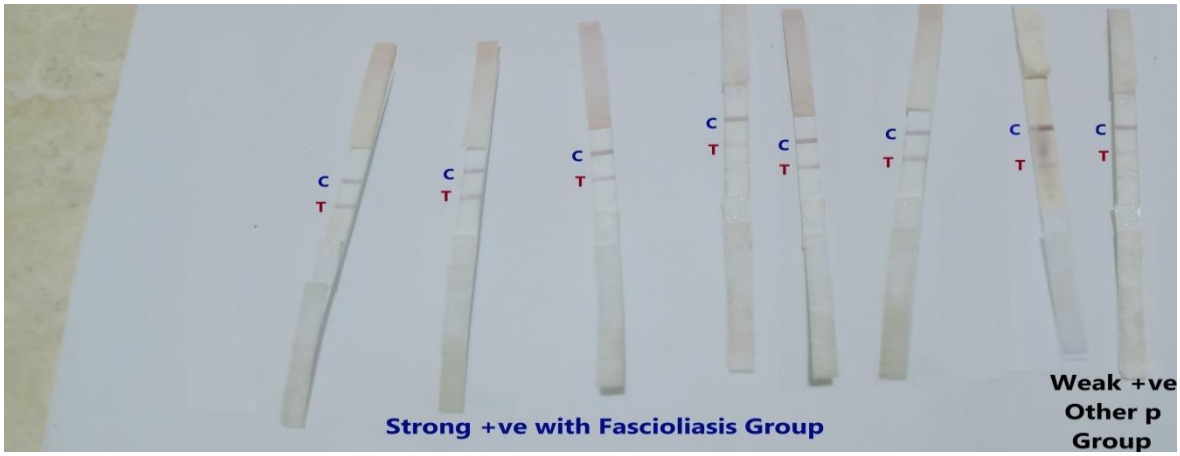
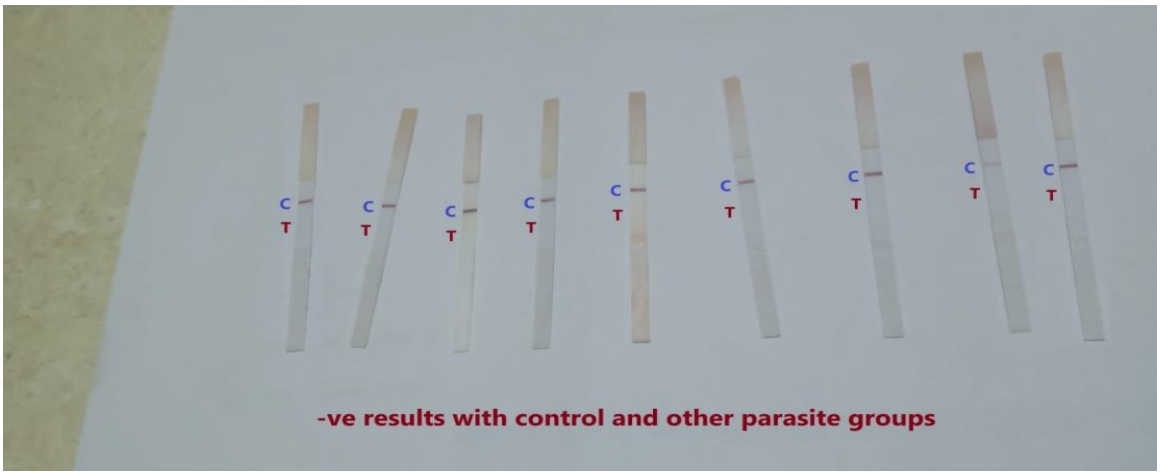


Figure 6. Assessment of *Fasciola* antigen by using homemade LFA using fascioliasis positive (+ve), OP and HC negative (-ve) groups human sera. (A) True positive fascioliasis group and other parasite false positive, (B) true negative other parasite and control groups. C letter in blue color describes the control line and the T letter in red color describes the test line of positive sera.



(A)



(B)

Figure 7. Scatter diagram for determine the accuracy of sandwich ELISA with the three different studies groups, cutoff value = 0.254.

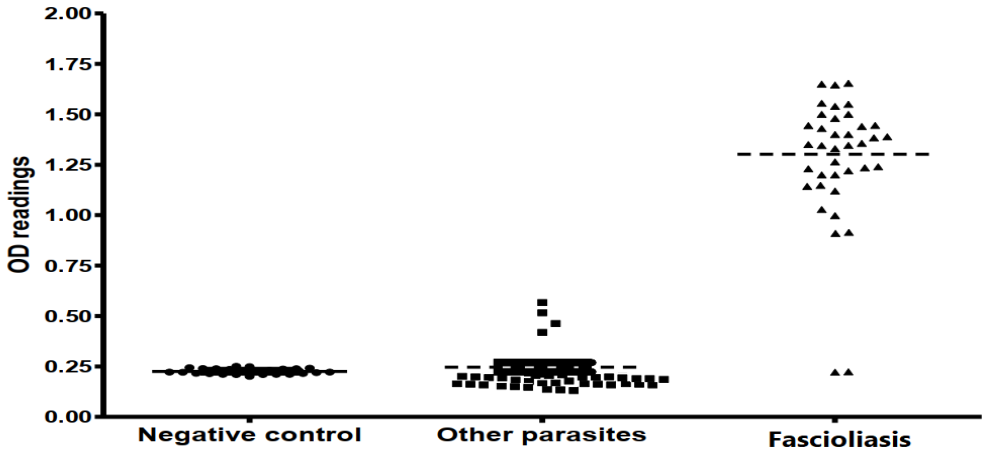
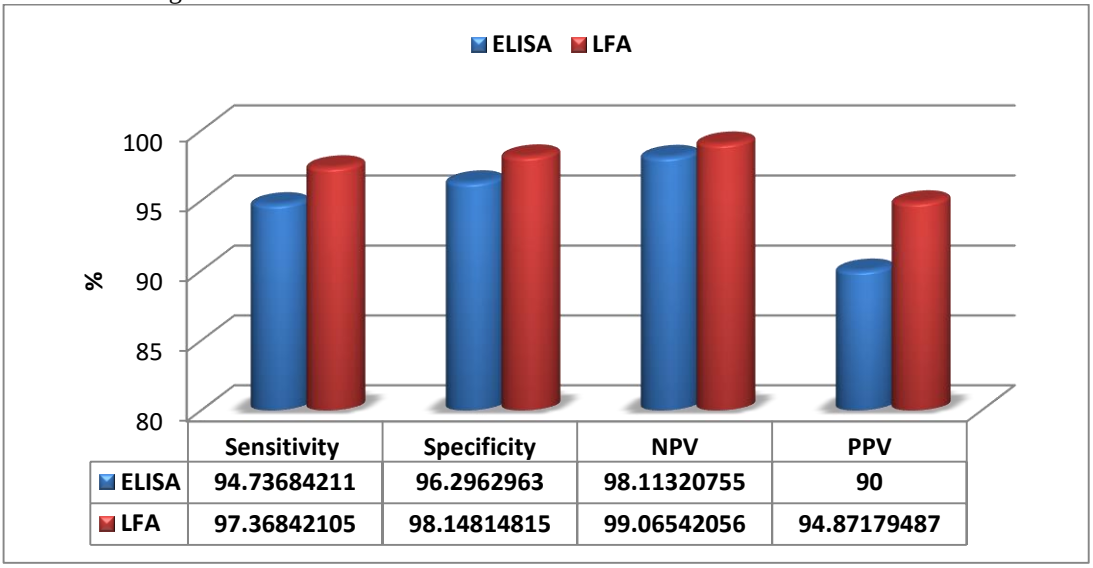


Figure 8. Comparison histogram for Se, Sp, NPV and PPV of sandwich ELISA and LFA in detecting fascioliasis antigen in human sera.



	Sensitivity	Specificity	NPV	PPV
ELISA	94.73684211	96.2962963	98.11320755	90
LFA	97.36842105	98.14814815	99.06542056	94.87179487

Discussion

Up to date, several studies have reported parasitological fecal analysis for *Fasciola* eggs is a trustworthy, easiest and cheapest test can be used in distant endemic regions, and although considered as the ideal standard method and the conformed method for differential and identification diagnosis in many studies, but one major disadvantage feature is that the sensitivity is very low and needs a specialized skill, the immunoassays techniques are more efficient for diagnosis of human fascioliasis [15]**.** At the same time, the parasitological technique lacks accuracy and sensitivity because of the widely differences in the number of eggs secreted especially with mild infections patients, needs an expert technicians to be able to detect eggs in stool samples and eggs appear in the definitive hosts feces quite late, approximately at sixteen weeks from the incubation period when metacercariae became adulthood stage [16]. Occasionally, with some patients, as in the acute or light chronic infection may present a long term febrile illness with disability that may remain undiagnosed for several days or weeks, the available parasitological diagnostic methods have low accuracy of specificity and sensitivity and that lead to inaccurate assessment of widespread and severity of infection [17]. To control parasitological diagnostic methods defects, immunological diagnostic methods are presented. Detection of anti-*Fasciola* antibodies in blood plays an important role in diagnosing fascioliasis only two weeks after infection and before the appearance of fascioliasis eggs in the stool. Nevertheless, Antibody detection methods have inaccuracy of sensitivity and specificity, an inability to distinguish between previous and current infection and may persevere for long time after the parasites disappear or being killed [16]. It was noted the antigen diagnostic methods may provide the ideal diagnostic method for early stage infection rather-than antibody detection methods, because the antibody detection method takes a long time to produce the immunoglobulin detectable level. Assessment of fascioliasis antigens, both in sera and stool, is currently the convenient, validated and the high accuracy method for diagnosis of human fascioliasis [18]. A study conducted by **Rokni *et al.*** [19] to compare the diagnostic potential of crude antigen with Excretory/Secretory (E/S) antigen using enzyme linked immunotransfer blot (EITB) assay. The sensitivity and specificity values for crude antigens were 91.0% and 96.2% respectively, compared with sensitivity and specificity E/S antigen were 95.2% and 98.0% respectively. Another study conducted by **Yamasaki *et al.*** [20] expressed high percentage of sensitivity and specificity by using an ELISA with Excretory/Secretory antigens of *Fasciola* with only a single case of cross-reactivity with *Schistosomiasis japonicum* infected serum patient. On other hand, Another study by **Maleewong *et al.*** [21] demonstrated 25.9 % cross-reactivity between patients with cholangiocarcinoma and *Fasciola* antigen**.**

The main purposes of this study are to develop and compare a novel diagnostic lateral flow assay and sandwich ELISA after conjugation with

gold nanoparticles AuNPs, which uses a larger surface area, to achieve higher sensitivity for detection of human fascioliasis antigen. The techniques were evaluated by comparing the diagnostic accuracy of homemade LFA with Nano-sandwich ELISA.

In this study, concerning assessment of *Fasciola* antigen in human blood samples using a sandwich ELISA conjugated with AuNPs, 2 out of 38 fascioliasis infected samples showed false negative results with 1.302±0.339 mean OD value noticeably higher than the cut off, it was noted the average OD of the two false negative samples was approximately equal to the cut off value compared to the average OD of the rest of the fascioliasis infected group that expressed a very high OD values. The sensitivity of the sandwich ELISA using tegumental *F.gigantica* antibody conjugated with AuNPs was 94.7 %. All 25negative healthy control samples were below the cut off value with mean OD value 0.226 ±0.014, while 4 out of 81 of other parasites were false positive giving 96.3% specificity, PPV was 90% and NPV was 98.1%. meanwhile, the average OD of the 2 out of 4 that gave false positive results was very high in those with schistosomiasis infection and the avargae OD of the other two false positive samples infected with *hydatid* and *filarial* was very low average OD almost equal to the cut off value.

It was noted that application of the covalent conjugation of AuNPs with fascioliasis tegumental Ag in homemade LFA remarkably enhanced and increased the sensitivity, specificity and the incidence of positivity of LFA towards higher antigen detection in patients with mild and moderate infection, 1 out of 38 fascioliasis infected samples showed false negative results giving sensitivity 97.4 % , 2 out of 81 were gave false positive within other parasite infection group giving specificity 98.2 %. All of the healthy control patients tested negative giving 94.8% PPV and 99.1 % NPV. The test line colors of both false positive samples were very pale in color, showing weak positive results compared to the fascioliasis infection group.

Unlike our previous study [22] In order to create a novel lateral flow strip for detection of human Fascioliasis, the strip was prepared and developed which contains sample pad, adjustment pad, conjugation release pad, nitrocellulose membrane, absorption pad, and semirigid polyethylene sheet, and a self-adhesive polyethylene protective film. Moreover, the conjugation of tegumental fascioliasis Abs with AuNPs was developed and evaluated by using EDC/Sulfo NHS covalent conjugation to increase the attraction force between Abs and AuNPs, and to prevent the random orientation attachment of the Abs, 17-β-estradiol was used to reinforce the orientation attachment of antibodies to use the smallest amount of antibodies [14]. To increase the flow rate we added the adjustment pad. At the end, we have noticed loose of adhesion during the manual assembly of lateral flow strip in the pads and membrane overlapping, that were not properly pressed, this loose adhesion resulting misdirection and an incomplete flow during the evolution of the test trials. As a result, we added a self-adhesive polyethylene tape to act as protective films which in turn will promote the correct directed flow of conjugate solutions.

In our study, showed improvement in Se, Sp, PPV and NPV indicates by 2.7%, 1.9%, 4.8% and 1% between homemade sandwich ELISA and LFA, respectively. Similar results were obtained by **Sadaow *et al.*** [23] they used an excretory-secretory E/S and recombinant cathepsin L (RCL) antigens based LFA for diagnosis of fascioliasis, they showed values of Se, Sp, NPV and PPV of E/S LFA were 100%, 98.9% 96.8%, 100%, and 99.2%, respectively, meanwhile with RCL were 86.7%, 93.7%, 81.3%, 95.7%, and 92.0%, respectively, E/S based LFA expressed more accurate and significant results compare to RCL.

Conclusions

In this work, we have successfully developed both sandwich ELISA and LFA techniques that use fascioliasis tegumental antibodies to detect fascioliasis antigens in human serum. These techniques can be used easily (they did not need a fully trained technicians) and quickly (the results appears after 7 minutes). In addition, after a year from fabrication and storage in a well-sealed plastic envelope with desiccants, the detection efficiency of LFA strips were still consistent. More importantly, the detection of Fascioliasis by gold nanoparticles based LFA technique has shown significant improvement in accuracy and test duration. it could be concluded that gold nanoparticles based LFA model was more efficient and time saving than sandwich ELISA model. Using covalent AuNPs-conjugated IgG antibody increases the diagnostic efficacy of ELISA and LFA

techniques gave higher results (sensitivity and specificity). Sandwich ELISA and LFA models could be used as Ideal diagnostic techniques in evolution of drug treatment, sero-epidemiological screening and the rapid detection of fasciolosis in different regions, which in turn could contribute to the surveillance and control of the disease.

Competing interests:

The authors declare that they have no competing interests

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