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EVALUATION OF GOLD NANOPARTICLES COATED SANDWICH ELISA AS INNOVATIVE DIAGNOSIS FOR HUMAN BANCROFTIAN FILARIASIS

By
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Abstract

This study compared the traditional sandwich ELISA and nano-sandwich ELISA methods for diagnosis of lymphatic filariasis. With the ability to interact with matter at the nanoscale, the development of nanotechnology architecture and materials could potentially extend molecular detection beyond the limits of conventional diagnostic modalities. This study included 142 subjects who were classified into; filarial diseased, other parasitic diseased and healthy control groups. Firstly, thick blood film was done for all subjects under study and their sera were obtained and preserved. A prepared sitarial antigen was injected into a rabbit to obtain polyclonal antibodies. That rabbit serum was purified, then pure IgG was obtained and a part of it was conjugated with gold nanoparticles. Conventional ELISA and nano-ELISA were done for patient's serum to detect circulating filarial antigen. Examination of blood film for microfilaria resulted in that; 5.1% of all patients were positive however 94.9% were negative. In comparison between ELISA and nano-ELISA readings, it was obvious that nano-ELISA is advantageous than traditional ELISA in all cases. The sensitivity, specificity, positive predictive value and negative predictive value of ELISA were 90.7%, 75%, 84.8% and 84% respectively. While by using nano-ELISA, they were 95.1%, 87.5%, 92.9% and 91.3% respectively. It was concluded that using nano-sandwich ELISA of the serum samples for detection of circulating filarial antigen in patients infected with *W. bancrofti* gave higher sensitivity and specificity than conventional ELISA, as proved by statistical study.

Key words: Filariasis, Blood, ELISA, Gold, Nanoparticles.

Introduction

Filariasis is a disease caused by filarial worms, which are microscopic roundworms that live in lymphatic tissues of humans. The most critical form for humans is elephantiasis as it is a serious complication of this disease (Kabatereine *et al,* 2010). Lymphatic filariasis (LF) is caused by three species; *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*. It was considered as one of the neglected tropical health problems of the 21st Century (Simonsen and Mwakitalu, 2013). In Egypt, filariasis has been reported long ago (Halawani, 1951) and its insect-vector (El-Bashier *et al,* 2006). Diagnosis of this disease can be based on the history of being in an endemic area and clinical examination of the affected limb. The most common presentation is unilateral or bilateral asymmetrical swelling of the limbs, which is of long duration and associated with thickening of the skin and history of repeated acute attacks of lymphangitis (Shenoy, 2008). Definitive diagnosis can be made by detection of microfilariae in blood film, circulating filarial antigen in patient's serum, filarial DNA using PCR in the blood or adult worms in lymphatics. In rare cases, microfilariae or adult worms can be found in tissue biopsies (Kumar *et al,* 2011).

Nanoscience is the study and manipulation of materials at the nanoscale, where properties differ significantly from those at a larger scale. In nanotechnology, a particle is defined as a small object that behaves as a whole unit and effective nanoparticles are sized between 1 & 100 nanometers (Buzea *et al,* 2007). Nanomedicine is the nanotechnology used in diagnosis of diseases, drug design, drug delivery, treatment, monitoring

and control of biological systems (Shelton *et al,* 2007). In disease diagnosis, it increases sensitivity, specificity and early detection as nanoparticles have a large surface area that enables attachment of large number of target specific molecules of interest for accurate detection (Padmavathy *et al,* 2010). Many forms of nanomaterials are found; liposomes, polymeric nanoparticles, dendrimers, solid lipid nanoparticles, carbon nanotubes and metallic nanoparticles (gold and silver) (Buzea *et al,* 2007). Gold nanoparticles (GNPs) were used in immunochemical studies for identification of the protein molecules (Tomar and Garg, 2013), as they exhibited unique physicochemical properties allowing surface modification and use in different biomedical applications (Shukla *et al,* 2005).

This study aimed to compare between conventional sandwich ELISA and Nano sandwich ELISA using gold nanoparticles in diagnosis of lymphatic filariasis.

Subjects and Methods

This cross-sectional study was carried out from January 2016 to October 2016 on patients from endemic areas of filariasis in Menoufia Governorate. Written informed consents were obtained from all patients. The work was performed in Theodor Bilharz Research Institute (TBRI), Giza, Egypt.

Study population: The study included 142 subjects who were classified according to clinical disease into 3 groups; GI (n=78, suspected filarial patients; based on their history, clinical picture and data obtained from Ministry of Health "filarial center"), GII (n=54, other parasitic patients infected with other parasites e.g. *S. mansoni, F. gigantica* and hookworms) and GIII (n=10, healthy control persons). They represented both sexes; 62 males and 80 females of different age groups. They were selected from endemic areas for filariasis which were; Delehmo village (Ashmoun Center), Sirs El-Liayyan and villages around Qweisna and Berkat Al-Saba Centers (Menoufia Governorate).

All were subjected to the following after their consent; history taking, clinical and laboratory investigations, which included: examination of blood samples for microfilaria using thick blood film and serological tests for detection of the circulating filarial antigen (CFA) using sandwich ELISA and nano-sandwich ELISA methods.

Parasitological examination (Thick blood film): Fresh blood samples by finger-prick were collected at daytime from all subjects under study an hour after taking a single dose of 100mg diethylcarbimazine citrate (DEC) (Hetrazan, Wyeth Limited co.) for adults and 50mg for children (one tablet) as a provocative sample test (Garcia and Bruckner, 2001). Blood samples were examined microscopically for detection of microfilariae in thick blood films stained with Ge-imsa stain (Eberhard and Lammie, 1991).

Serological tests (ELISA & Nano-ELISA): Venous blood samples about 4ml were taken from all individuals in EDTA tubes. Serum was separated by centrifugation at 2000g for 10 minutes, fractionated into small Eppendorf tubes and stored at -20°C until used (Gounoue-Kamkumo *et al,* 2015).

Animals: White New-Zealand male rabbit, weighing approximately 1.5 Kg and about 2 months age, purchased from rabbit research unit (RRU), Faculty of Agriculture, Cairo University, Egypt. It was examined before the start of the experiments and used in the production of the antibodies (Abdel-Megeed and Abdel-Rahman, 2004). It was housed in the animal house in Theodor Bilharz Research Institute (TBRI), Giza, Egypt.

Parasite: *Sitaria digitata* (*S. digitata*) worms were recovered from diseased animals (donkeys) in the Faculty of Veterinary Medicine, Cairo University, Egypt. They were transferred to the laboratory in Theodor Bilharz Research Institute (TBRI) in Hanks' Balanced Salt Solution (HBSS) to stimulate normal ion concentration under physiological conditions (Waizy *et al,* 2012).

Preparation of antigen: The adult female worms of *S. digitata* were washed extensive-

ly in phosphate buffer saline (PBS), and ultrasonicated for 1 minute. Then, they were collected and dialyzed overnight in 50mM acetate buffer (pH 4·5) and treated with 25mM sodium meta-periodate (Sigma) for 1 h. The reaction was stopped by treating with 50mM sodium borohydride for 30 min at room temperature and the sodium meta-periodate oxidized antigen was dialyzed extensively against PBS (Mohanty *et al*, 2001). Protein content was estimated by a Bio-Rad protein assay (Bradford 1976).

Purification of antigen by Diethyl [2-hydroxypropyl] aminoethyl (DEAE)-Sephadex A-50 (Smith *et al*, 1993): It is an effective method for separating proteins based on their charge. Sephadex A-50 powder was swelled in 0.5M Tris buffer. Then, it was poured in 30 x 2.5 cm column and covered with the binding buffer, then the sample was dialyzed versus the binding buffer and its protein content was calculated. The buffer above the beads was removed. The protein content < 10% of column bed capacity was applied to the column using Pasteur pipette. The outlet tubing was opened till the sample penetration to the beads then closed again for 10 minutes for antigen binding to the beads. The outlet tubing was then opened and the beads were washed by 5 bead volumes binding buffer. The protein was eluted by 20mM Tris 150mM NaCl under gravity by collecting 2ml of each fraction. Absorbance at 280nm of each fraction was measured using spectrophotometer (Perkin-Elmer Lambda 1A). Fractions exhibited high absorbance at first peak was pooled together. The protein content was estimated by a Bio-Rad protein assay (Bradford, 1976).

Purification of antigen by gel filtration chromatography on Sephacryl-S-200 high resolution (HR) column (Sun *et al*, 2004): It is a technique for separating proteins on the basis of molecular size. The fractions collected from sephadex A-50 ion exchange chromatography were further purified by DEAE sephacryl S-200 gel filtration column chromatography. The outlet of the column was opened for buffer penetration in the gel surface. The sample was layered on the beads surface and left to penetrate into the beads. The inlet tubing was filled with buffer and connected into the column. The column outlet was connected to an automatic fraction collector and one ml of each fraction was collected. The absorbance of each fraction was measured at 280 nm for protein content determination. Fractions exhibiting high absorbance at fist peak were collected together and under gone to estimation of protein content and then analyzed by SDS-PAGE under reduction conditions.

Characterization of the antigen by sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE) (Harlow and Lane, 1988; Myers, 1995): The eluted protein fractions resulted from the different purifications methods were analyzed by 12.5% SDS-PAGE. The gel and sample were prepared under reducing condition then the current was turned on and the run was started by 120 volt and continued until the samples reached the bottom of the gel (about 2 h). The gel was fixed and stained in 0.1% Coomassie Blue, then excess dye was removed from the gel by destaining solution to visualize the protein bands. Protein bands were appeared at 2 locations; 18 kilodalton (kDa) and 66.6 kDa which represent purified protein antigen (Fig.1). The parasite antigen was kept frozen at -20 °C until further use..

Production and purification of polyclonal Antibodies: Rabbit anti-filarial serum was obtained by immunizing the rabbit with *sitaria* purified protein antigen. The rabbit received priming dose intramuscular injection [1 mg antigen mixed 1:1 in Freund's complete adjuvant (Sigma)]. Three booster doses were given, each was 0.5 mg antigen emulsified in incomplete Freund's adjuvant. The first booster dose was two weeks after the priming dose. The following two booster doses were given at weekly intervals.

Test blood samples were withdrawn from rabbit before the injection of each immunizing dose. They were tested for the presence

of specific anti-filarial antibodies by indirect ELISA. The rabbit was bled for collection of serum one week after the last booster dose. The total protein content of crude rabbit serum containing anti filarial antibody was 16.3 mg/ml. It was purified by 50% ammonium sulfate precipitation method where the protein content was 10mg/ml (Odutayo and Odunuga, 2013). More purification of IgG pAb was performed by 7% caprylic acid method, the protein content dropped to 6.4 mg/ml (Mckinney and Parkinson, 1987). Rabbit serum which contained anti-filarial polyclonal antibodies was fractionated and kept at -20°C (Fagbemi *et al*, 1995).

Conjugation of the antifilarial antibodies with gold nanoparticles: Gold nanoparticles were purchased from Nano tech Egypt Company (6 October City) to be conjugated with the prepared polyclonal antibodies. This depends on three types of interactions; hydrophobic interactions, ionic interactions and dative binding. Hydrophobic interactions are due to attraction between hydrophobic parts of the antibody and the metal surface, resulting in the formation of a non-covalent bond. Ionic interactions are formed between positively charged groups in antibodies, (positively charged amino acids and the N-terminal) and the negatively charged surface of the nanoparticles. Dative binding is the formation of a covalent bond between the gold nanoparticles and free sulfhydryl groups of the antibody (Hermanson, 2008).
To thirty mL of the prepared AuNPs, 45µL of 1mM mercaptoundecanoic acid (MUA) in ethanol (Sigma Aldrich, Germany) was added. The solution was mixed and left overnight at 4^{o}C. Two nm of AuNPs-MUA solution was prepared from stock solution by diluting with deionized water. The pH was adjusted to 7.4 using 0.1M NaOH (Pronalab, Germany). Two mg of anti-*filaria* pAb was added to 5ml of AuNPs-MUA (2nm; pH = 7.4). In trying to obtain more robust AuNPs-pAb conjugates, covalent linkage between pAb and AuNPs-MUA was done by using N-hydroxysuccinimide/1-Ethyl-3-[3dimethylaminopropyl] carbodiimide (NHS/EDC) cross-linkers (NHS: Fluka, Germany). Five mL of a mixture of 5mM sodium phosphate buffer (pH =7), 1.2mM NHS & 2.8mM EDC were added to 5ml of AuNPs-pAb conjugates. Mixture was mixed and left to incubate overnight at 4^{o}C that allowed the electrostatic binding between pAb & AuNPs - MUA. The conjugate was prepared in gla-ss containers to prevent aggregation to plastic vials walls (Mukherjee *et al*, 2005).

Detection of circulating filarial antigen by traditional sandwich ELISA: The microtitration plates were coated with 100µl/well of anti-filarial IgG pAb, incubated overnight at room temperature and washed 3 times with 0.1M PBS/T, pH 7.4. Wells were blocked with 100µl/well of 2.5% fetal calf serum per phosphate buffer saline per tween (FCS/ PBS/T), incubated for 2hr at 37°C and washed 3 times with PBS/T. 100µl of each human serum sample was pipetted into the wells in duplicate, incubated for 2hr at 37°C and washed 3 times. Then, 100µl/well of peroxidase-conjugated pAb of 1/100 for IgG was then added, plates were incubated for 1 hr at room temperature. Plates were washed 5 times with washing buffer. Substrate solution 100µl was added to each well and plates were incubated in the dark at room temperature for 30 min, 50µl/well of 8 N H2SO4 was added to stop the enzyme substrate solution. Absorbance was measured at 492nm using ELISA reader (Nutman, 2013)

Detection of circulating filarial antigen by nano-sandwich ELISA: For diagnosis of filariasis by nano-sandwich ELISA, the same steps were adopted by using conjugated anti-filarial antibodies with gold nano-particles instead of the non-conjugated ones.

Statistical methods: Data were collected and entered to the computer using SPSS pro-program, version 20; Inc., Chicago (Snedecor and Cochran, 1981). Data were entered as numerical or categorical, as appropriate.

Two types of statistics were done; descriptive included mean, SD, range, frequency percent and analytical included Chi-square

test, risk ratio, student t-test, ROC curve, sensitivity, specificity, positive and negative predictive values. The P-value less than 0.05 was considered statistically significant.

Results

The results were given in tables (1, 2, 3, 4, 5 & 6) and figures (1, 2, 3, 4 & 5).

Table 1: Demographic data (n= 142)

			Filariasis (n=78)	Others (n=54)	Control (n=10)	Total	P value
Resi-dence	Rural	Frequency	68	27	8	103	.001
		%	88 %	50 %	80 %	72.5%	
	Ur-ban	Frequency	10	27	2	39	
		%	12 %	50 %	20 %	27.5%	

Distribution of filariasis in rural areas (88%) was statistically higher than in urban ones (12%). In parasitic group, distribution was equal in both areas (50%) while in healthy control, distribution was 80% in rural and 20% in urban areas.

Table 2: Classification of subjects according to clinical picture

			Filariasis (n=78)	Others (n=54)	Control (n=10)
Clinical picture	Normal legs	Frequency	17	54	10
		%	21.8%	100 %	100 %
	Elephantiasis	Frequency	17	0	0
		%	21.8%	0 %	0 %
	Lymphedema	Frequency	36	0	0
		%	46.2%	0%	0 %
	Redness + long streaks	Frequency	8	0	0
		%	10.2%	0 %	0 %

In filariasis group, the most prominent clinical manifestation was lymphedema (46.2%) followed by elephantiasis and normal legs with equal percent (21.8%), while redness was least clinical finding (10.2%). However, in other parasite and healthy control groups, 100% of the subjects were normal without clinical manifestations.

Table 3: Detection of microfilariae in blood film in different groups

Groups		Positive cases (microfilaraemic)		Negative cases (amicrofilaraemic)	
		No.	Frequency	No.	frequency
I	Filariasis (n=78)	4	5.1%	74	94.9%
II	Other parasites (n=54)	-	-	54	100%
III	Healthy control (n=10)	-	-	10	100%

Microfilariae were detected in thick blood films (5.1%) filarial cases whereas 94.9% were negative. But, in other parasites and in the healthy control no microfilariae were detected in thick blood films (100%).

Table 4: Mean± SD of OD readings for circulating filarial antigen in groups by sandwich ELISA of sera.

Groups		Positive cases		Negative cases	
		No.	(M ± SD)	No.	(M ± SD)
I	Filariasis (n=78)	64	(0.68 ±0.114)	14	(0.065±0.23)
II	Other parasites (n=54)	8	(0.57± 0.16)	46	(0.184 ±0.03)
III	Healthy control(n=10)	-	----	10	(0.161 ± 0.0539)

By ELISA, 64 were positive (0.68±.114), and 14 were negative (0.065±0.23). Patients with other parasites, 8 were positive (0.57±0.16) and 46 were negative (0.184± 0.03). Control was negative (0.161±0.0539).

The mean OD reading of negative controls ±2SD was estimated. Cut off value was 0.263 OD reading. Tested samples showed OD values > cut off value were positive while those showed OD values < cut off value were negative for all patients by using traditional sandwich ELISA. Cut off value was 0.263 to detect circulating filarial antigen by sandwich ELISA in subjects' sera. But, the cut off value of nano-ELISA was 0.448.

Table 5: Mean± SD of OD readings for circulating filarial antigen in groups using nano-sandwich ELISA of sera:

Groups		Positive cases		Negative cases	
		No.	(M±SD)	No.	(M ±SD)
I	Filaria (n=78)	72	(0.826±0.115)	6	(0.006 ±0.007)
II	Other parasites (n=54)	2	(0.546±0.019)	52	(0.159±0.07)
III	Healthy control (n=10)	----	----	10	(0.0256±0.102)

By using nano-sandwich ELISA**,** 72 were positive (0.826±0.115), and 6 were negative (0.006±0.007) in filariasis patients. Patients with other parasites, 2 were positive (0.546± 0.019) and 52 were negative (0.159±0.07). Control was negative (0.0256±0.102). By using nano-ELISA, other parasites were low compared to traditional ELISA that proved higher sensitivity and specificity than nano-ELISA.

Table 6: Sensitivity, specificity, PPV and NPV of sandwich ELISA and nano-sandwich ELISA methods of serum samples for circulating filarial antigen in patients

Techniques	Sensitivity%	Specificity%	PPV%	NPV%
Sandwich ELISA	90.7%	75%	84.8%	84%
Nano-Sandwich ELISA	95.1%	87.5%	92.9%	91.3%

In ELISA, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 90.7%, 75%, 84.8% & 84% respectively. In nano-sandwich ELISA showed sensitivity, specificity, PPV and NPV were 95.1%, 87.5 %, 92.9% & 91.3% respectively**.**

Table 7: Incidence of positivity for filarial detection using sandwich ELISA and nano sandwich ELISA in sera of groups.

Groups		Sandwich ELISA		Nano sandwich ELISA	
		Positive No.	% positivity	Positive No.	% positivity
I	Filaria (n=78)	64	82%	72	92.3%
II	Other parasites (n=54)	8	14.8%	2	3.7%
III	Control (n=10)	-	-	-	-

By sandwich ELISA, 64 were positive (82%) in filariasis patients while in patients with other parasites, 8 were positive (14.8%). By nano-ELISA, 72 were positive (92.3%) in filariasis patients and patients with other parasites 2 were positive (3.7%).

Discussion

Lymphatic filariasis is a mosquito-borne infection. The adult worms live in the afferent lymphatics (and/or lymph nodes) while the microfilariae circulate in the peripheral blood where they are available to infect mosquito vectors when they feed. lymphatic filarial disease is the second leading parasitic cause of disability. Although two thirds of the 120 million infected people have subclinical infections, around 40 million have episodic adeno-lymphangitis, lymphedema, hydrocele and elephantiasis (Nutman, 2013). New diagnostic tools that have been developed for detection of infection by *W. bancrofti* in human population have opened up new possibilities in terms of interrupting transmission and consequently the elimination of filariasis (Molyneux e*t al*, 2000). Until the early 1990s, the only available parasitological method for the evaluation of individuals or populations infected with *W. bancrofti* was the thick blood smear (Eberhard and Lammie, 1991). However, due to its low sensitivity, this method does not allow accurate determination of the true prevalence of infection (McCarthy, 2000). Moustafa *et al.* (1998) reported that antigen detection assays may facilitate earlier diagnosis than antibody tests, as production of detectable levels of specific immunoglobulin needs time. Antigen detection assay in serum is generally performed by sandwich ELISA (Abraham *et al*, 2003). Nano-diagnostics can be defined as the use of man-sized materials, devices or systems for diagnostic purposes. They were available for the clinical diagnosis with increased sensitivity at lower cost (Jain, 2003).

In the present study, 78 blood samples were collected from patients of lymphatic filariasis, 54 from other parasitic infected patients and 10 from healthy control per-

sons. Most of the studied filarial cases were from the rural areas (88%), while urban cases were 12% of all cases. There was a statistically significant difference among examined cases in relation to their residence ($P < 0.05$). However, another study showed that the majority of people live in remote rural communities were associated with lymphatic filariasis-vector-risk environment (Ivoke *et al,* 2015). In contrast, Sherchand *et al.* (2003) showed that most of cases occurred in urban areas as well as rural areas. Crowded living conditions, housing quality, inadequate waste disposal and sanitation facilities combined with seasonal migration between endemic rural areas and non-endemic urban areas shown to contribute to the growing "urbanization" of the disease (Schweinfurth, 1983; Mak, 1986).

In the current study (among endemic patients); lymphedema was the commonest clinical manifestation (46.2 %), elephantiasis (21.8%), normal legs (21.8%), and redness was the least detected manifestation (10.2%). Also, lymphedema of the legs was the commonest clinical manifestations reported (Ivoke *et al,* 2015). This might be due to age of cases, as lymphatic obstruction increases with time, so lymphedema develops in older patients (most of patients were old).

In contrast to Weil *et al*. (1999), where there was very little clinical filariasis, the study detected only one case of lymphedema among the sample population.

Thick blood film was done to all persons after an hour of taking 100 mg DEC to detect microfilaria. 5.1% of filarial diseased persons were positive for microfilaria, while 94.9% were amicrofilaraemic. The explanation of this result is that most of the patients were chronic obstructive cases (lymphedema and elephantiasis), so lymphatics were obstructed which prevented microfilariae appearance in the peripheral blood. In another study, a rate of 38% microfilaria positivity was detected amongst the night blood of ICT (immunochromatographic card test) and 57.4% microfilaria positivity was detected amongst the samples from the DEC provocation test when ICT was compared to night blood samples and day samples after DEC provocation (Faris *et al,* 1993).

The present study showed that sensitivity, specificity, positive predictive value and negative predictive value of sandwich ELISA were 90.7%, 75%, 84.8% and 84% respectively. Faris *et al.* (1993) detected circulating filarial antigen by ELISA with a sensitivity of 97.5%. ELISA gave 94% sensitive and 70% specific for detection of microfilariae antigen. Wattal *et al.* (2007) found that ELISA gave 100% sensitivity and 94.12% specificity. Hoti *et al.* (2002) by ELISA found specificity (100%) and sensitivity (96.8%) of samples collected at night.

In the present study, sensitivity, specificity, positive predictive value and negative predictive value of sandwich nano-ELISA were 95.1%, 87.5%, 92.9% and 91.3% respectively. Therefore, sandwich ELISA conjugated with gold nanoparticles was found to provide higher specificity, sensitivity, positive predictive value and negative predictive value compared to traditional ELISA technique. A similar study used the conjugation of gold nanoparticles (with different sizes; 20, 30 & 40 nanometer and different synthesis methods) to mouse anti-human IgG was evaluated for an immunochromatographic (ICG) strip test to detect brugian filariasis, it was found that conjugated IgG with gold nanoparticles (Au-NPs) synthesized using seeding-growth method had faster detection times, as compared with the Au-NPs synthesized using the citrate reduction method. Also, 30nm AuNPs demonstrated the best performance because it displayed the best sensitivity and the highest specificity when tested with serum samples from brugian filariasis patients and controls (Makhsin *et al,* 2012). Also, Moharm *et al*. (2014) reported that the novel nano-diagnostic assay for diagnosis of human giardiasis showed higher sensitivity, specificity, PPV and NPV (95.8%, 95%, 97.2% & 92.6%) compared to traditional sandwich ELISA (93%, 92.5%, 95.7%

& 88%, respectively). Sandwich ELISA with paramagnetic nanoparticles detected echinococcosis (Koura *et al*, 2015) with sensitivity, specificity, PPV and NPV of 95.2%, 95.5%, 97.6% &b 95.5% respectively. Those of sandwich ELISA were 90.48%, 91.3%, 95% & 91.3% respectively.

Conclusion

Using nano-sandwich ELISA technique for detection of circulating filarial antigen in *W. bancrofti* patients gave higher sensitivity and specificity than the traditional ELISA technique. The better diagnostic parameters of nano-gold particles were due to high binding capacity and rapid reaction kinetics of solutions. So, sandwich ELISA with paramagnetic nanoparticles gave higher specificity and sensitivity compared to a microplate-based ELISA technique.

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Explanation of figures

Fig. 1: Characterization of antigen by SDS-PAGE gel electrophoresis: Lane 1: Molecular weight of standard protein. Lane 2: Crude filarial antigen before purification. Lane 3: Partially purified. Lane 4: Target antigen eluted from DEAE Sephadex A-50 ion exchange chromatography. Lane 5: Target antigen eluted from gel filtration chromatography on Sephacryl-S-200 column.

Fig. 2: Thick blood film showing microfilaria of *W. bancrofti* with unstained sheath and bluish green nuclei. Giemsa stain (x100).

Fig. 3: Cut off point of OD readings for sandwich ELISA

Fig. 4: Cut off point of OD values for nano-Sandwich ELISA.
Fig. 5: Sensitivity, specificity, PPV & NPV of sandwich ELISA and nano-sandwich ELISA in sera for circulating filarial antigen in patients