COMPARATIVE STUDY OF DOT-IMMUNOGOLD SILVER STAINING AND DOT-ELISA FOR THE DETECTION OF SERUM ANTIBODIES AGAINST *WUCHERERIA BANCROFTI*

Liu Yi-sheng[1] Du Wen-ping[1] Chen Ming[2] and Wu Zhong-xing[3]

[1]Department of Parasitology, Xuzhou Medical College, Xuzhou, Jiangsu 221002, China; [2]Department of Infectious Diseases, Xuzhou Medical College; [3]Institute of Parasitic Diseases of Jiangsu Province, Wuxi, Jiangsu 214064, China

Abstract. Dot-immunogold silver staining (Dot-IGSS) and Dot-ELISA, using the soluble antigen of *Brugia malayi*, were employed to detect anti-*Wuchereria bancrofti* antibodies in 50 cases of *Wuchereria bancrofti* microfilaremia. The positive rates were 100% and 90% in Dot-IGSS and Dot-ELISA respectively. The average titer in the 45 positive cases was 1:184 (1:10-1:2560) for Dot-IGSS and 1:150 (1:10-1:2560) for Dot-ELISA, with 30 cases showing the same titer in both tests, 13 cases showing higher titer in Dot-IGSS than in Dot-ELISA and 2 cases in the former showing lower titers than in the latter. There was a linear relationship between the titers of antibodies detected by Dot-IGSS and by Dot-ELISA ($r = 0.8443$). Dot-IGSS, similar to Dot-ELISA, is easy to carry out and the result is easy to read. It is seen that Dot-IGSS is highly sensitive and specific and is practicable for immunodiagnosis and surveillance of filariasis.

INTRODUCTION

The detection of specific antibody is important in the diagnosis and epidemiological survey of filariasis, especially in regions where the disease has been basically eliminated. Immunological methods, such as indirect fluorescent antibody test (IFAT;Sethumadhavan *et al,* 1988), indirect hemagglutination test (IHAT; Kaliraj *et al,* 1981) and enzyme-linked immunosorbent assay (ELISA; Chantea *et al,* 1991) have been used for diagnosis. Recently an immunoenzyme staining test (IEST; Chen *et al,* 1992) and Dot-ELISA (Yuan *et al,* 1992) were also used to detect serum antibody against filaria. Dot-immunogold silver staining (Dot-IGSS) has been reported as a diagnosis method for some parasitic diseases, including clonorchiasis (Wu *et al,* 1989), schistosomiasis (Wu *et al,* 1991) and cysticercosis (Du *et al,* 1993). In order to further study the value of Dot-IGSS in serodiagnosis and surveillance of filariasis, we compared Dot-IGSS with Dot-ELISA in the detection of serum anti-filarial antibodies of patients infected with *W. bancrofti.*

Correspondence: Liu Yi-Sheng, Department of Parasitology, Xuzhou Medical College, 84 West Huai Hai Road, Xuzhou, Jiangsu 221002, People's Republic of China.

MATERIALS AND METHODS

Antigen : The soluble antigen of *B. malayi* adult was provided by the Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine. Its nitrogen concentration was 2.1mg/ml and its working dilution was 1:10.

Sera : Six sets of sera were examined: (1) Sera from 50 cases of *W. bancrofti* microfilaremia, who lived in Guzhen County, Anhui Province, with the number of microfilariae 1–186 per 60 μl peripheral blood (provided by the Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine); (2) Sera from 40 schistosomiasis japonica patients with stools positive for *Schistosoma japonicum* ova by hatching method (provided by the Institute of Parasitic Diseases of Jiangxi Province); (3) sera from 40 patients with cysticercosis diagnosed by CT, x-rays or clinically, and confirmed by Dot-ELISA; (4) Sera from 40 clonorchiasis patients with the eggs found in their feces by Stoll's method; (5) Sera from 35 healthy blood donors; and (6) Sera from staff members of our medical college. The sera of patients with microfilaremia were diluted 1:40–1:2560 in the test, while the other sera were tested at 1:40 dilution.

Sheep anti-human IgG labeled with chloroauric acid (GSAHIgG): The sheep anti-human IgG was labeled according to Slot's method (1985) in our laboratory with colloidal gold 5 nm in diameter. The GSAHIgG was used 1:40 dilution.

Solutions used in Dot-IGSS: The blocking solution was pH 8.2 0.02 mol/l Tris-HCl buffer saline (TBS) containing 1% bovine serum albumin (BSA) and 10% sheep serum (SS). The diluting solution was pH 8.2 0.02 mol/l TBS containing 10% calf serum, which was used to dilute serum samples and GSA HIgG. The developer was prepared with 3 solutions: (i) citrate buffer (pH 3.5), containing citric acid 25.5 g and sodium citrate 23.5 g in double distilled water 100 ml; (2) hydroquinone 1.7 g in double distilled water 88 ml; (3) silver nitrate 0.044 g in double distilled water 2 ml. Ten ml of solution A and 88 ml of solution B were mixed carefully and 2 ml of solution C was added immediately before use (Danscher, 1981).

Dot-IGSS procedurer: Mixed cellulose ester micropore filter membrane (MCE, pore size 0.22 μm) was divided into small squares (0.4 × 0.4 cm) by drawing with a pencil. Two μl of *B. malayi* antigen was spotted on to the center of each small square and the paper was dried at 37°C for 15 minutes. The MCE paper was then cut into squares and blocked for 10 minutes at room temperature. The blocked MCE squares each were placed in the wells on a 40-well flat-bottom polystyrene plate containing the diluted sera to be tested. The plate was incubated at 37°C for 2 hours and then washed 3 times for 5 minutes each with pH 8.2 0.02 mol/l TBS. Blocking was repeated once at room temperature for 15 minutes. Fifty μl of GSAHIgG was added to each well and the plate was incubated for 1 hour at 37°C, then washed 5 times, twice with pH 8.2 TBS, twice with deionized water and once with double distilled water sequentially. The developer was added and the plate put aside at room temperature to react. Color developed in 5 to 10 minutes. The plate was then washed twice with deionized water and once with double distilled water, then left to dry. The positive reaction was decided by the appearance of brown-yellow or brown-grey dots at the center of MCE squares. The intensity of color was arbitrarily judged with the naked eye as 1 +, 2 +, 3 + or 4 +.

Dot-ELISA: The techique was developed by modifying Hawkes' method (1982). The MCE was used as a vechicle to replace nitrocellulose filter and pH 7.4% 0.05 mol/l TBS containing 10% calf serum was used as blocking and diluting solution. The substrate was 4-chioro-l-naphthol. The peroxidase-conjugated anti-human IgG obtained from Sino-American Biotechnology Company Shanghai Branch was diluted 1:40 before use.

RESULTS

The results of Dot-IGSS and Dot-ELISA for anti-*W. bancrofti* antibodies in 225 human sera are presented in Table 1. All of the 50 microfilaremic subjects gave

Table 1

Results of anti-*Wuchereria bancrofti* antibody detected by Dot-IGSS and Dot-ELISA.

Sources of sera	No. cases detected	Dot-IGSS		Dot-ELISA	
		No. Positive	Positive rate (%)	No. positive	Positive rate (%)
Microfilaremia	50	50	100.0	45	90.0
Clonorchiasis	40	0	0	3	7.5
Schistosomiasis	40	0	0	2	5.0
Cysticercosis	40	6	15.0	7	17.5
Healthy donors	35	0	0	1	2.9
Staff nembers of the college	20	4	20.0	4	20.0

a positive reaction in Dot-IGSS, while only 45 of them were positive in Dot-ELISA. In the control groups, the positive rates in Dot-IGSS lower than in Dot-ELISA, except in the staff member group.

The distribution of titers of the microfilaremic sera detected by Dot-IGSS and by Dot-ELISA is shown in Fig 1. All of the microfilaremic sera were positive to Dot-IGSS at 1:10 dilution and the mean - titer was 1:184 (range 1:10-1:2560), while to Dot-ELISA only 45 showed posititive reaction, with a mean titer of 1:150 (range 1:10-1:2560).

The difference between the titers determined by the 2 methods was not significant. Among the 45 serum specimens which were positive in both assays, 30 had the same titer in both assays, while the other 13 had titers higher in Dot-IGSS than in Dot-ELISA by 4, 16 and 64 fold in 11, 1 and 1 case respectively, and 2 had titers lower in the former than in the latter by 4 fold.

The relationship between the titers of sera from microfilaremic subjects detected by two methods is shown in Fig 2. The correlation betwen the two assays was highly significant ($r = 0.8445$, $p<0.001$). The rectilineal regression equation was $Y = lg^{-1}$ (0.9712 lgx – 0.1333), where Y is the reciprocal titer of the sera detected by dot-IGSS; X, the reciprocal titer of the sera detected by Dot-ELISA. There was no correlation between the serum titers and the blood microfilariae counts.

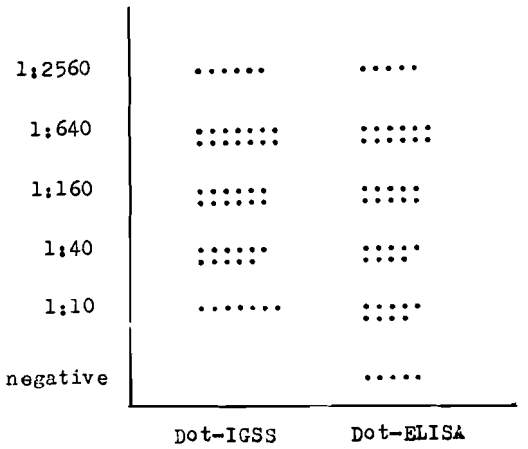

Fig 1–Distribution of titers of the sera from 50 cases of *Wuchereria bancrofti* microfilaremia assessed by Dot-IGSS and Dot-ELISA.

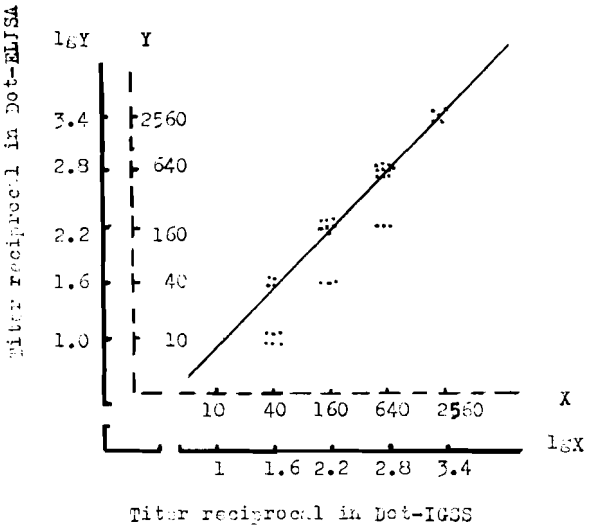

Fig 2–Relationship between the titers of sera in 45 cases of *Wuchereria bancrofti* microfilaremia assessed by Dot-IGSS and Dot-ELISA.

DISCUSSION

Since Wu *et al* (1989) used Dot-IGSS to detect antibodies against *Clonorchis sinensis*, we have usd it to study the serum specific antibodies in schistosomiasis patients and cysticercosis patients separately and have found that Dot-IGSS has higher specificity and sensitivity than Dot-ELISA (Wu *et al*, 1991; Du *et al*, 1993). In the present study, the positive concordance rate between Dot-IGSS and examination of peripheral blood microfilariae was 100%, as against 90% in the case of Dot-ELISA. The positive rate of Dot -IGSS obtained was higher than that of Dot-ELISA, as well as higher than that of ELISA reported by Sumati *et al*, (1990) (80%-95%) and El-Ganayni (1992, 92.8%-95.3%). The antibody titer determined by the 2 immunoassays showed a highly significant correlation, indicating that Dot-IGSS is very reliable for detection of anti-filaria antibodies. It was noticed that some normal staff members showed positive results. It is possible that these people had been infected with filaria or had a latent infection, for Xuzhou has been an epidemic region of bancroftian filariasis, although the disease has been basically eliminated. The clonorchiasis patients, schistosomiasis patients and other healthy donors were all negative to Dot-IGSS, but positive to Dot-ELISA in 3, 2 and 1 case

respectively, showing that specificity of Dot-IGSS is higher than that of Dot-LISA.

Among the 50 cases of *W. bancrofti* microfilare-mia, the blood microfilaria count was less than 5 microfilariae/60 μl of blood in 24 cases, with only one microfilaria found in 60 μl of blood in 10 of them, so false negatives can hardly be avoided if blood examination is used as a unitary surveillance means in regions where the infection is mild or the disease has been under control. However this shortcoming is now overcome by immunoassays, especially the Dot-IGSS, which is specific and sensitive.

Dot-IGSS is similar to Dot-ELISA in procedure, yet has other advantages: (1) the labeling of IgG with colloidal gold is simple; (2) the solutions used in Dot-IGSS are stable; (3) the substrate is harmless to the operator; (4) the results can be judged with the naked eye. Besides, only a tiny amount of blood is required for a Dot-IGSS and a 20 μl blood sample can easily be taken from the ear lobe or finger tip as we did in an epidemiological survey of clonorchiasis in rural areas (Liu *et al*, 1993).

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