

Original Article

Study on application of filarial specific IgG4 kit in disease surveillance of lymphatic filariasis

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Abstract: Objective: To investigate the filarial specific IgG4 kits in disease surveillance of basic filariasis eliminated and filariasis eliminated area. Methods: filarial specific IgG4 kit was applied in filariasis endemic areas in China. While in some cases, blood were taken at night for microfilaria examination under microscope. Results: In high and middle filariasis endemic areas, results of filarial specific IgG4 in residents were consistent with the results of microfilaria examination results ($\chi^2 = 2.42$, $\chi^2 = 1.57$, $P > 0.05$), indicating the detection of filarial-specific IgG4 can reflect local filariasis prevalence levels and the status quo. We detected filarial specific IgG4 in people who birthed before and after substantially eliminated filariasis periods, and the results showed significant difference ($\chi^2 = 17.15$, $P < 0.05$), indicating that in basic filariasis eliminated areas people with positive rate of filarial-specific IgG4 were existed and it showed an increasing trend with aging. We detected filarial specific IgG4 levels in filariasis substantially eliminated areas at different times, and the results showed that through years of prevention and monitoring after filariasis substantial elimination, in former filarial endemic areas the filariasis specific IgG4 level decreased in different populations, while the preventive and control effects were solid. We detected duration and propagation effects of specific filarial IgG4 in heartworm microfilaria hyperlipidemia patients, and the results showed that there were medium-density and high-density of microfilaria hyperlipidemia patients in basic filariasis eliminated areas with low efficiency transmission effect. We detected filarial specific IgG4 levels of floating population and entry-exit personnel in filariasis substantially eliminated areas, and the results showed that the positive came from former endemic areas and infected areas. Therefore we should strengthen monitoring floating population who came from former endemic areas and entry-exit personnel who came from the affected areas. Conclusion: Filarial specific IgG4 kits in disease surveillance of basic filariasis eliminated and filariasis eliminated area have good value.

Keywords: Lymphatic filariasis, specific IgG4, migrants, disease surveillance

Introduction

China was once among the countries with the most severe prevalence of lymphatic filariasis, involving 330 million people in 864 counties (cities) of 16 provinces, autonomous regions and municipalities directly under the central government. After the founding of New China, lymphatic filariasis was listed as one of the diseases causing greatest harm to people's health and deserving close attention. Under joint efforts, large-scale epidemiological surveys along with prevention and control measures have been launched in various parts of China. Shandong Province was the first to meet the standard for the eradication of filariasis in

1983. By 1994, 864 endemic counties (cities) had reached the standard. After that, the focus of the prevention and control work was shifted to disease monitoring and total eradication. With extremely low detection rate and density of microfilariae, it is not reasonable to continue the use of microscopy for epidemiological surveys and evaluation of prevention and control effect. More importantly, the obscure sources of infection cannot be timely identified by the old method. In addressing this issue, Expert Advisory Committee on Parasitosis of the Ministry of Health recommends nationwide application of filaria-specific IgG4 ELISA kit for total eradication of filariasis. Because of its high sensitivity, specificity and application

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value, the Ministry of Health included the filaria-specific IgG4 ELISA kit in Diagnostic Criteria for Filariasis (WS260-2006) in 2006. The following is a report of the application study of the kit in disease monitoring.

Materials and methods

Materials

Samples for detection: Serum samples Venous blood or peripheral blood was collected and centrifuged, and supernatant was collected. The blood samples were transported at low temperature and stored at 4°C. The blood samples were still valid for detection 2 years after collection if stored at -20°C. Repeated freezing and thawing was prevented to reduce the impact on antibody titer.

Filter-paper blood samples Blood was collected from the earlobes or fingertips using quantitative filter paper (Xinhua). The diameter of blood drop was 1.1-1.2 cm, and the blood volume was about 20 µl (or 10 µl serum). After numbering and air dry, the samples were preserved at low temperature in plastic bags containing a desiccant. The samples were still valid for detection 6 months after collection if stored at 4°C or 2 years after collection if stored at -20°C.

ELISA kit: Filaria-specific IgG4 ELISA kit joint developed by Shandong Institute of Parasitic Disease Prevention and Control and Shenzhen Kangbaide Biotech Co., Ltd. The detections were all performed within the period of validity of the kit.

Methods

Filaria-specific IgG4 detection: Dried blood samples on filter paper (1.2 cm) were added with 200 µl of diluent and frozen at 4°C in a fridge overnight. The volume of 100 µl was taken and added into the reaction well. For serum sample detection, 2 drops of sample diluent were added into each well, and 10 µl of serum was added into each sample well and properly mixed. Negative, positive and blank controls were set up. After treatment at 37°C for 90 min, the wells were washed and 2 drops of alkaline phosphatase conjugation anti-human IgG4 McAB were added into each well for cell culture at 37°C for 90 min. Then the wells were washed and 2 drops of developer were added into each well. The reaction was

terminated by adding 1 drop of stopping solution after color development at 37°C for 30 min.

Result interpretation: OD value of each well was detected using microplate reader at 405 nm. The OD value larger than 2.1 times that of the negative control was considered as positive. When the OD value was below 0.07, it was taken as 0.07.

Etiological examination: Routine thick blood film method (60 µl/film) was used for blood sampling during the night. Microfilariae were observed by microscopy after Giemsa stain.

Immunochromatographic card test (ICT): Wuchereria bancrofti ICT card was provided by WHO and manufactured by Binax Corporation (USA). Test was carried out according to the instructions. Two lines (T and C) observed from the window indicated positive test; any pink line in T region indicated positive test; the test was positive even when the T line was lighter or darker than C line; appearance of C line alone indicated negative test. To ensure sufficient reaction time for weakly positive samples, the results were not recorded until 10 min after closing the card. No observation of C line indicated invalid test result, and a second test was performed.

Results

ELISA kit detection and microscopy for different endemic regions

Zhaoguai administrative village in Loudian Town of Fengyang County, Anhui Province was once a highly filaria endemic region. Before the start of prevention and control work, the microfilarial positive rate was 35.40%. This village reached the standard for basic eradication of filariasis in 1993. A total of 91 filter-paper blood samples were detected for the village in 1997, and 24 samples were positive for filaria-specific IgG4. The specific IgG4-positive rate was 26.37%, and that in etiological monitoring was 18.73% (50/267). Statistical analysis ($\chi^2 = 2.42$, $P > 0.05$) showed that there was no significant differences in positive rate between ELISA kit detection and microscopy. See **Table 1**.

Daxu Village in Qinji Town of Bubang City, Anhui Province used to be a moderately endemic

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Table 1. Comparison of ELISA kit detection and microscopy of microfilariae for different endemic regions after years of efforts in disease prevention and control

Endemic region	ELISA kit detection			Microscopy		
	Cases detected	Positive	Positive rate (%)	Cases detected	Positive	Positive rate (%)
Highly endemic	91	24	26.37	267	50	18.73
Moderately endemic	260	2	0.77	1463	2	0.14
Total	351	26	7.41	1730	52	3.01

Table 2. ELISA kit detection after basic eradication of filariasis in different endemic regions

Population	Cases detected	Positive	Positive rate (%)
People in endemic regions	200	19	9.50
Children born after basic eradication of filariasis	230	2	0.87

Table 3. ELISA kit detection shortly after basic eradication of filariasis

Population	Cases detected	Positive	Positive rate (%)
People from previously highly endemic region	48	6	12.50
Microfilaremia cases turning negative	61	11	18.03
Children born after basic eradication of filariasis	34	6	17.65

region. The microfilarial positive rate was 11.91% before the start of prevention and control work. The village reached the standard for basic eradication of filariasis in 1984. Among local residents, 260 filter-paper blood samples were detected in 1997, and 2 samples were positive for filaria-specific IgG4. The specific IgG4-positive rate was 0.77%, and that by etiological examination was 0.14% (2/1463). According to statistical analysis ($\chi^2 = 1.57$, $P > 0.05$), there were no significant differences in positive rate between ELISA kit detection and microscopy. See **Table 1**.

ELISA kit detection for populations born at different time after basic eradication of filariasis

Twelve counties in Linyi City, Shandong Province were once the moderately endemic regions. Before the start of prevention and control work, the average microfilarial positive rate was 15.27%. By 1983, filariasis had been basically eradicated, and the average microfilarial positive rate decreased to 0.16%. In 1990, 200 samples were collected from local population born before the basic eradication of filariasis (1983), and 19 samples were positive for filaria-specific IgG4. The positive rate was 9.50%. For children born after basic eradication of fila-

riasis, 230 samples were collected, and 2 samples were positive, with positive rate of 0.87%. The results are shown in **Table 2**. No significant differences were detected by statistical analysis ($\chi^2 = 17.15$, $P < 0.05$).

ELISA kit detection in different endemic regions at different time after basic eradication of filariasis

ELISA kit detection shortly after basic eradication of

filariasis: Tengzhou City and Pingyi County of Shandong Province were the highly endemic regions in previous years. The average microfilarial positive rate was above 20% before the start of prevention and control work. The two regions reached the standard for basic eradication of filariasis in 1974 and 1977, with microfilarial positive rate declining to 0.17% and 0.16%, respectively. For Jiangtun Town of Tengzhou City (provincial-level filariasis monitoring spot), 61 serum samples were collected from microfilaremia cases turning negative (30 from Jiangtun Town and 31 from Pingyi County). A total of 48 serum samples were collected, including 26 from Pingyi County and 22 from Tengzhou City. In Jiangtun Town, 34 samples were collected from children born after basic eradication of filariasis (aged 6-12 years old). All samples were positive for filaria-specific IgG4. The positive rate was 18.03%, 12.50% and 17.65% in ELISA kit detection, respectively. The results are shown in **Table 3**.

ELISA kit detection some time after basic eradication of filariasis: Zaozhuang City and Tancheng County were once the highly endemic regions. The average microfilarial positive rate was above 20% before the start of prevention and control work. The two regions reached the

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Table 4. ELISA kit detection some time after basic eradication of filariasis in different endemic regions

Population	Cases detected	Positive	Positive rate (%)
People from previously highly endemic region	760	6	0.97
People from previously lowly endemic region	1095	3	0.27
Microfilaremia cases turning negative	69	1	1.45
Late-stage filariasis cases	77	0	0

Table 5. ELISA kit detection long after basic eradication of filariasis in different endemic regions

Population	Cases detected	Positive	Positive rate (%)
Children born after basic eradication of filariasis	312	0	0
Late-stage filariasis cases	48	0	0
Microfilaremia cases turning negative	76	0	0

standard for basic eradication of filariasis in 1980, with average positive rate declining to 0.14% and 0.48%, respectively. Jinxiang County of Jining City used to be the lowly endemic region, with positive rate below 5%. This region basically eradicated filariasis in 1979, with average positive rate reducing to 0.23%. In 1995, 760 filter-paper blood samples were collected from residents in Yicheng District, Zaozhuang City; 1095 samples were collected in Jinxiang County; 69 and 77 filter-paper samples were collected from microfilaremia cases turning negative and late-stage filariasis cases in Tancheng County and Yicheng District of Zaozhuang City, respectively. The ELISA kit detection results are shown in **Table 4**.

ELISA kit detection long after basic eradication of filariasis: Zaochuang City in Shandong Province was once the highly endemic region, which basically eradicated filariasis in 1980 and radically eradicated filariasis in 2004. In June 2003, 312 filter-paper blood samples were collected from children born after basic eradication of filariasis (aged 6-12 years old), 76 samples from microfilaremia cases turning negative and 48 samples from late-stage filariasis cases. All samples were negative for specific IgG4. See **Table 5**.

ELISA kit detection and serological test after basic eradication of filariasis in different endemic regions

Baojing, Guiyang and Longshan County in Hunan Province were all the moderately filaria-

sis endemic regions. The average microfilarial positive rate before the start of prevention and control work was 6.79%-11.77%. The two regions had basically eradicated filariasis in 1981 and 1985, respectively, with positive rate declining to 0-0.27%. In these 3 counties, a total of 343 filter-paper blood samples were collected from microfilaremia cases turning negative in 2002, including 6 specific IgG4-positive samples. The specific IgG4-positive rate was 1.75%, and etiological examination was negative for

all cases. For 4 primary schools in the 3 counties, 542 students were sampled and all were IgG4-negative. In 2004, 76 filter-paper blood samples were collected from microfilaremia cases turning negative in Guiyang County. All were negative for specific IgG4 and in etiological examination. There was no positive case among 143 children aged 6-12 years old. For non-endemic Guidong County, 200 dried blood samples on filter paper were collected and all were negative for specific IgG4. A total of 33 serum samples were collected from cases with late-stage filariasis, acute lymphangitis, schistosomiasis, paragonimiasis and clonorchiasis sinensis. All were negative for specific IgG4. See **Table 6**.

ELISA kit detection after eradication of filariasis

Dongping County of Shandong Province was previously the lowly endemic region. The average microfilarial positive rate was below 5% before the start of prevention and control work. Filariasis was basically eradicated in 1977, with microfilarial positive rate reducing to 0.17%. Jiaxiang County was once the moderately endemic region and reached the standard for basic eradication of filariasis in 1979, with microfilarial positive rate declining to 0.10%. In June 2004, 2744 filter-paper blood samples were collected from residents in Yicheng District and Tai'erzhuang District of Zaochuang City in total. In July of the same year, 2243 filter-paper blood samples were collected in

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Table 6. Comparison of ELISA kit detection and etiological test after basic eradication of filariasis for different endemic regions

Time	Population	Cases detected	ELISA kit		Etiological test	
			Positive	Positive rate (%)	Positive	Positive rate (%)
2002	Microfilaremia cases turning negative	343	6	1.75	0	0
	Children born after basic eradication of filariasis	542	0	0	0	0
2004	Microfilaremia cases turning negative	76	0	0	0	0
	Children born after basic eradication of filariasis	143	0	0	-	-
	Children from non-endemic regions	200	0	0	-	-
	Late-stage filariasis cases	3	0	0	0	0
	Acute lymphangitis	1	0	0	0	0
	Other parasitic diseases	29	0	0	-	-

Table 7. ELISA kit detection after eradication of filariasis for different endemic regions

Population	Cases detected	Positive	Positive rate (%)
People from previously lowly endemic region	2744	0	0
People from previously moderately endemic region	800	0	0
People from previously highly endemic region	2243	0	0
Total	5787	0	0

Dongping County, and all were negative for specific IgG4. In 2013, 800 samples were collected in Jiexiang County and all were negative for specific IgG4. See **Table 7**.

Tracking of cases with low-density and high-density microfilaremia

Located in the mountainous region in the west of Hunan Province, Jishou City was once the moderately filariasis endemic region. The city reached the standard for basic eradication of filariasis in 1985. Erketuo Stronghold in Jibi Village of Majing'ao Town was monitored. This stronghold, surrounded by mountains on all sides, was inhabited by Tujia ethnic minority people and had underdeveloped economy and poor sanitation and transportation facilities. The total household number of the stronghold was 27 with a population of 116 people. Different households lived separately from each other, and there were a total of 14 residential sites. The microfilarial positive rate was 19.0% during a survey in 1984. Diethylcarbamazine citrate treatment was given alone for 6 months without other prevention and control measures after 1985. The residents of the stronghold were tracked. In 1991 IFAT was performed for 88 residents, and 4 people had

positive anti-microfilarial antibody $\geq 1:20$, so the positive rate was 4.55%. Of 96 residents detected in 1996, 2 were positive for anti-microfilarial antibody ($\geq 1:20$), and the positive rate was 2.08%. However, these 2 cases were negative for micro-

filaremia by etiological examination. Among 77 residents detected in 2002, 7 were positive for specific IgG4. In 2004, 78 residents were detected, and 8 of them were positive for specific IgG4. Only 1 out of 63 residents detected in 2008 was positive for specific IgG4. Moreover, during ICT for a natural village near Erketuo Stronghold in 2008, all 491 residents detected were negative (**Table 8**).

ELISA kit detection for migrant populations after eradication of filariasis

ELISA kit detection for migrant population in Dongguan city: Dongguan City was once the lowly filariasis endemic region, with microfilarial positive rate reaching 1.70% before the start of prevention and control work. By 1986, filariasis was basically eradicated, with microfilarial positive rate reducing to 0.13%. The standard for eradication was met in 2001. However, Dongguan City is currently the focus of filariasis prevention because of high mobility of population. Migrant populations who came from filariasis endemic regions and had lived in Dongguan City for over one peak season were subject to serological test. Some migrant workers at 4 factories who came from endemic regions such as Anhui Province were sampled in 2004. Filter-

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Table 8. Serologic test of filariasis for Erketuo natural village, Jishou City in 1990-2008

Year	Detection method	Cases detected	Positive	Positive rate (%)
1990	IFAT	88	4	4.45
1996	IFAT	96	2	2.08
2002	IgG4	77	7	9.09
2004	IgG4	78	8	10.26
2008	IgG4	63	1	1.58
2008	ICT	491	0	0

Table 9. ELISA kit detection among migrant population in Dongguan City

Source region	Cases detected	Positive	Positive rate (%)
Anhui	659	1	0.15
Jiangxi	812	0	0.00
Hubei	509	0	0.00
Henan	92	0	0.00
Other	53	0	0.00
Total	2125	1	0.05

Table 10. ELISA kit detection among migrant population in Shanghai

Source region	Cases detected	Positive	Positive rate (%)
Anhui	1640	5	0.30
Jiangxi	218	0	0.00
Henan	128	0	0.00
Hubei	52	0	0.00
Other	94	0	0.00
Total	2132	5	0.23

paper blood samples were detected for specific IgG4. Among 2125 samples, only one was positive for specific IgG4. Blood test during the night also indicated no microfilariae (**Table 9**).

ELISA kit detection for migrant population in Shanghai: By 2002, 11 provinces (cities and municipalities directly under the central government) had passed the provincial-level appraisal for eradication of filariasis, including Guangxi, Guizhou, Shanghai, Sichuan, Chongqing, Hunan, Jiangsu, Hubei, Guangdong, Zhejiang and Fujian. Filariasis monitoring in recent years shows that individual cases are still positive in previously endemic regions, and these cases are usually left out. For 5 districts of Shanghai (Nanhui, Baoshan, Jiading, Fengxian and

Songjiang), filariasis monitoring program was launched among migrant population from previously filariasis endemic regions. Dried blood samples on filter paper were collected from 2134 migrant workers, and 5 samples were positive for specific IgG4. All 5 cases came from Anhui Province. The overall positive rate was 0.23% (**Table 10**). However, these cases were negative for microfilaremia by etiological examination.

ELISA kit detection for entry-exit personnel in previously endemic regions

Nearly all provinces in China had reached the standard for basic eradication of filariasis by 1994. Only 1 case was positive for microfilariae by etiological examination in 16 endemic provinces (districts, municipalities directly under the central government) in 2002. China had achieved the goal of eradicating filariasis by 2006. However, frequency international exchanges may have an adverse impact on filariasis control. In 2006, 398 entry-exit personnel who had lived in other filariasis endemic countries were detected for specific IgG4. Three cases were positive for filaria-specific IgG4, including 2 from India and 1 from Japan, and the positive rate was 0.75%. Entry-exit personnel were predominately of Asian origin, and those of African origin accounted for the second largest proportion (**Table 11**).

Discussion

Since one year after that Shandong province reached filariasis elimination standard for the first time in 1983, China's Filariasis prevention entered the monitoring phase until eliminated filariasis across the country [1].

In the early stages of monitoring, the main purpose of filariasis surveillance was to find and remove residual source of infection. The study was originated in 1996 from filariasis super higher endemic area of Zhaoguai administrative village (provincial longitudinal monitoring points). 91 cases of filter blood were collected, and 24 cases of filarial specific IgG4 were positive, with the positive rate of 26.37%. Compared with the result of microscope detection of microfilaria, the statistical processing ($\chi^2 = 2.42$, $P > 0.05$) showed no significant difference; at the same time, 260 cases of filter blood were collected from the residential in

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Table 11. Nationality distribution of entry-exit personnel in previously endemic regions

	Country	Cases		Country	Cases
Asia	Japan	125*	Africa	Egypt	2
	Indonesia	25		Mauritius	1
	India	72**		Libya	1
	Korea	88		Mozambique	1
	Burma	1		Tanzania	2
	Thailand	7		Angola	1
	Malaysia	17		Cameroon	1
	The Philippines	27		Sudan	1
	Pakistan	6		Sri Lanka	1
America	Brazil	3	Oceania	Nigeria	12
	Costa Rica	1		Australia	3
Total	398	Positive		3	

Note: *one positive case; **two positive cases.

middle endemic area of Daxu administrative Village (it met the basic filariasis elimination standard in 1984). Two cases of filarial specific IgG4 were positive with the positive rate of 0.77%. Compared with the result of microscope detection of microfilaria, the statistical processing ($\chi^2 = 1.57$, $P > 0.05$) showed no significant difference [2]; Studies have shown that the results of filarial-specific IgG4 detection was consistent with that of microscope microfilaria detection, and the detection of filarial-specific IgG4 can reflect local filariasis prevalence levels and the status quo.

When the filariasis were basically eliminated, in addition to that detection of residents' filariasis special IgG4 levels in epidemic area for evaluation of filariasis levels and status quo, the detection of filariasis special IgG4 levels in children who were born in epidemic area after filariasis were basically eliminated, will be more sensitive to reflect the local filariasis conditions. In 1990, local residents' filariasis special IgG4 positive rate was 9.50% in Linyi City, Shandong Province; the positive rate was 0.87% in children who were born after basic filariasis elimination, and the difference between the two showed statistical significance ($\chi^2 = 17.15$, $P < 0.05$), indicating that in basic filariasis eliminated areas the residential with positive rate of filarial-specific IgG4 were existed and it showed an increasing trend with aging [3].

With the continuous advance of filariasis control and surveillance, surveillance in basic elim-

inated filariasis areas in different periods of different populations can reflect the local control effect of filariasis. In this study, the filariasis special IgG4 positive rate of filter blood samples from highly endemic area residents, patients with negative microfilaremia, and children who were born after basic filariasis elimination were 12.50%, 18.03%, 17.65% at an early stage (1989) of basic filariasis eliminated areas [4]; The filariasis special IgG4 positive rate of filter blood samples from highly endemic area residents, patients with negative microfilaremia, and children who were born after basic filariasis elimination were 0.97% and 0.27%, 1.45% at middle

stage of basic filariasis eliminated areas and it was negative in patients with advanced filariasis [5]. The filariasis special IgG4 of filter blood samples from highly endemic area residents, patients with negative microfilaremia, children who were born after basic filariasis elimination, and patients with advanced filariasis were all negative at late stage of basic filariasis eliminated areas, indicating that through years of prevention and monitoring after filariasis substantial elimination, in former filarial endemic areas the filariasis specific IgG4 level decreased in different populations, while the preventive and control effects were solid. In filariasis eliminated areas, it is difficult to find the residents with positive filariasis special IgG4.

In addition, in 2004 filariasis specific IgG4 tests were performed for the floating population in Shanghai city and Dongguan city which were filariasis eliminated areas. In the two cities, a total of 4257 copies of filter samples were detected, and there were 6 cases of positive filariasis special IgG4, who were mainly come from endemic areas of filariasis in Anhui Province [6]; In 2006, 398 cases of the immigrated who live in filariasis endemic areas were test for filarial specific IgG4 in Zhejiang Province and 3 people showed filariasis special IgG4 positive. Two of them come from India and one was from Japan [7]. Test results showed that the disease surveillance in filariasis eliminated areas were still needed, first for the people who come from filariasis endemic areas and especially for floating population who come from

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highly endemic area and the entry-exit personnel from infected areas.

Filarial specific IgG4 detection not only has high sensitivity and specificity, but also shows no significant difference ($P > 0.05$) compared with ICT card detecting bancrofti antigen through study method comparisons carried out by studying team, indicating that the two methods can be used for filariasis diagnosis and epidemiological investigation, but the practice showed that filarial specific IgG4 detection is more suitable for group detection, epidemiological investigations and disease surveillance, while ICT cards are more suitable for detection of individual case. In particular, the test kits are available for dried blood testing, which save manpower, material and are easily accepted by the mass. The results of the tests are objective, accurate, and can be used for batch inspection, especially suitable for epidemiological investigations and disease surveillance control in advanced filariasis prevention. Especially for the surveillance in filariasis eliminated areas of filariasis, this method can be used for screening. For patients with positive filariasis specific IgG4, etiological blood tests should be performed to review. This will not only ensure the quality of monitoring, but also save a lot of manpower and material resources. Therefore, the detection of filarial specific IgG4 show great significance in eliminating filariasis and disease surveillance in filariasis basically eliminated areas.

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Disclosure of conflict of interest

None.

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