STUDY ON DETECTING ANTIBODIES TO *TOXOPLASMA GONDII* IN POOLED SERUM OF BLOOD DONORS BY DOT-IGSS

Liu Yisheng[1], Zheng Kuiyang[2], Chen Ming[2], Fu Linlin[1], Du Wenping[1], Shi Zhixu[3]

[1]Department of Parasitology, Xuzhou Medical College, Xuzhou, Jiangsu 221002, PR China; [2]Department on Infectious Diseases, Xuzhou Medical College, Xuzhou, Jiangsu 221002, PR China; [3]Xuzhou Red Cross Blood Service, Xuzhou, Jiangsu 221002, PR China

Abstract. To prevent post-transfusion toxoplasmosis, the sera of blood donors, per six of which were mixed as a pool, were examined for anti-*Toxoplasma gondii* antibiodies by Dot-immunogold silver staining (Dot-IGSS) with the single serum specimens examined simultaneously. The results showed that the sensitivity and specificity of serum pool method were 92.31% and 99.96% respectively. The consistent rate between the two methods was 99.73% and kappa value was 0.947 ($p<0.01$). Considering the mean infection rate of *Toxoplasma gondii* being 4.86% in China, if the serum pool method be adoped, with pool size k=5, a 57% reduction in the number of tests, as well as the cost of the screen, can be expected. Beside the social benefit, consequent upon the interruption of the *Toxoplasma gondii* infection spread through blood transfusion also can be expected.

Toxoplasma gondii (T. gondii) is an opportunistic protozoon, which parasites in the nucleated cells of host. The protozoon may cause serious toxoplasmosis with clinical manifestations when the host in immunocompromised. The infection can be transmitted through blood and some cases of post-trasfusion toxoplasmosis have been reported at home and abroad (Holliman *et al*, 1991, Wang *et al*, 1994). The infective rate of *T. gondii* was estimated at 4.86% in China (Cui, 1991). A similar rate should be expected in the population of blood bonors. Hence the Ministry of Health of PR China has listed toxoplasmosis as one of the important blood transmitted diseases.

For lack of practicable testing methods, screening for *T. gondii* infection in blood donors has not been practised in most area of China. The urgent task is therefore to establish a simple, economical and highly efficient method for this purpose, which can be routinely used by most blood servicse, so as to prevent post-transfusion toxoplasmosis.

It is known that the serum pool strategy is feasible to screen the population of blood donors for antibodies to human immunodeficiency virus (anti-HIV) (Ji *et al*, 1992) and antibodies to human hepatitis C virus (anti-HCV) (Liu *et al*, 1994, 1997a), and that the Dot-immunogold silver staining (Dot-IGSS) is effective for detecting antiobdies to *T. gondii* and other pathogenic parasites (Wu *et al*, 1991; Liu *et al*, 1994b, 1995, 1996a, 1997b). We tried to integrate the serum pool method with Dot-IGSS to screen *T. gondii* infected subjects in the population of blood donors and obtained satisfactory results.

MATERIALS AND METHODS

Antigen to *T. gondii*

The nitrogen concentration of soluble antigen of *T. gondii* tachyzoite, which was porvided by the Institute of Animal Husbandary and Veterinary of Jiangsu Academy of Agro-

Correspondence: Prof Liu Yisheng, Department of Parasitology, Xuzhou Medical College, 84 West Huai hai Road, Xuzhou, Jiangsu 221002, People's Republic of China.
Tel. 86-0516-5748449; Fax: 86-0516-5748429
E-mail: jsc@xzmc.edu.cn

Sciences, was 4.0 mg/ml and it was diluted 1:40 before use.

Serum samples

The sera from 2,589 local voluntary donors were provided by the Xuzhou Red Cross Blood Service. There were 1,616 (62.4%) men and 973 (37.6%) women, aged 18 to 53 years, total 2,589.

Dot-IGSS

Sheep anti-human IgG labeled with chloroauric acid (GSAHIgG): The sheep anti-human IgG was provided by SINO-American Biotechnology Co. The colloidal gold was 5 nm in diameter. The sheep anti-human IgG was labeled in our laboratory according to Slot and Genuze's (1985) method. After being labeled and concentrated, the IgG was preserved at -20ºC. Its best dilution was 1:40.

Dot-IGSS procedure: The test was carried out according to Wu *et al* (1989). A drop of the antigen (about 1 μl) was added with a pin onto mixed cellulose ester micropore filter membrane (pore size 0.22 μm), which had been divided into small squares (0.4 cm x 0.4 cm) by drawing with a pencil. After being dried, the paper was cut into squares. pH 8.2 TBS containing 10% sheep serum and 1% bovine serum albumin (BSA) was used as the blocking solution. After being blocked (at room temperature for 15 minutes), the squares, one for each, were placed in the wells on a 40-well flat-bottom polystyrene plate containing the sample sera properly diluted. The plate was inclubated at 37ºC 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. Fifty μl of GSAHIgG was added to each well and the plate was incubated for 1 hour at 37ºC and 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 would be developed in 5 to 10 minutes. The plate was then washed twice with dieonized and once with double distilled water before being left to dry. The positive reaction was decided by the appearance of brown-yellow or brown-grey dots at the center of the filter membrane. The intensity of color was arbitrarily judged with the naked eye as 1+, 2+, 3+ or 4+.

Single serum method and serum pool method was carried out simultaneously along with the positive control, negative control and blank control. The results of assays were judged by 2 experimenters independently.

Dilution of serum samples: In single serum test, 2 μl serum sample was mixed with 100 μl pH 8.2 0.02 mol/l PBS containing 10% calf serum in each well. The dilution of the serum to be detected was 2:102. The total volume of serum was 12 μl (containing 10 μl calf serum). In the serum pool tests, 6 serum samples as a group, 2 μl of each, were pooled in a well. The pool was then mixed with 90 μl of the PBS (containing no calf serum), to make the final volume 102 μl and also a serum dilution of 2:102. The total volume of serum was also 12 μl, which was human serum.

RESULTS

The sensitivity and specificity of the serum pool method for detecting anti-*T. gondii* antibodies

From Table 1 it was calculated that, using the single serum method as control, the sensitivity and specificity of the serum pool method were 92.31% and 99.96% respectively. The consistent rate between the results of the 2 method was 99.73% and kappa value was 0.947 ($p<0.01$).

Comparision between titers of single serum and serum pool method samples

Table 2 showed that among the positive sera, 34 produced same titers in both assay, 40 produced titers higher in the single serum tests, but only 5 showed lower titer in the single serum tests than in the serum pool tests.

Size of serum pool and cost-benefit analysis (Liu *et al*, 1997b)

Let the seroprevalence of *T. gondii* be P, the size of the pool be k, the size of the

population to be screened be n, and the number of tests required for one pool be a_i (where i=1, 2, ..., n/k), the probability that k single sera are all negative, (*ie* only 1 test is needed for this pool) will be $(1-P)^K$, while the probability that the one pool is positive (*ie* k=1 tests are needed for the pool), will be $1-(1-P)^K$. The expected number of tests for 1 serum pool and that for the whole population will respectively be Ea_i and Ea:

$$Eai=1 \times (1-P)^K+(k+1) \times [1-(1-P)^K] \quad (1)$$

$$Ea=n/k \times Eai=n[1-(1-P)^K+1/k] \quad (2)$$

Let L be the percentage of reduction of number of tests expected in serum pool method, then:

$$L=(n-Ea)/n=[(1-P)^K-1/k] \times 100\% \quad (3)$$

In the present study, the seroprevalence of *T. gondii* in local blood donors was estimated by referring to that of the pregnant women in the same city of Xuzhou (Liu *et al*, 1996b), about 3.54%. According to Liu *et al* (1994b), in such a case, the pool size was set at 6, and the best L value of 63.89% would be achieved. In other words, only 37 tests would have been performed for every 100 serum samples in the survey.

If the cost of one test is W, the cost saved by practising the pool method will be S:

$$S = W \times n \times L \quad (4)$$

Considering that 1 Dot-IGSS test costs RMB ¥10 and there are some 30,000 doners to be screened in Xuzhou each year, the use of serum pool method will save RMB ¥198,000, a sum equivalent to over US$21,000 per year. Evidently, be the serum pool Dot-IGSS adopted for screening donors nationwide (where the mean seroprevalence p=4.86% and the pool size k=5), at least 57% of laboratory labor and corresponding expenses can be saved.

Table 1
Results of anti-*T. gondii* IgG detected by single serum and serum pool method.

Serum pool method	Single serum method		Total
	Positive	Negative	
Positive	72	1	73
Negative	6	2,510	2,516
Total	78	2,511	2,589

DISCUSSION

According to the statistical principle, when kappa value ≥0.75, the agreement in results between the 2 methods will be very satisfactory (Ni, 1990). In the present study, kappa value was 0.974, indicating that the 2 methods used were highly consitent ($p<0.01$) and it is of sound rationale theoretically to use serum pool Dot-IGSS to screen anti-*T. gondii* positive subjects from blood donors.

From equation (3) and (4), it can be noticed that the variables P and n, especially the former, are related to the number of tests required and the benefits produced economically and socially. The lower the seroprevalence p, the less the tests required and the higher the benefits. For instance, when p=10%, 40 tests can be

Table 2
The reaction intensity of positive specimens detected by single serum and mixed serum method.

Mixed serum method	Single serum method					Total
	-	+	++	+++	++++	
-	0	4	2	0	0	6
+	1	7	11	3	1	23
++	0	2	22	8	4	36
+++	0	0	1	4	7	12
++++	0	0	1	0	1	2
Total	1	13	37	15	13	79

reduced for 100 serum samples; when p=20%, the reduction becomes 17 at most; but when p=30%, the number of tests can not be reduced at all. Hence a proper estimation of p-value is very important before the serum pool protocol is adopted for screening purposes.

Analysis of the reaction titers revealed that about 50% of the serum pools produced titers lower than the single serum samples (Table 2). This phenomenon may be an explanation to the appearance of 6 false negative results in the serum pool tests. Virtually, the lowering of titers is related to the blocking effect of human sera, as demonstrated in our previous study in developing the Dot-IGSS method. We had noted that human serum had stronger blocking effect than calf serum, reducing nonspecific reaction, and could thus diminish the occurence of false positive results (while conversely, increase the chance of "false negatives" appearance). In the present study, the reacting system in single serum test contained 2 μl of sample serum and 10 μl of calf serum plus 90 μl of PBS, while in the serum pool test, contained 12 μl of human serum and 90 μl of PBS. Though the ratio of serum to the PBS was 12:90 in both methods, the difference in species of the sera should have some bearing on the intensity of the titers obtained.

However, our experiment demonstrated that the performance of serum pool Dot-IGSS was less time-consuming than the single serum method, and the results obtained by the 2 methods were in satisfactory agreement. Cost-benefit analysis showed the economic benefit is definite. If the serum pool Dot-IGSS method for screening anti-*T. gondii* positive donors nationwide, the economical benefit will be remarkable, beside the tremendous social significance consequent upon the cut-off of the route transmitting post-transfusion toxoplasmosis.

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