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Evaluation of a Commercial Kit for *Toxoplasma* Direct Agglutination Test

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Abstract

A *Toxoplasma* direct agglutination test using a commercial kit (Toxo-Screen DA/bioMérieux, France) was evaluated by comparing it with the dye test (DT) and indirect latex agglutination test (LA) (Toxo-MT/Eiken Chemical Co., Tokyo). The frequencies of respective titers of the direct agglutination test (AG) on 104 human sera revealed a bimodal distribution curve, thereby titers above 1:64 were regarded as positive. High agreements were qualitatively shown between AG and DT (95.2%) and AG and LA (99.0%), respectively. The AG titers obtained by testing sera from general outpatients were about thirty times as high as DT titers. Both AG and LA did not give positive results to 7 sera with various titers of HBe and HBs antigens but without DT antibody. From these results, the AG kit is considered to be useful for screening of toxoplasmosis.

Key words: toxoplasmosis, serodiagnosis, direct agglutination test

Introduction

The toxoplasma direct agglutination test (AG) was first described by Fulton and Turk (1959). This method using formalin-fixed tachyzoites is very simple but lacks sensitivity and specificity. Desmonts and Remington (1980) reported a modified AG method with increasing sensitivity and specificity. The improved method could minimize occurrence of non-specific reaction by a certain device in preparing antigen and the use of a buffer containing 2-mercaptoethanol (2ME). McCabe *et al.* (1983) reported that the AG was useful to diagnose cerebral toxoplasmosis in patients with acquired immunodeficiency syndrome. The modified AG is now available (Toxo-Screen DA/bioMérieux) and is routinely used in France. In the present study, we evaluated this kit by comparing it with the DT and indirect latex agglutination test (LA).

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Materials and Methods

Sera

One hundred four sera tested were chosen at random from general outpatients at Jikei University Hospital in Tokyo. In addition, 7 sera containing hepatitis B antigens, HBe and HBs, were also used. HBe and HBs antigens were detected by radioimmunoassay (RIA) and reversed-passive hemagglutination test (RPHA), respectively. Those sera were completely negative for DT ($<$1:4) but contained HBe antigen (RIA count indices, 3—6) as well as HBs antigen (RPHA titers, 1:64—1:1024).

Performance of serologic tests

The AG was performed using the commercial kit product (bioMérieux, France) as follows: 25 μl of the buffer solution was applied to each well of a hard U-type microplate. 25 μl each of test sera was added to the first well and mixed to make 1:2 dilutions. This was followed by two-fold dilution technique to make a series of serum dilutions on each serum. 25 μl of 0.2M 2ME was then added to each well. Finally, 50 μl of the suspension of the toxplasma antigen was added to each well. The plate was agitated to secure

reaction mixture. After standing the plate overnight at room temperature, the agglutination patterns were read. Antibody titers were expressed in terms of the initial serum dilution before addition of the antigen suspension.

The DT was performed by a modification (Kobayashi *et al.*, 1968) of the technique described by Frenkel and Jacobs (1958).

The LA was performed as previously described (Kobayashi *et al.*, 1977) using a commercial kit product (Eiken Chemical Co., Tokyo).

Results

Frequency distribution of AG

The frequencies of respective titers of AG on 104 sera revealed a bimodal distribution curve, thereby titers above 1:64 were regarded as positive (Fig. 1).

Qualitative agreements between AG and DT or LA

The qualitative correlation between AG and DT on 104 sera is shown in Table 1. Both tests agreed in 95.2% of the sera. In the remaining sera (4.8%), DT was negative and AG was positive.

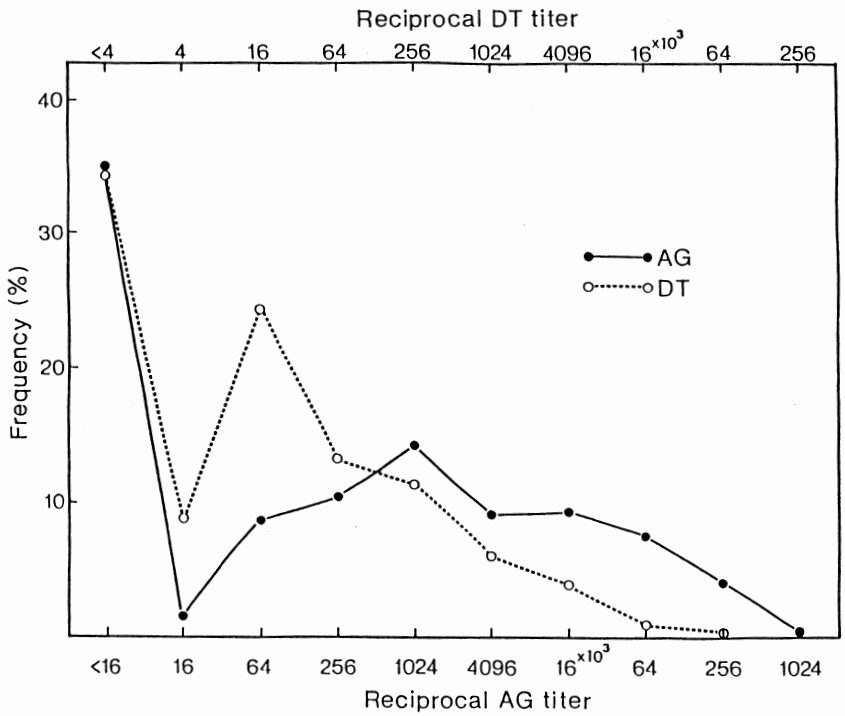

Fig. 1. Frequency distribution curves of AG and DT titers in 104 sera.

Table 1 Qualitative agreement between DT and AG with 104 sera

Agreement			Disagreement			
DT+ AG+ (%)	DT− AG− (%)	Total (%)	DT+ AG− (%)	DT− AG+ (%)	Total (%)	Grand total (%)
61 (58.7)	38 (36.5)	99 (95.2)	0 (0)	5 (4.8)	5 (4.8)	104 (100)

A higher agreement (99%) of the sera was also observed between AG and LA with only one discrepancy case, AG positive and LA negative (Table 2).

Quantitative correlations of AG with DT and LA

Quantitative studies were carried out on 104 sera and AG titers were compared with those of DT and LA. As shown in Tables 3 and 4, AG titers were shown thirty times higher than DT

Table 2 Qualitative agreement between LA and AG with 104 sera

Agreement			Disagreement			
LA+ AG+ (%)	LA− AG− (%)	Total (%)	LA+ AG− (%)	LA− AG+ (%)	Total (%)	Gaand total (%)
65 (62.5)	38 (36.5)	103 (99.0)	0 (0)	1 (1.0)	1 (1.0)	104 (100)

Table 3 Anti-toxoplasma antibody titers observed by DT and AG in 104 sera

Reciprocal of DT titers	Reciprocal of AG titers																		(% Posi. by AG)
	<4	4	8	16	32	64	128	256	512	1024	2048	4096	8192	16×10³	32	64	128	256	Total	
<4	32		2																34	(0)
4	1	1			2		3	2											9	(55.6)
16						3	3	5	2	4	4	1				1	1		24	(100)
64									2	1	5	1	2	3					14	(100)
256										2		2		2	1	3		2	12	(100)
1024														3		2	1		6	(100)
4096													1		1			2	4	(100)
16384													1						1	(100)
Total	33	1	2	2	3	6	7	4	7	9	4	4	8	2	6	2	4	104		(63.5)
(% Posi. by DT)	(0)	(0)	(0)		(0)	(100)	(50)	(71.4)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(58.7)	

Table 4 Anti-toxoplasma antibody titers observed by LA and AG in 104 sera

Reciprocal of LA titers	Reciprocal of AG titers																		(% Posi. by AG)
	<4	4	8	16	32	64	128	256	512	1024	2048	4096	8192	16×10³	32	64	128	256	Total	
<2	2																		2	(0)
2	7		1																8	(0)
4	14	1	1																16	(0)
8	7				1														8	(0)
16	3				1	1													5	(20)
32						1	2	3	1				1		1				9	(100)
64						1	3	3	1		3			1					12	(100)
128							1	1		5	1			2				1	11	(100)
256										1	3	1		2		1		1	9	(100)
512									2	1		1	1	2	1	2	1		11	(100)
1024											2	2		1	1		1		7	(100)
2048													1						1	(100)
4096													1			1			2	(100)
8192																1		1	2	(100)
16384																		1	1	(100)
Tatal	33	1	2	0	2	3	6	7	4	7	9	4	4	8	3	5	2	4	104	(63.5)
(% Posi. by LA)	(0)	(0)	(0)	(0)	(0)	(66.7)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(67.3)	

Table 5 Results of AG and LA tests on 7 sera with various titers of HBe and HBs antigens

Serum No.	HBe antigen titer	HBs antigen titer	Reciprocal antibody titer		
			DT	AG	LA
1	3.03	256	<4	<4	2
2	4.95	128	<4	<4	2
3	5.05	256	<4	<4	2
4	5.48	256	<4	<4	2
5	5.56	1024	<4	<4	2
6	6.09	64	<4	<4	2
7	6.40	64	<4	<4	2

HBe and HBs antigen titers are expressed as count indices by RIA and reciprocal titers by RPHA, respectively.

titers and fifteen times higher than LA titers.

AG and LA titers in sera containing HBe and HBs antigens

Ise *et al.* (1981) reported that sera which contained HBe and HBs antigens reacted non-specifically in LA. To check a possibility of such a non-specific reaction in AG, 7 sera which were negative in DT but contained HBe and HBs antigens were tested. The results showed that none of those sera had false-positive reactions in both tests (Table 5).

Discussion

The direct agglutination test (AG) was first described by Fulton and Turk (1959). The modified AG by Desmonts and Remington (1980) is characterized by eliminating false-positive reactions as seen in the original AG. The present study was aimed to compare the modified AG (Toxo-Screen DA, bioMérieux) with LA kit which is now commonly used in Japan. A qualitative agreement rate of 95.2% was observed between AG and DT, which was similar to that (98.4%) described by Desmonts and Remington (1980). High agreement (99%) was observed between AG and LA, suggesting that both tests are qualitatively almost similar. Thus, the combined use of the two tests will not be meaningful. On the other hand, there exists a difference in titers among AG, DT and LA. The AG titers were markedly high as compared with those of DT and LA. In their comparative test of AG with DT, Desmonts and Remington (1980) also reported previously a higher sensitivity of AG over DT on sera from chronically infected persons.

Ise *et al.* (1981) reported an occurrence of non-specific reaction by LA in HBe-positive sera with high titers of HBs antigen. In the present study, the result indicated that none of 7 sera tested (DT-negative, HBe and HBs-positive) showed false-positive reaction in both AG and LA. However, it remains unclear for the possible occurrence of the non-specific reactions in sera with much higher HBs antigen titers, i.e., above 1:2048. Further studies for their specificities would be necessary.

Thus, the AG kit is qualitatively very similar to the LA and would provide a useful tool for the screening of toxoplasmosis.

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