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Research Note

A Simple Method for the Titration of Anti-*Toxoplasma* Antibodies with a Small Volume of Blood of Mice Using PKU Filter Paper and an Indirect Latex Agglutination Test

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The primary *Toxoplasma* infection in a pregnant woman occasionally causes a congenital infection with the organisms in the infant (Remington and Desmonts, 1976). An early diagnosis is needed for the newborn infants with congenital toxoplasmosis, however, there are still many problems on the serodiagnosis of the congenital toxoplasmosis. For the animal model to study the serodiagnosis of the congenital toxoplasmosis, mice are considered to be suitable, because a large number of animals can be easily used and their genetical background is clear. In the present study, we tried to establish a simple method to measure anti-*Toxoplasma* antibody titers with a small volume of the blood of mice in order to make possible the titration of anti-*Toxoplasma* antibodies in newborn mice.

Outbred female ddY, inbred female C57BL/6 and BALB/c mice were purchased from Shizuoka Agricultural Cooperative Association for Laboratory Animals (Hamamatsu).

Bradyzoites of the avirulent Fukaya strain of *T. gondii* were used for infection. The bradyzoites were obtained from the brains of the chronically infected mice by treatment with 0.25% trypsin as described previously (Suzuki *et al.*, 1981). Released bradyzoites were suspended in phosphate-buffered saline at 2.5×10^4 organisms/ml, and mice were inoculated with 0.2 ml of the suspension intraperitoneally.

The blood obtained from normal and infected mice were dropped on PKU filter paper (Kitasato Institute, Tokyo), and the filter papers were dried in air at room temperature. The dried paper was used as blood-absorbed filter paper. The eluate from the filter paper was prepared by the method described previously (Kobayashi *et al.*, 1978). Two pieces of the disks of 3 mm diameter from the blood-absorbed filter paper were shaked in 50 μl of 0.2 M aminoethylpropanol-HCl buffer (pH 8.0) by a micromixer for 30 min.

An indirect latex agglutination (ILA) test with a commercial kit (Eiken Chemical Co. Ltd., Tokyo) was performed for the titration of anti-*Toxoplasma* antibodies. An equal volume (0.025 ml) of latex particle suspension was added to each of doubling dilutions of sera or the eluates from the filter papers in a microtiter tray (Tomy Seiko Co. Ltd., Tokyo), and the pattern of agglutination was read after the trays had stood at room temperature overnight.

The sera from 107 normal mice were subjected to ILA test. The greater part of the sera showed the ILA test titer at less than 1 : 2 (Fig. 1). Only 11 sera showed a non-specific agglutination in ILA test, however, their ILA titers were 1 : 8 and less.

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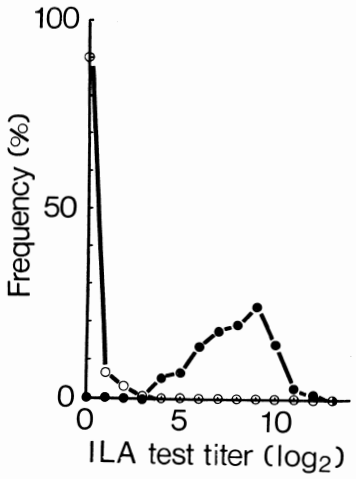

Fig. 1 Frequency distribution curves of ILA test titers in mice. One hundred and seven of normal, and 144 of infected mice were bled, and their sera were subjected to ILA test. Symbols: ○, normal mice; ●, infected mice.

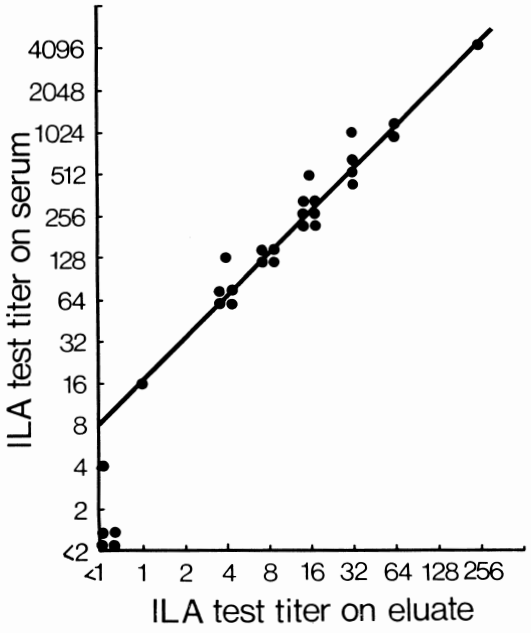

Fig. 2 Relationship between the ILA test titers of the serum and eluate from the blood-absorbed filter paper.

One hundred and forty-four *Toxoplasma*-infected mice were bled 3 weeks to 1 year after infection, and the sera were tested by ILA test. Their ILA test titers distributed from 1 : 16 to 1 : 4096 with an average titer of 1 : 395 (Fig. 1). Based on these results, sera with titers of ≧1 : 16 were classified as positive in anti-*Toxoplasma* antibodies in ILA test.

In humans, we have reported that sera with ILA test titres of ≧1 : 32 are regarded as positive in anti-*Toxoplasma* antibodies (Kobayashi *et al.*, 1977). Thus, the minimum ILA test titer to decide a presence of *Toxoplasma* infection is different between humans and mice.

Next, we examined whether or not anti-*Toxoplasma* antibody titers could be measured with the eluates from the blood-absorbed filter papers. Five normal and 25 infected mice were bled, and sera and blood-absorbed filter papers were obtained. Fig. 2 shows anti-*Toxoplasma* antibody titers of the sera and the eluates from the filter papers. The antibody titers of the eluates were one sixteenth of those of the sera, indicating that the eluates are equivalent to sixteen-fold dilution of sera. These results indicate that anti-*Toxoplasma* antibody titers of mice can be determined with the eluate from the filter paper containing a small volume of blood. The relationship between antibody titers of the sera and eluates in mice was the same as that in humans (Kobayashi *et al.*, 1978).

The blood-absorbed filter papers were stored at different temperatures, and an effect of the storage on their anti-*Toxoplasma* antibody titers was examined. When the filter papers were kept at 4°C in a refrigerator, ILA test titers of the filter papers did not change for 8 weeks (Table 1). In contrast, at a higher temperature, 27°C, the ILA test titers decreased gradually during the course of the storage (Table 1). The decrease in ILA test titers was already observed on the second week of the storage in some of the filter papers. On the other hand, when the blood-absorbed filter papers were stored in a desiccator at 27°C, the ILA test titers did not change for at least 4 weeks (Table 2). These results demonstrate that the blood-absorbed filter paper can be stored without a change

Table 1 Effects of storage of blood-absorbed filter papers at different temperatures on ILA test titer of the filter paper

Storage temperature	Sample No.	ILA test titer after storage for weeks of			
		0	2	4	8
4°C*	1	1 : 256	1 : 256	1 : 256	1 : 512
	2	1 : 512	1 : 1024	1 : 512	1 : 512
	3	1 : 512	1 : 512	1 : 512	1 : 512
	4	1 : 1024	1 : 1024	1 : 1024	1 : 1024
	5	1 : 2048	1 : 2048	1 : 2048	1 : 2048
27°C	1	1 : 256	1 : 128	1 : 128	1 : 64
	2	1 : 512	1 : 512	1 : 128	1 : 64
	3	1 : 512	1 : 512	1 : 256	1 : 128
	4	1 : 1024	1 : 1024	1 : 1024	1 : 256
	5	1 : 2048	1 : 512	1 : 512	1 : 256

* The blood-absorbed filter papers were stored in a refrigerator.

Table 2 Effect of storage of blood-absorbed filter papers in a desiccator on ILA test titer of the filter paper

Storage condition	Sample No.	ILA test titer after storage for weeks of			
		0	2	4	8
27°C in a desiccator	1	1 : 512	1 : 512	1 : 512	1 : 512
	2	1 : 1024	1 : 1024	1 : 1024	1 : 512
	3	1 : 2048	1 : 2048	1 : 2048	1 : 2048
	4	1 : 4096	1 : 4096	1 : 4096	1 : 2048
	5	1 : 4096	1 : 4096	1 : 4096	1 : 2048
27°C in air	1	1 : 512	1 : 512	1 : 512	1 : 128
	2	1 : 1024	1 : 1024	1 : 512	1 : 256
	3	1 : 2048	1 : 2048	1 ··1024	1 : 512
	4	1 : 4096	1 : 4096	1 : 2048	1 : 512
	5	1 : 4096	1 : 2048	1 : 2048	1 : 1024

of anti-*Toxoplasma* antibody titers for at least 1 month, if the filter papers are kept in a refrigerator or a desiccator.

A simple method for the titration of anti-*Toxoplasma* antibodies with a small volume of blood of mice was established by using PKU filter paper and ILA test. In this method, anti-*Toxoplasma* antibody titers can be exactly measured with only 10 μl of blood. Therefore, anti-*Toxoplasma* antibody titers of newborn mice can be easily determined by this method.

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短　報

PKU 濾紙と間接ラテックス凝集反応を用いたマウス微量血液からの抗トキソプラズマ抗体価簡易測定法

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PKU 濾紙にマウス血液を吸着させ，その濾紙からの溶出液につき間接ラテックス凝集反応（ILA）で抗体価を測定することにより，微量の血液から容易にかつ正確に抗トキソプラズマ抗体価を測定することができた．マウスにおける ILA 陽性判定基準は抗体価 1：16 であった．マウス血液吸着濾紙は，冷蔵庫またはデシケーター内に置けば，抗体価の変化なく少なくとも 1 ケ月間の保存に耐えることが判明した．