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# Micro-agglutination Test for Toxoplasma Antibodies

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**Summary.** A micro direct agglutination test has been devised for the detection of *Toxoplasma* antibodies. For this purpose a pure suspension of *Toxoplasma gondii*, the causative organism, was used as antigen. The test is easily and rapidly performed with the Takatsy micro-agglutination kit and very small amounts of antigenic material are required. The results obtained agree well with those of other serological tests in common use.

## INTRODUCTION

A number of serological tests have been described for the detection of *Toxoplasma* antibodies since Nicolau and Ravelo (1937) first used that of complement fixation. This test and the dye test of Sabin and Feldman (1948) have probably been the most widely used up to the present. In a preliminary report a macro direct agglutination test for *Toxoplasma* antibodies was described in which a pure suspension of *Toxoplasma gondii* free from other cells was used (Fulton and Turk, 1959). This test has subsequently been shown to be specific and sensitive and gives results in good agreement with those obtained in other serological tests such as the dye test, complement fixation and fluorescent antibody test (Fulton and Voller, 1964). In order to conserve antigenic material a micro-agglutination technique has now been devised and compared with other tests commonly used for estimating *Toxoplasma* antibodies.

## EXPERIMENTAL METHODS

### *Antigen*

The virulent RH strain of *T. gondii* was used to prepare a pure suspension of parasites from the peritoneal exudates of infected cotton rats essentially as described previously. In order to shorten the time of preparation one centrifugation procedure is now avoided by adding cotton rat red-cell antiserum while the white cells of the exudate are sedimenting before filtration through a sintered glass funnel. At the final stage of preparation 50 ml of a parasite suspension are allowed to sediment in a boiling tube for 30–60 minutes according to density so that any small clumps present may settle out and the suspended organisms are then used in the agglutination test. Previously it was found that the antigen could be kept for a period of 6 months or longer in 1 : 10,000 formol saline. However, infection with bacteria or fungi sometimes occurred and this material was rendered unsatisfactory for use in the agglutination test. For that reason a concentration of 0·2 per cent formalin is now used for preservation.

### *Micro techniques*

The Takatsy micro serial dilution kit was used (Sever, 1962). It consists of plates of Plexiglass approximately $13 \times 7 \times 0{\cdot}6$ cm in which six rows of twelve cups are drilled. Loops of coiled heat-resisting wire pick up—by capillary attraction—either 0·025 or 0·05 ml saline solution, for making the appropriate serum dilutions. Glass pipettes calibrated with

mercury to hold 0·025 ml can also be used for this purpose but it is a much slower process than when loops are used. On completing the serum dilutions the antigen suspension in 0·025 ml amounts is added to each cup from a calibrated dropper consisting of a metal tube for delivery and Perspex holder.

*Conditions of test*

The antigen suspension is first adjusted to give an optical density of approximately 0·50 in a Hilger Biochem Absorptiometer using a $\frac{1}{2}$ cm cell and Ilford $RO_2$ filter. Such a suspension contains approximately $5 \times 10^8$ parasites per ml and was used in the present experiments either undiluted or diluted with an equal volume of saline. Results are best read by placing the plate on a dark background and were sometimes available 6–8 hours after adding the antigen but generally readings are made on leaving overnight. To prevent evaporation of reagents the Perspex plate is covered with glass or sealed over with transparent tape. A 50 per cent agglutination was selected as the end-point and the reciprocal of the serum dilution giving this result is regarded as the antibody titre. For comparative purposes different antigen preparations were used and the macro-agglutination test was carried out at the same time so that the number of parasites used in each test could be compared directly.

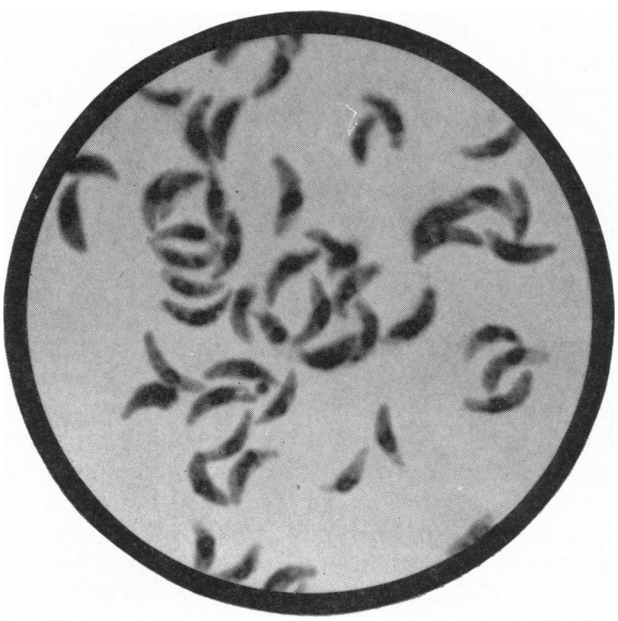

FIG. 1. Suspension of *Toxoplasma gondii* free from other cells used as antigen (×1760).

## RESULTS

The nature of the suspension of parasites used as antigen is shown in Fig. 1. When carrying out the macro-agglutination test one volume of antigen of the optical density indicated was added to 2·2 volumes of saline and 0·4 ml of the mixture was used per tube, equivalent to 0·125 ml of the original preparation. Since 0·025 ml of the preparation

undiluted or after dilution with an equal volume of saline is used in the micro test, that is equivalent to 1/5 or 1/10 the number of parasites used in the macro test. The saving in antigenic material is thus considerable.

The pattern of agglutination is shown in Fig. 2 and differs from that in the macro test previously described. In each case the serum dilutions start at 1/4 and extend by two-fold dilutions to a maximum dilution of 1/512. Each well on the right, containing saline instead of serum, serves as a control, and some of the wells contained no reagents. In the absence of serum or at dilutions beyond the agglutinating range a compact button of parasites is formed at the bottom of the well. As the end-point is approached on moving left there is a spread of parasites to form a thin carpet and button formation is absent. At the 50 per cent end-point the transition is relatively sharp. In Fig. 2, plate 2, the same antigen suspension diluted with an equal volume of saline was used. In Fig. 2, plate 3, the undiluted antigen was again used after serum dilutions had been carried out with glass pipettes calibrated at 0·025 ml instead of with metal loops. The results obtained were very similar on all three plates and the titres determined are shown in Table 1.

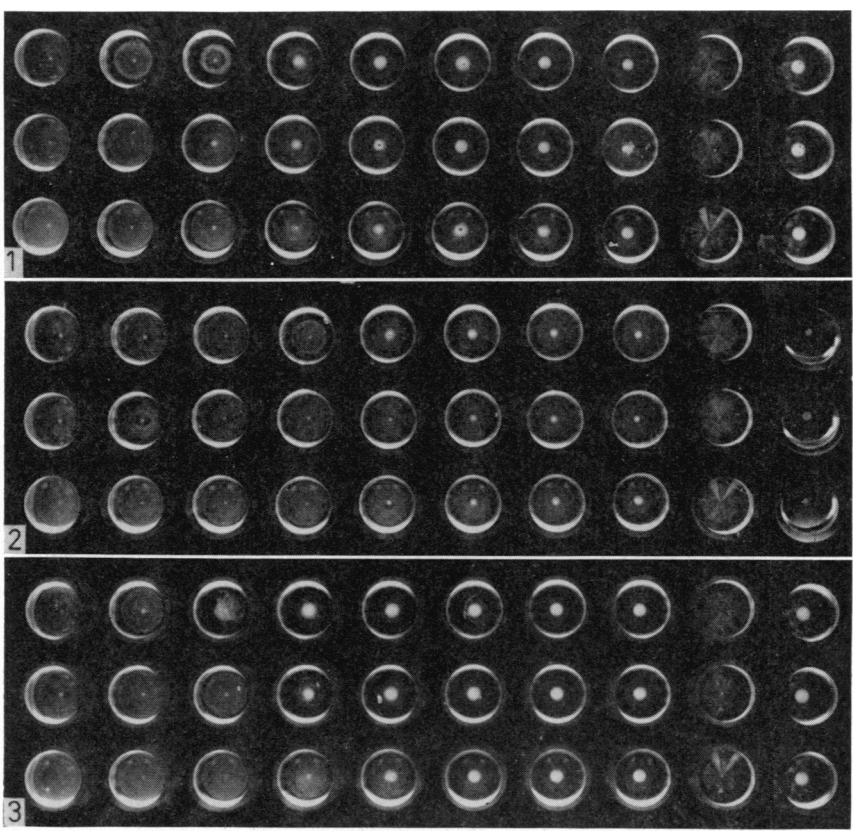


FIG. 2. Pattern of agglutination in the micro test. In plates 1 and 3 the antigen is undiluted and diluted with an equal volume of saline in plate 2. Dilutions were made by metal loops on plates 1 and 2 and with calibrated pipettes in 3. Some wells contain no reagents and the right-hand column in each plate serves as a control in absence of serum. The initial dilution is 1 : 4 and increases by two-fold steps to a maximum of 1 : 512. A 50 per cent agglutination is used as the end-point.

TABLE 1

DIRECT AGGLUTINATION TITRES OF THREE SERA DETERMINED BY THE MICRO METHOD

The patterns obtained are shown in Fig. 1 (Plates 1, 2 and 3).

| No of serum | Serum titre | | |
|---|---|---|---|
| | Plate 1 | Plate 2 | Plate 3 |
| 3 | 16 | 32 | 16 |
| 4 | 16 | 64 | 16 |
| 5 | 32 | 64 | 32 |

The results of the three tests are in satisfactory agreement. There is a tendency for the titres to be raised slightly when the antigen is diluted to half the normal concentration. The end-points are more easily determined directly than from a photograph.

In Table 2 are shown the titres of nine sera determined by the dye test, and by macro- and micro-agglutination tests with three different antigen preparations.

TABLE 2

SERUM TITRES DETERMINED BY THE DYE TEST AND BY THE MACRO- AND MICRO-AGGLUTINATION TEST USING THREE DIFFERENT ANTIGEN PREPARATIONS

| Serum No. | Dye test | Serum titre | | | | | |
|---|---|---|---|---|---|---|---|
| | | A | | B | | C | |
| | | Macro | Micro | Macro | Micro | Macro | Micro |
| 1 | <8 | 4 | <4 | 4 | <4 | 8 | 4 |
| 2 | 512 | 512 | 512 | 256 | 512 | 512 | 1024 |
| 3 | 1024 | 256 | 512 | 256 | 1024 | 512 | 1024 |
| 4 | 8 | 64 | 8 | 64 | 32 | 32 | 16 |
| 5 | <8 | <4 | <4 | <4 | <4 | <4 | <4 |
| 6 | 256 | 128 | 64 | 64 | 64 | 256 | 128 |
| 7 | 32 | 64 | 16 | 32 | 64 | 64 | 64 |
| 8 | 4096 | 2048 | 2048 | 2048 | 1024 | 4096 | 2048 |
| 9 | 4096 | 1024 | 2048 | 2048 | 4096 | 4096 | 2048 |

The results of all three tests are again in good agreement and do not diverge from one another by more than one or two dilutions. This divergence invariably occurs in all serological tests and when titres are high is of no significance. Slight differences in the quality of the antigen may have contributed to these results. It has already been shown (Fulton and Voller, 1964) that the macro test is specific in character and that there are no cross-reactions with serum of patients suffering from other protozoal or helminth infections. These findings were also verified in the case of the micro test.

## DISCUSSION

The macro direct agglutination test was previously shown to give results comparable with other serological tests commonly used in detection of *Toxoplasma* antibodies. A micro agglutination test is now described in which only 1/10 to 1/5 of the amount of antigen used in the macro test is required. The micro test possesses a number of advantages over some others in that it can be easily and rapidly performed and effects a marked saving of antigenic material. The end-points are sharp and can be readily detected by the naked eye. Comparison of results obtained with standard serological methods established

the value of the micro method for detection of antibodies to *Toxoplasma*. Moreover, in the test a suspension of dead parasites is used which can be kept for many months and the danger of infection is thus avoided.

## ACKNOWLEDGMENTS

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