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IMPROVED TECHNIQUE OF INDIRECT IMMUNOFLUORESCENCE FOR SEROLOGICAL DIAGNOSIS OF TOXOPLASMOSIS

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SUMMARY

Minor technical modifications as incubation of slides at 37°C for longer periods (one hour) and use of conjugates devoid of inespecific staining in lower dilutions have led to results in the indirect fluorescent antibody test comparable to those of the Sabin-Feldman dye test. They were identical as to positivity and negativity and the titers were the same or differed at most by one dilution. In general, the fluorescence test has shown higher titers than the dye test.

Results of the indirect fluorescence antibody test applied to the serology of toxoplasmosis, published to the present, have shown a lower percentage of reactivity of sera, as compared to that of the Sabin-Feldman dye test [4, 5]. The differences lie mainly in low titred sera which react only in the dye test. This fact has thrown some doubt on the specificity of such results.

By introducing minor modifications in the technique, we have been able to improve the fluorescent technique sensibility so as to obtain results identical to those of the dye test. Same dilutions of 140 inactivated sera were titrated by the two methods. Fourfold dilutions in saline ran from 1:16 to 1:4000 and from here, in twofold dilutions when necessary. Non-reactive sera at 1:16 were considered negative. The dye test was done as follows: peritoneal exsudate of mice, inoculated two days before with toxoplasma, were obtained injecting intraperitoneally about 2 ml of sodium citrate 3.8%. The resulting suspension of parasites was diluted 1:5 with "accessory factor" (normal undiluted human serum kept at −20°C). One tenth ml of this mixture was added to 0.1 ml of each serum dilution in 12×75 tubes. After one hour in 37°C water bath, one drop (0.035 ml) of alcaline methylene blue [6] was added to every tube which remained at room temperature for about 15 minutes. A small drop of each was microscopically examined and the proportions of stained and unstained parasites determined. The highest serum dilution still able to modify the staining of at least 50% of the toxoplasma was given as the dye test titer. Results were very good as to reproductibility (eventual variations of one tube, always in the same direction, in different days) and sensibility (constantly checked with a standard serum from the C.D.C., Atlanta, USA, kindly sent by Drs. Brooke and Kagan).

The fluorescent test was done in slides prepared and kept as described by GOLDMAN [3]. Each dried antigen drop (five per slide, isolated from each other by lines of nail polish) was covered with a small volume (about 0.01 ml) of one dilution of serum and incubated for one hour at 37°C in wet chamber. After washing twice in buffered saline pH 7.2, the slides were blotted dry and each smear covered with diluted anti-human-globulins serum conjugated to fluorescein isothiocyanate. The slides were incubated at 37°C for another hour, washed, blotted and mounted with buffered glycerine pH 8.0 and coverslip. They were examined in darkfield under oil immersion (obj. 40×, oc. 12.5×), HBO 200 as the light source, exciting filter BG 12 and barrier filter 50 (Zeiss). The highest serum dilution still giving an evident fluoerscence (1+) of most parasites was

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Results of indirect fluorescent antibody test on 109 dye-test positive sera

Dye test titers	Indirect fluorescent test: number of sera with titers of								
	1:16	1:64	1:250	1:1000	1:4000	1:8000	1:16000	1:32000	1:64000
1:16	2	3	2	—	—	—	—	—	—
1:64	—	4	12	7	—	—	—	—	—
1:250	—	1	16	14	—	—	—	—	—
1:1000	—	—	2	18	15	—	—	—	—
1:4000	—	—	—	—	4	—	1	—	—
1:8000	—	—	—	—	—	1	1	—	—
1:16000	—	—	—	—	—	—	1	1	—
1:32000	—	—	—	—	—	—	1	1	2

taken as the fluorescent test titer. Controls of antigen and conjugate have never shown any fluorescence. Antiglobulins conjugate was prepared by slowly adding the fluorochrome using a dialysis technique, modified from CLARK & SHEPARD [2], after salting out the globulins with half saturated ammonium sulphate [1]. Free dye was removed by exhaustive dialysis against phosphate buffered saline. Fluorescein: protein ratio was about 6×10^{-3}. The conjugate was used diluted at 1:60.

In 140 sera, 31 were negative or non reactive at 1:16 and 109 were reactive. Every positive serum reacted in both tests.

In 109 reactive sera, the titers were the same in 47, differed of one tube in 52 and of two tubes in 10 sera, the fluorescent test giving most of the higher titers.

These initial results suggest the routine use of the indirect fluorescent antibody test, an easier technique than the dye-test as to handling of antigen, speed of reading and no need of "accessory factor".

RESUMO

Técnica aperfeiçoada de imunofluorescência indireta para diagnóstico sorológico da toxoplasmose.

Introduziram-se pequenas modificações técnicas na reação de imunofluorescência indireta, aplicada à sorologia da toxoplasmose. Em conseqüência os resultados obtidos foram comparáveis aos da reação de Sabin-Feldman. Houve total concordância quanto à positividade ou negatividade e os títulos em ambas reações coincidiram ou divergiram em geral de apenas uma diluição de sôro.

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