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# DIAGNOSTIC INFORMATION FROM SEROLOGICAL TESTS IN HUMAN TOXOPLASMOSIS

## I — A comparative study of hemagglutination, complement fixation, IgG- and IgM-immunofluorescence tests in 3,752 serum samples

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### SUMMARY

Four different tests for toxoplasma antibodies, the anti-IgG immunofluorescence (IF-IgG), anti-IgM immunofluorescence (IF-IgM), hemagglutination (HA) and complement fixation (CF) tests were comparatively studied in serum samples from 3,752 individuals. IF-IgG was the most sensitive one as indicated by positive tests in 54.8% of the sera. A sensitivity of 0.975 for HA test and 0.704 for CF test was shown by respective co-positivity indices to the IF-IgG test. Anti-toxoplasma IgM antibodies were found in 5.3% of sera, corresponding to 9.7% of reactive samples. Low titers were predominant in reactive sera and a frequent equivalence of HA titers and of CF titers to IF-IgG titers could be disclosed. This permitted to define a range of "equivalent titers" for HA and CF tests, as compared to IF-IgG titers. However when studying sera with a positive IF-IgM test, higher IF-IgG and CF titers were the rule, with even higher CF titer values than the expected "equivalent" ones. On the contrary, HA titers were much lower than the expected "equivalent" titers. Thus 2 different serologic patterns could be distinguished: one related to old infections, with similar IF-IgG and HA titers and not higher than 1:4,000, CF titers of 1:80 or less and frequently negative, and a negative IF-IgM test; the other related to recent infections, presenting besides a positive IF-IgM test, low HA titers, frequently of 1:4,000 or less, IF-IgG titers of 1:8,000 or more and CF titers of 1:160 or more.


### INTRODUCTION

Sensitive serologic procedures, such as the Sabin-Feldman dye test [16], immunofluorescence [3] and hemagglutination [8] tests, very frequently detect antibodies to *Toxoplasma gondii* in a high percentage of adult individuals. In our country, different surveys in urban and rural areas [6, 9, 12, 14] and even in indian tribes [2] have shown positive results varying from 30% to 95% of the samples studied. Thus the mere finding of such antibodies is of little help for diagnostic purposes. Although acute cases of toxoplasmosis usually show high titers, as compared to those found in the majority of reactive samples in a population, the large range of overlap and the frequent permanence of high titers for months or years after clinical evidences of the disease have entirely subsided [1], reduce the diagnostic value of test titers. Serologic procedures such as the complement

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fixation [10] and the IgM-antibody [15] tests have been recommended as good indicators to distinguish between acute toxoplasmosis and a quiescent or past toxoplasmic infection.

In this paper we present our observations from performing routinely the anti-IgG immunofluorescence (IF-IgG), passive hemagglutination (HA), complement fixation (CF) and anti-IgM immunofluorescence (IF-IgM) tests in 3,752 serum samples from patients sent for toxoplasmosis tests.

## MATERIAL AND METHODS

### Serum samples

Blood samples from 3,752 patients were either collected at the laboratory or sent to it and sera were usually processed the same day or sometimes kept frozen at −20°C for 1 or 2 days before testing. Fresh sera or thawed frozen sera were heat-inactivated at 56°C for 30 minutes just before testing.

### Immunofluorescence tests

Were performed with serum diluted in saline phosphate buffer (PBS: NaCl 0.15 M, phosphates 0.01 M, pH 7.2) from 1:16 to 1:4,096 in four-fold dilutions and when necessary in doubling dilutions from 1:4,000 on. Usually the 1:64 dilution was not included in the IF-IgG test. Fluorescent conjugates were obtained from Hyland, Travenol Laboratories, USA, and diluted for maximal reactivity. Two conjugates were used, anti-IgG and anti-IgM, according to the test. Reactivity in the IF-IgM test was seen to become negative after treatment of sera with 2-mercaptoethanol, which resulted in no significant differences for IF-IgG titers. This ensured that anti-IgM conjugates were specific for IgM antibodies. Recently high IF-IgM titers (1:4,000) with negative results in the IF-IgG test were found in cases of very recent toxoplasma infections, not included in the present study. This observation underlined the specificity of the anti-IgG conjugate showing that it did not react with IgM antibodies. For some time in the beginning of the study, an anti-human globulin conjugate was used instead of the anti-IgG conjugate. Tests were performed as described earlier [4]. Positive standards were always included in the tests. For the IF-IgG test, a chloroform-clarified [19], freeze-dried pool of positive sera, was used. It showed 670 I.U. when compared to the W.H.O. International Standard Serum for Toxoplasmosis, for which a titer: unit ratio of 0.15 was found, similar to that reported by NIEL et al. [13] for immunofluorescence tests. For the IF-IgM test, a positive serum with a 1:320 titer, gently sent by Dr. J. S. Remington, was used as reference.

### Hemagglutination tests

Were performed in V-shaped wells of plastic plates (Cooke Engineering Co., USA), with doubling dilutions of sera in PBS from 1:64 to 1:4,096, and from 1:4,000 on, when necessary. To 2 drops (0.05 ml) of diluted serum, one drop (0.025 ml) of sensitized cells suspension was added and the sedimentation pattern read as usual, after 1 to 2 hours or even overnight when convenient, the plates kept at room temperature. The cell suspension was prepared as described for other systems [5, 7] by sensitizing human, formalin treated erythrocytes with a soluble extract of toxoplasma, diluted so as to give the highest possible titers for positive sera and at the same time clear-cut negative results for non-reactive sera. To prepare this extract, peritoneal exsudates from 70 to 80 mice inoculated with the parasite two days before were filtered through nylon-wool [18]. The purified toxoplasma cells were washed in saline solution, the sediment suspended in about 6 ml of distilled water and submitted to ultrasonic oscillations at 40 Hz for 2 or 3 minutes (Sonifier Cell Disruptor, Heat Systems-Ultrasonics Inc., USA) in a ice-bath. After centrifuging for 15 minutes at 10,000 g the supernatant extract was isotonized with a concentrated NaCl solution, distributed in 0.5 ml aliquots and stored at −70°C. The sediment was kept to prepare antigen for the complement fixation test. In general, maximal sensitization of cells was obtained with 1:40 to 1:80 dilutions of the extract. Sensitized cells were fixed again with formalin at 3% or glutaraldehyde

at 0.1% and preserved by lyophilization. When reconstituted with distilled water this reagent could be used for at least 10 days when maintained at 4°C.

#### Complement fixation tests

Were done in U-shaped wells of plastic plates, with one drop volumes (0.025 ml) of dilutions in triethanolamine buffer [11], of serum, complement and antigen. Doubling serum dilutions from 1:20 on, 4 50% hemolytic complement units and dilutions of antigen giving maximal fixation were used. After incubating plates for about 18 hours at 4°C and 30 minutes at 37°C, one drop (0.025 ml) of hemolysin-sensitized sheep erythrocytes was added to each well, the plates left for 1 hour at 37°C with occasional agitation, centrifuged and the tests immediately read. Standard positive and negative sera were always included, as well as tests for complement and for anticomplementary activity of 1:20 dilutions of sera. Hemolysis of 50% or less in the tests was taken as a positive result. Antigen was prepared by suspending sediment of sonicated toxoplasma, prepared as described above, in about 3 ml triethanolamine buffer. The suspension was homogeneized by ultrasonic treatment for a few seconds, one part of glycerin added to one part of the suspension and the mixture kept at −20°C. Dilutions for use corresponded to about 1:80 of this final mixture. The frequent occurrence of antibodies to toxoplasma in guinea-pig serum used for complement represented a difficulty, simulating anticomplementarity of antigens. A fluorescent antibody test with an anti guinea-pig globulin conjugate was used to select serum from non-infected animals.

## RESULTS

#### Frequency of positive results and observed titers

IF-IgG test showed positive results in 2,055 samples (54.8%). It was positive whenever any of the other tests was positive and sometimes appeared as an isolate positive test.

Percentages of positive results were 53.4% for the HA test, 38.6% for the CF test and 5.3% for the IF-IgM test. These tests showed co-positivity indices of 0.975, 0.704 and 0.097 respectively for the IF-IgG test, taken as a reference test.

Results presented under "group A" in Tables I and II show the frequency of titers observed for the 2,055 reactive samples for the IF-IgG and HA tests and for the CF-test respectively. It can be observed that titers of 1:4,000 or less were seen in 82.1% of samples for the IF-IgG test, and in 87.8% for the HA test. Titers of 1:80 or less, or negative results, were seen for the CF-test in 86.4% reactive sera. Titer frequency curves for these tests are shown in Fig. 1.

The IF-IgM test was positive in 200 (9.7%) of the reactive sera, with titers from 1:16 to 1:8,000 in the following percentages: 1:16-8.5%; 1:64-18.0%; 1:256-21.0%; 1:1,024-17.5%; 1:4,000-31.5%; 1:8,000-3.5%.

#### Comparative study of titers observed in IF-IgG, HA and CF tests

In order to compare IF-IgG and HA titers, sera were distributed according to the results obtained as indicated in Table III. When searching for any possible correspondence of titers in both tests, a range of "equivalent" HA titers for each IF-IgG titer value was looked for by trial and error as those furnishing the highest possible agreement percentages. For the observed disagreements, percentages and 95% confidence intervals were also calculated (Table IV).

The same was done for respective serum titers in IF-IgG and CF tests (Tables V and VI).

#### Positivity in the IF-IgM test and titers in the different anti-toxoplasma tests

Sera were distributed according to IF-IgM reactivity and to titers in the IF-IgG, HA and CF-tests. Table VII shows the percentages of IF-IgM positive sera for such titers.

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TABLE I

Titer frequency (and 95% confidence intervals) for IF-IgM and HA tests. A — in 2,055 reactive samples; B — in 200 reactive samples presenting a positive IF-IgM test

| Titers | IF-IgG test | | HA test (*) | |
|---|---|---|---|---|
| | A | B | A | B |
| < 16 | 0.0% (0.0% \|—\| 0.2%) | 0.0% (0.0% \|—\| 1.8%) | 2.5% (1.8% \|—\|3.2%) | 12.5% (9.3% \|—\| 17.2%) |
| 16 | 1.4% (0.9% \|—\| 1.9%) | 0.0% (0.0% \|—\| 1.8%) | 8.1% (6.9% \|—\| 9.3%) | 12.0% (7.9% \|—\| 17.4%) |
| 256 | 18.5% (16.8% \|—\| 20.2%) | 0.5% (0.0% \|—\| 2.8%) | 28.6% (26.6% \|—\| 30.5%) | 14.0% (9.5% \|—\| 19.6%) |
| 1,024 | 25.9% (24.0% \|—\| 27.8%) | 5.5% (2.8% \|—\| 9.7%) | 27.6% (35.5% \|—\| 39.7%) | 30.0% (23.8% \|—\| 36.9%) |
| 4,000 | 36.3% (34.2% \|—\| 38.4%) | 25.0% (19.2% \|—\| 31.6%) | 11.0% (9.6% \|—\| 12.3%) | 7.5% (4.3% \|—\| 12.1%) |
| 8,000 | 10.4% (9.1% \|—\| 11.7%) | 36.5% (30.0% \|—\| 42.8%) | 7.0% (5.9% \|—\| 8.1%) | 9.0% (5.4% \|—\| 13.9%) |
| ≥ 16,000 | 7.5% (6.4% \|—\| 8.6%) | 32.5% (26.1% \|—\| 39.5%) | 5.2% (4.2% \|—\| 6.1%) | 15.0% (10.4% \|—\| 20.7%) |

(*) Titers accumulated for the same intervals used for the IF-IgG test

TABLE II

Titer frequency (and 95% confidence intervals) in the CF-test: A — in 2,055 reactive samples; B — in 200 reactive samples presenting a positive IF-IgM test

| CF titers | A | B |
|---|---|---|
| < 20 | 29.6% (27.6% \|—\| 31.6%) | 10.5% (6.6% \|—\| 15.6%) |
| 20 | 27.7% (25.8% \|—\| 29.6%) | 4.0% (1.7% \|—\| 7.7%) |
| 40 | 19.1% (17.4% \|—\| 20.8%) | 6.0% (3.1% \|—\| 10.3%) |
| 80 | 10.1% (8.8% \|—\| 11.4%) | 10.0% (6.2% \|—\| 15.0%) |
| 160 | 5.7% (4.7% \|—\| 6.7%) | 14.5% (9.9% \|—\| 20.2%) |
| 320 | 4.8% (3.9% \|—\| 5.7%) | 30.0% (23.8% \|—\| 36.7%) |
| ≥ 640 | 3.2% (2.4% \|—\| 4.0%) | 25.0% (19.2% \|—\| 31.7%) |

**Comparative study of titers in IF-IgM positive sera**

Titer distributions in IF-IgG and HA-tests for the 200 sera presenting a positive IF-IgM test are shown in Tables I and II, under "group B", and in Fig. 2. While IF-IgG titers under 1:4,000 occurred in only 6.0% of samples, 69.0% had titers over this value. However, in the HA-test, titer distribution did not differ significantly from that observed for the whole group of reactive samples, except for a somewhat larger percentage both of negatives and of very high titers (1:16,000 or more), at the expenses of medium titers. Distribution of titers for the CF-test was significantly different from that of the whole group of reactive samples, since in 69.5%, titers of 1:160 or more were found.

To compare IF-IgG, HA and CF-test titers observed for same sera, IF-IgM positive samples were distributed according to such titers (Tables VIII and IX) and percentages of agreement calculated between IF-IgG and "equivalent" HA and CF titers (Tables X and XI).

## DISCUSSION

The IF-IgG test was the most sensitive of several tests for toxoplasma antibodies includ-

ed in this study. It showed the highest percentage of reactive samples (54.8%), and when any of the other tests were positive, IF-IgG test was positive. Thus, positivity in the IF-IgG test was sufficient to distinguish between reactive and non-reactive sera. As widely demonstrated [4, 20], results of the immunofluorescence toxoplasma test are qualitatively and quantitatively very similar to those of the Sabin-Feldman dye-test. It must be emphasized, however, that sensitivity of immunofluorescence tests depends on several factors, related for example to characteristics of fluorescent conjugates, as intensity of labeling and dilution of use, as well as of the fluorescence microscopy optical systems. Thus, sensitivity of the test must be always checked with the help of standard positive reference sera.

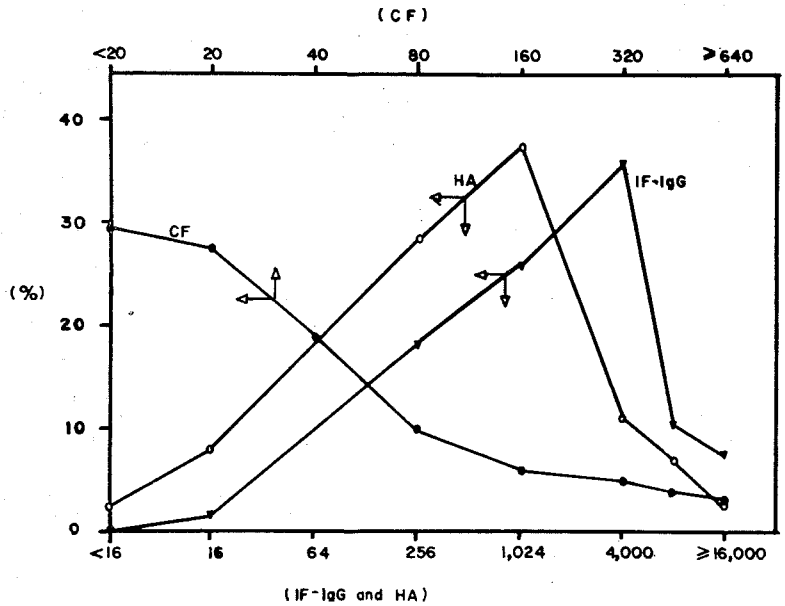


Fig. 1 — Titer frequency curves for 2,055 reactive serum samples in IF-IgG, HA and CF tests.

TABLE III

Reactive sera distributed according to IF-IgG and HA titers

| IF-IgG titers | HA titers | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|
| | < 64 | 64 | 256 | 1,024 | 4,000 | 8,000 | ≥ 16,000 | |
| 16 | 11 | 10 | 6 | 1 | 0 | 0 | 0 | 28 |
| 256 | 11 | 93 | 239 | 37 | 1 | 0 | 0 | 381 |
| 1,024 | 4 | 29 | 231 | 256 | 12 | 1 | 0 | 533 |
| 4,000 | 10 | 15 | 84 | 415 | 172 | 48 | 1 | 745 |
| 8,000 | 9 | 14 | 18 | 41 | 31 | 66 | 34 | 213 |
| ≥ 16,000 | 7 | 4 | 10 | 23 | 11 | 29 | 71 | 155 |
| Total | 52 | 165 | 588 | 773 | 227 | 144 | 106 | 2,055 |

Our previous observations in sera from residents of São Paulo had shown most reactive sera to have titers of 1:256 or higher. In the present study, these account for 98.6% of all reactive samples. Thus, for practical purposes, while maintaining 1:16 as the limit of positivity, dilution 1:64 was omitted from the IF-IgG test, so that dilutions up to 1:8,000 could be routinely included in the set of 5 dilutions initially tested for each serum sample.

As shown in Table I, titers more frequently found were 1:256, 1:1,024 and 1:4,000. It should be remembered, however, that sera here included may not represent a random sample of residents in São Paulo, but only of patients sent for toxoplasmosis serological tests.

It is to be remarked that "polar" staining of toxoplasma [17], frequently observed for non-reactive and even for weakly reactive sera

TABLE IV

Percentages of agreement or disagreement (and respective 95% confidence intervals) between IF-IgG and "equivalent" HA titers in 2,055 reactive sera

| IF-IgG titers | "Equivalent" HA titers | Percent of | | |
|---|---|---|---|---|
| | | Agreement with "equivalent" HA titers | Disagreement due to titers | |
| | | | HA < IF-IgG | HA > IF-IgG |
| 16 | < 64 or 64 | 75.0% (55.1% \|—\| 89.3%) | — | 25.0% (10.7% \|—\| 44.9%) |
| 256 | 64 or 256 | 87.1% (83.4% \|—\| 90.4%) | 2.9% (1.4% \|—\|5.2%) | 10.0% (7.1% \|—\| 13.5%) |
| 1,024 | 256 or 1,024 | 91.4% (88.4% \|—\| 93.4%) | 6.2% (4.3% \|—\| 8.5%) | 2.4% (1.3% \|—\| 4.1%) |
| 4,000 | 1,024 or 4,000 | 78.8% (75.7% \|—\| 81.7%) | 14.6% (12.0% \|—\| 17.3%) | 6.6% (4.9% \|—\|8.6%) |
| 8,000 | 4,000 or 8,000 | 45.5% (38.3% \|—\| 51.9%) | 38.5% (32.1% \|—\| 43.8%) | 16.0% (11.4% \|—\| 21.7%) |
| ≥ 16,000 | 8,000 or ≥ 16,000 | 64.5% (56.5% \|—\| 72.0%) | 35.5% (28.0% \|—\|43.5%) | — |

TABLE V

Reactive sera distributed according to IF-IgG and CF titers

| IF-IgG titers | CF titers | | | | | Total |
|---|---|---|---|---|---|---|
| | < 20 | 20 | 40 | 80 | ≥ 160 | |
| 16 | 27 | 1 | 0 | 0 | 0 | 28 |
| 256 | 279 | 88 | 13 | 0 | 1 | 381 |
| 1,024 | 209 | 239 | 72 | 12 | 1 | 533 |
| 4,000 | 76 | 230 | 276 | 121 | 42 | 745 |
| 8,000 | 11 | 8 | 27 | 65 | 102 | 213 |
| ≥ 16,000 | 6 | 1 | 4 | 10 | 134 | 155 |
| Total | 608 | 567 | 392 | 208 | 280 | 2,055 |

in tests done with anti-globulin conjugates was absent but in very exceptional cases when using in the tests gamma-chain specific anti-IgG conjugates, although particularly frequent and strong in tests with anti-$\mu$ chain conjugates. Polar staining could be entirely removed by previously treating sera with 2-mercapto-ethanol, and thus seems to be due in most cases to IgM antibodies cross-reacting with certain toxoplasma structures.

HA-test also showed a high sensitivity, with positive results (1:64 or higher) for 97.5% of reactive sera. When stable HA reagents are prepared and lyophilized as described, the test is easy to perform and also economical, since antigenic material from 70 to 80 infected mice was sufficient for about 10,000 qualitative tests. For epidemiological studies as in prevalence surveys or for routine pre-natal investigation of the infection, the test may present other advan-

TABLE VI

Percentages of agreement or disagreement (and respective 95% confidence intervals) between IF-IgG and "equivalent" CF titers, in 2,055 reactive sera

| Serum IF-IgG titers | "Equivalent" CF-titers | Percent of | | |
|---|---|---|---|---|
| | | Agreement with "equivalent" CF titers | Disagreement due to titers | |
| | | | CF > IF-IgG | CF < IF-IgG |
| 16 | < 20 | 96.4% (81.7% \|—\| 99.9%) | — | 3.6% (0.1% \|—\| 18.3%) |
| 256 or 1,024 | < 20 or 20 | 89.2% (86.9% \|—\| 91.6%) | — | 10.8% (8.4% \|—\| 13.1%) |
| 4,000 | 20 or 40 | 67.9% (64.5% \|—\| 71.3%) | 10.2% (8.0% \|—\| 12.4%) | 21.9% (18.9% \|—\| 24.9%) |
| 8,000 | 80 or 160 | 56.3% (49.6% \|—\| 63.0%) | 21.6% (16.1% \|—\| 27.1%) | 22.1% (16.5% \|—\| 27.7%) |
| ≥ 16,000 | ≥ 160 | 86.5% (81.1% \|—\| 91.9%) | 13.5% (8.1% \|—\| 18.9%) | — |

TABLE VII

Percentages of IF-IgM positive sera for different titers in IF-IgG, HA and CF tests

| Titers | < 1,024 | 1,024 | 4,000 | ≥ 8,000 |
|---|---|---|---|---|
| IF-IgG test | 0.2% (0.0% \|—\| 1.4%) | 2.1% (1.2% \|—\| 4.1%) | 6.9% (5.2% \|—\| 9.1%) | 37.2% (32.0% \|—\| 42.8%) |
| HA-test | 9.7% (0.8% \|—\| 12.3%) | 7.5% (5.7% \|—\| 9.6%) | 7.1% (4.1% \|—\| 11.3%) | 19.2% (14.5% \|—\| 24.7%) |
| Titers | ≥ 20 | 40 | 80 | ≥ 160 |
| CF-test | 2.6% (1.7% \|—\| 3.5%) | 3.1% (1.6% \|—\| 5.4%) | 9.6% (5.9% \|—\| 14.2%) | 48.9% (43.0% \|—\| 56.1%) |

tages, as the possibility of automation or semi-automation, and of employing eluates of finger-tip blood samples collected on filter paper, as is also the case for immunofluorescence tests.

CF-test was less sensitive than IF-IgG and HA-tests, with positive results (1:20 or more) in about 70% of reactive samples.

Immunofluorescence test for IgM antibodies was positive (1:16 or higher) in about 10% of reactive sera. Since such antibodies are especially related to recent infections [15], this high percentage certainly expresses the concentration in our material of samples from clinically suspected cases of acute toxoplasmosis.

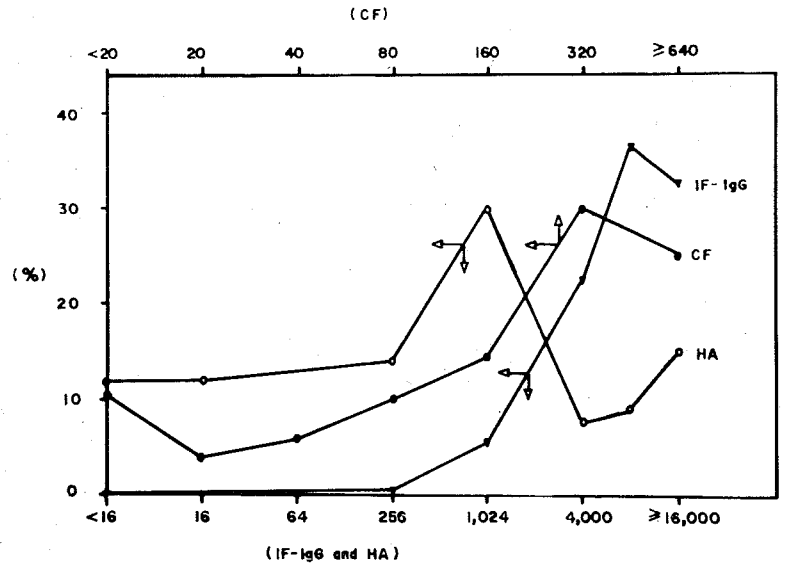


Fig. 2 — Titer frequency curves for 200 reactive samples presenting a positive IF-IgM test

TABLE VIII

Reactive sera presenting a positive IF-IgM test, distributed according to IF-IgG and HA test titers

| IF-IgG titers | HA titers | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|
| | < 64 | 64 | 256 | 1,024 | 4,000 | 8,000 | ≥ 16,000 | |
| 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 256 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| 1,024 | 3 | 1 | 2 | 4 | 0 | 0 | 0 | 10 |
| 4,000 | 9 | 7 | 9 | 19 | 6 | 1 | 0 | 51 |
| 8,000 | 8 | 13 | 10 | 19 | 4 | 11 | 8 | 73 |
| ≥ 16,000 | 5 | 3 | 6 | 16 | 6 | 7 | 22 | 65 |
| Total | 25 | 24 | 28 | 58 | 16 | 19 | 30 | 200 |

In order to disclose eventual serologic differences between recent and old toxoplasma infections, serum samples presenting a positive IF-IgM test were compared to the whole group of reactive sera, among which samples from ancient infection cases are certainly the most frequent ones. It was found that, while about 80% of *reactive samples* had IF-IgG titers of 1:4,000 or less, 70% of *IF-IgM positive sera* presented a IF-IgG titer of 1:8,000 or more. Also, CF titers of 1:80 or less occurred in 86% of *reactive sera* and negative results in 30% of these, but CF-test showed titers of 1:160 or higher in almost 70% of *IF-IgM positive samples*. No such marked differences were seen for the HA-test between both groups of sera; thus, hemagglutination titers of

TABLE IX

Reactive sera presenting a positive IF-IgM test, distributed according to IF-IgG and CF-test titers

| IF-IgG titers | CF titers | | | | | Total |
|---|---|---|---|---|---|---|
| | < 20 | 20 | 40 | 80 | ≥ 160 | |
| 16 | 0 | 0 | 0 | 0 | 0 | 0 |
| 256 | 1 | 0 | 0 | 0 | 0 | 1 |
| 1,024 | 3 | 3 | 2 | 1 | 1 | 10 |
| 4,000 | 7 | 3 | 7 | 13 | 21 | 51 |
| 8,000 | 8 | 2 | 2 | 5 | 56 | 73 |
| ≥ 16,000 | 2 | 0 | 1 | 1 | 61 | 65 |
| Total | 21 | 8 | 12 | 20 | 139 | 200 |

TABLE X

Percentages of agreement or disagreement (and respective 95% confidence intervals) between IF-IgG and "equivalent" HA titers in 200 reactive sera with a positive IF-IgM test

| IF-IgG titers | "Equivalent" HA titers | Percent of | | |
|---|---|---|---|---|
| | | Agreement with "equivalent" HA titers | Disagreement due to titers | |
| | | | HA > IF-IgG | HA < IF-IgG |
| 16 | < 64 or 64 | 0% | — | 0% |
| 256 | 64 or 256 | 100% | 0% | 0% |
| 1,024 | 256 or 1,024 | 60.0% (26.2% \|—\| 87.8%) | 40.0% (12.2% \|—\| 73.8%) | 0% |
| 4,000 | 1,024 or 4,000 | 49.0% (34.8% \|—\| 63.4%) | 49.0% (34.8% \|—\| 63.4%) | 3.9% (0.5% \|—\| 13.5%) |
| 8,000 | 4,000 or 8,000 | 20.6% (12.0% \|—\| 31.6%) | 68.5% (56.6% \|—\| 78.9%) | 11.0% (4.9% \|—\| 20.5%) |
| ≥ 16,000 | 8,000 or ≥ 16,000 | 44.6% (32.3% \|—\| 57.5%) | 55.4% (42.5% \|—\| 67.7%) | — |

TABLE XI

Percentages of agreement or disagreement (and respective 95% confidence intervals) between IF-IgG and "equivalent" CF titers in 200 reactive sera with a positive IF-IgM test

| IF-IgG titers | "Equivalent" FC titers | Percent of | | |
|---|---|---|---|---|
| | | Agreement with "equivalent" CF titers | Disagreement due to titers | |
| | | | CF < IF-IgG | CF > IF-IgG |
| ≤ 1,024 | < 20 or 20 | 60,0% (26.2% \|—\| 87.8%) | — | 40.0% (12.2% \|—\| 73.8%) |
| 4,000 | 20 or 40 | 19.6% (9.8% \|—\| 33.1%) | 13.7% (5.7% \|—\| 26.3%) | 66.7% (52.1% \|—\| 79.2%) |
| 8,000 | 80 or 160 | 9.6% (3.9% \|—\| 18.8%) | 13.7% (6.7% \|—\| 23.7%) | 76.7% (65.3% \|—\| 85.8%) |
| ≥ 16,000 | ≥ 160 | 93.8% (84.9% \|—\| 98.3%) | 6.1% (1.7% \|—\| 15.0%) | — |

1:1,024 or less were found for 77% of reactive sera and for 69% of IF-IgM positive sera.

When correlations between test titers in both serum groups were investigated, clear differences were found. An equivalence between HA and IF-IgG titers was frequently observed in the reactive serum group, but in the IF-IgM positive group, HA test titers were in general much lower than IF-IgG titers, as became evident when comparing agreement percentages between IF-IgG and "equivalent" HA titers, as indicated in Tables IV and X. A comparison between CF and IF-IgG titers also disclosed differences, as can be observed in Tables VI and XI. In IF-IgG positive sera, CF titers were in most cases higher than the expected "equivalent" values observed for the reactive serum group, especially when IF-IgG titers of 1:4,000 or 1:8,000 were considered.

The described differences could be observed also when distributing IF-IgM positive sera according to titers in the other tests. Percentages increased steadily with increasing IF-IgG and CF titers, but not with HA titers (Table VII).

In this way, 2 serologic patterns could be distinguished corresponding to different titer distributions in both groups of sera (Figs. 1 and 2). The first one, related to recent infections, was characterized by high IF-IgG and CF titers, low HA titers and presence of IgM antibodies. The second pattern, found in old infections, showed a negative IF-IgM test, low IF-IgG and HA titers usually under 1:4,000 and presenting similar values, and a CF titer not exceeding 1:80 or even negative.

As a common rule, tests with antigens related mainly to less soluble parasitic wall components, as the immunofluorescence and complement fixation tests, usually showed an earlier rise in titers than the HA-test, in which more soluble antigenic cytoplasmic constituents are involved and a longer time seems to be necessary for a full antibody response to develop.

## RESUMO

*Significado diagnóstico de testes sorológicos na toxoplasmose humana. I — Estudo comparativo entre testes de hemaglutinação, fixação do complemento e imunofluorescência anti-IgG e anti-IgM, em 3.752 soros*

CAMARGO, M. E.; LESER, P. G. & LESER, W. S. P. — Diagnostic information from serological tests in human toxoplasmosis. I — A comparative study of hemagglutination, complement fixation, IgG- and IgM-immunofluorescence tests in 3,752 serum samples. *Rev. Inst. Med. trop. São Paulo* **18**:215-226, 1976.

Diferentes testes sorológicos para a toxoplasmose, as reações de hemaglutinação, fixação do complemento, imunofluorescência anti-IgG e imunofluorescência anti-IgM, foram aplicados a 3.752 soros humanos, comparando-se, em seguida, os resultados obtidos. Observou-se maior sensibilidade para o teste IF-IgG, positivo em 54,8% dos soros. Os índices de co-positividade em relação a IF-IgG, de 0,975 para HA e 0,704 para CF, traduzem as sensibilidades destes testes.

Encontraram-se anticorpos IgM anti-toxoplasma em 5,3% dos soros, correspondentes a 9,7% das amostras reagentes.

Quanto a títulos, valores considerados como baixos foram predominantes. Observou-se freqüente correspondência entre títulos dos diferentes testes nas amostras de soro, tendo sido possível estabelecer para os testes de HA e CF níveis de títulos "equivalentes" para cada valor observado para o teste IF-IgG.

Para soros IF-IgM positivos, os títulos de IF-IgG e FC foram, em média, significantemente mais elevados do que para soros IF-IgM negativos, com títulos CF ultrapassando os níveis "equivalentes". Ao contrário, os títulos HA mostraram-se muito inferiores aos "equivalentes". Os dados permitiram distinguir dois perfis sorológicos diferentes, que parecem relacionados respectivamente com infecções antigas e recentes.

O primeiro apresenta títulos semelhantes para IF-IgG e HA, de até 1:4.000, CF até 1:80, mas com freqüência negativo, e IF-IgM negativo. O segundo perfil é caracterizado por IF-IgM positivo, títulos CF acima de 1:80, IF-IgG acima de 1:4.000, porém com HA em baixos níveis, de 1:4.000 ou menos.

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