

Specificity of the *Treponema pallidum* haemagglutination test*

Analysis of results

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SUMMARY The automated haemagglutination assay using *Treponema pallidum* antigen (AMHA-TP) and the Venereal Disease Research Laboratory (VDRL) test were used to examine 330 163 sera. Reactive results were checked by the fluorescent treponemal antibody-absorption (FTA-Abs) test. When isolated reactivity or non-reactivity in the AMHA-TP test was investigated an estimated margin of error of 0.07% probably wrongly non-reactive and 0.008% presumably false non-reactive results was found. These figures were confirmed by randomised FTA-Abs tests on 504 sera with repeat AMHA-TP tests. The latter is therefore still the most reliable and practicable method for mass screening for syphilis.

Introduction

The *Treponema pallidum* haemagglutination assay (TPHA) is at present the most specific method for the detection of antibodies against *T. pallidum*.¹ There is no difference between the results of the TPHA and the automated microhaemagglutination assay using *T. pallidum* as antigen (AMHA-TP)²; the latter is much more economical and suitable for mass screening.

The sensitivity of the test in early infections depends on the IgM-binding capacity of the reagents, which may vary between different kits from the same manufacturer. The IgG reactivity is usually very sensitive and therefore the AMHA-TP test is used for screening in routine serology for syphilis.

The determination of the margin of error of the haemagglutination method is consequently of major importance. A previous study³ found that 2262 (0.45%) of 503 848 samples were reactive in the AMHA-TP but non-reactive in the fluorescent *T. pallidum* antibody-absorption (FTA-Abs) and in the Venereal Disease Research Laboratory (VDRL) test. Eighty-five (0.02%) sera were non-reactive in the AMHA-TP test but reactive in the FTA-Abs and

VDRL tests. The specificity of isolated reactive AMHA-TP results could not then be investigated, but the evidence suggests that at least 20% of these 2262 patients had once had an infection with *T. pallidum*. This paper presents a further evaluation of the probability of the occurrence of false reactive and false non-reactive AMHA-TP results.

Material and methods

The computerised data of all reactive sera submitted for serological tests for syphilis between 1 January 1976 and 31 December 1979 were listed and the results compared with any history of syphilis given on patients' request forms for serological tests. Five hundred and forty-four sera from inpatients of the Krankenhaus der Stadt Wien-Lainz were investigated further as repeat tests could be performed and additional information collected.

All the 330 163 samples were examined by the AMHA-TP and the VDRL tests; reactive results in either of these were checked by the FTA-Abs test. The techniques used were those described.^{1,2}

Five hundred and four sera were selected at random from the daily intake and examined by the FTA-Abs and the AMHA-TP tests.

Results

The results of the serological tests are shown in the table. One or more tests gave a reactive result for 8988 sera, and of these 2572 (28.62%) were from

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TABLE Serological test results for syphilis

Test results			No of sera	No of patients with history of syphilis	% of reactive samples
AMHA-TP	FTA-ABS	VDRL			
R	NR	NR	1708	397	23·24
R	R	NR	5043	1462	28·99
R	R	R	1684	642	38·12
R	NR	R	57	21	36·84
NR	R	R	34	19	55·88
NR	NR	R	462	31	6·71
Total			8988	2572	28·62
NR	ND	NR	321 175	NA	
Grand total			330 163		

R = reactive; NR = non-reactive; ND = not done; NA = not available

patients with a history of syphilis. Data on the 321 175 specimens with negative AMHA-TP and VDRL test results have not yet been computerised, and the number with a possible history of syphilis in this group is not known.

Of the 1708 samples with isolated AMHA-TP reactivity 542 came from inpatients of the Krankenhaus der Stadt Wien-Lainz; 171 of these patients could not be re-examined but repeat tests were performed on the sera of 371 patients, 207 (55·79%) of whom gave a history of syphilis on further inquiry. The sera of 107 patients were consistently reactive in the AMHA-TP test while the FTA-ABS test was reactive at least once in a series of tests on different samples from the same donor. Specimens from 32 patients were repeatedly reactive in the AMHA-TP but non-reactive in the FTA-ABS test. These patients were reinterviewed and six then admitted to a history of syphilis while 26 said they could not remember. Twenty-five patients had at least one reactive and one non-reactive AMHA-TP result and consistently non-reactive results in the FTA-ABS test; none had a history of syphilis. Thus of the 371 sera 320 (86·25%) came from patients who had had an infection with *T pallidum*, while false AMHA-TP reactivity could not be excluded in the sera of 52 (13·75%).

Eight of the 34 patients whose sera were non-reactive in the AMHA-TP but reactive in the FTA-ABS and VDRL tests could not be traced for further investigations. Eight of the remaining 26 patients had primary syphilis and the AMHA-TP test became reactive after the FTA-ABS and the VDRL tests. Six patients' sera were reactive at least once in the AMHA-TP on subsequent occasions, and all gave a history of syphilis. The sera of 10 patients were repeatedly reactive in the FTA-ABS but non-reactive in the AMHA-TP test. Five had a history of syphilis; one of these had a secondary eruption but had a blocking factor which inhibited haemagglutination. The test was reactive after elimination of this substance, which has so far been discovered only in the

serum of this patient. The blocking factor consists of low-molecular-weight protein, whose chemical structure is not yet known.

Sera from two patients were reactive at least once in the AMHA-TP test on later occasions. Both patients denied ever having had syphilis. This gives a total of 19 (73%) of 26 sera from patients who had or probably have had an infection with *T pallidum* but whose sera were non-reactive in the AMHA-TP test. Seven (27%) of the 26 samples were probably non-syphilitic in origin.

Of the 504 randomised sera 480 were non-reactive in the AMHA-TP test; in the FTA-ABS 475 were non-reactive, four gave borderline results, and one (0·2%) was reactive. The AMHA-TP test was reactive in 24 sera; 21 were also reactive in the FTA-ABS test but three (0·6%) were non-reactive. One of these patients admitted to a history of syphilis and one, who had rheumatoid arthritis and a carcinoma with metastases, could not remember. This reduces the percentage of possible non-specific AMHA-TP results to, at most, 0·4%. The correlation between the FTA-ABS and AMHA-TP test results was 99·2%, whether or not borderline results are included.

Discussion

The percentage of patients with a history of syphilis is lower among the donors of sera showing an isolated reactivity in the AMHA-TP test compared with those showing reactive results in the AMHA-TP and the FTA-ABS tests and a non-reactive result in the VDRL test. The highest incidence of patients who remembered a former infection was among the 1684 whose sera gave reactive results in all three tests. The AMHA-TP test only was reactive in 23·24%; the AMHA-TP and FTA-ABS tests were reactive and the VDRL test negative in 28·99%; and all three tests were reactive in 38·12%. This gradual increase may be explained by the fact that reactivity ceases first in the VDRL test after adequate treatment (or

spontaneous cure) while the FTA-ABS result changes much later and the TPHA reverts to non-reactivity very late or never.

Reactivity in the AMHA-TP and the FTA-ABS tests indicates a former infection with *T pallidum*. However, less than one-third (31·28%) of the patients studied were reported to have had a history of syphilis. It can hardly be assumed that 68·72% of the donors had never known of a previous infection or had never noticed any symptoms of syphilis before their present examination. This seems to indicate that the record of a history of syphilis on the request forms accounts for only a small percentage of patients with a previous infection.

The evaluation of 371 patients with an isolated reactivity in the AMHA-TP test showed that 320 (86·25%) had a history or serological evidence of a previous syphilitic infection. Only 26 (7·01%) could not remember or denied having a history of syphilis. The reliability of these statements has been discussed above. Twenty-five (6·74%) gave non-reactive AMHA-TP test results at least once on later occasions. Several of these patients might have had a treponemal infection long ago and were just on the verge of permanent reversal of the AMHA-TP reactivity. False reactivity in the AMHA-TP test can therefore not be excluded in about 13·75% of the sera showing an isolated reactivity in this test. The actual figure is most probably much smaller but this cannot yet be proved.

The number of samples which were reactive in the AMHA-TP and the VDRL tests but non-reactive in the FTA-ABS test is very small (57). The percentage of donors with a history of syphilis (36·84%) is very similar to that of donors with serological evidence of a former syphilitic infection (31·28%). Further investigations on this point will be carried out.

Four hundred and sixty-two sera showed an isolated reactivity in the VDRL test; 31 (6·71%) of these patients gave a history of syphilis but could not be traced for further investigation. Most of these results in this group were probably biological false-positive results. Nevertheless, the sera of patients with an isolated VDRL reactivity and a history of syphilis need further consideration.

Special interest was focussed on the group of 34 sera which were non-reactive in the AMHA-TP but reactive in the FTA-ABS and VDRL tests, of which 26 could be reinvestigated. The non-reactive AMHA-TP results of eight patients with primary syphilis were due to the regrettable fact that the TPHA reagents became less sensitive for the detection of early infection. The manufacturers (Fujizoki) have been informed of this and are about to provide an antigen with improved IgM-binding capacity. A further 11

patients gave a history of syphilis. The sera of five of the seven who denied ever having had the disease were consistently non-reactive in the AMHA-TP but reactive in the FTA-ABS test, which might have been due to a false reactivity in the fluorescence test. The sera of two patients were repeatedly non-reactive in the AMHA-TP and at least once non-reactive in the FTA-ABS tests on several occasions. This may be because the FTA-ABS test tended towards non-reactivity in patients who had been infected and effectively treated (or spontaneously cured) many years ago; this, however, could not be proved. Thus 19 (about 73%) of the 26 sera with isolated non-reactivity in the AMHA-TP test were from patients with early syphilis or from persons with a history of previous treponemal infection; seven (27%) were probably non-syphilitic. These figures suggest that this pattern of results occurs very rarely.

Examination of the 504 randomised samples by the FTA-ABS test indicates an isolated AMHA-TP reactivity rate of 0·6%, which agrees with the results in the table (0·52%). The donor of the single sample showing a reactive FTA-ABS but non-reactive AMHA-TP result could not be traced. The discrepancy might be due to non-specific FTA-ABS reactivity or a false non-reactivity in the AMHA-TP test. Further investigations are being carried out.

In sera which gave reactive AMHA-TP but negative FTA-ABS and VDRL test results, 13·75% of the reactive AMHA-TP results were estimated to be non-specific. If this estimate is applied to the 1708 sera which showed this pattern (table), 235 (or 2·6% of the sera reactive by any test) gave false-positive AMHA-TP results. In sera which gave the pattern AMHA-TP negative, FTA-ABS and VDRL reactive, the AMHA-TP test result was considered to be falsely negative and so failed to detect syphilis in 73% of this group (or 0·28% of the sera reactive by any test). If these estimates are applied to the total 330 163 sera screened, the AMHA-TP test gave approximately 0·07% false-positive and 0·008% false-negative results. It is therefore still the most reliable and practical test for mass screening for syphilis.

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