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# *Treponema pallidum* haemagglutination test for syphilis
## *Comparison with the TPI and FTA-ABS tests*

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The *Treponema pallidum* haemagglutination (TPHA) test, the latest to be developed in the group of specific tests for syphilis, has been described by Tomizawa and Kasamatsu (1966), Rathlev (1967), Tomizawa, Kasamatsu, and Yamaya (1969), and Tringali (1970.) An automated microhaemagglutination technique, the AMHA-Tp test, has been evaluated by Cox, Logan, and Norins (1969) and Logan and Cox (1970). The results of the majority of these authors indicate that the TPHA test, carried out either by a manual or by an automated method, has, in general, a favourable specificity and sensitivity when compared with the *Treponema pallidum* immobilization (TPI) and fluorescent treponemal antibody absorption (FTA-ABS) tests in the various stages of syphilis.

Of the specific tests for syphilis, the TPI test is limited to a few laboratories because of the expense involved and the highly trained staff required to carry it out. On the other hand the FTA-ABS test can be performed in any laboratory with a suitable fluorescence microscope and staff trained in its use. As with the TPHA test, all the reagents for the FTA-ABS test are available commercially, but certain problems are involved such as borderline reactions and non-specific reactive test results due to failure of the sorbent to remove all group antibody from a proportion of sera (Király, Jobbágy, and Kováts, 1967). The TPHA test is easier both to perform and to read than either the TPI or the FTA-ABS. These reasons alone make it attractive as a substitute for the more complicated and initially expensive FTA-ABS test in laboratories where it is desired to carry out a simple specific test for syphilis.

This study was undertaken to compare the results of the TPHA test with those of the TPI and FTA-ABS tests on 'problem' sera referred to the laboratory for TPI and FTA-ABS testing. Problem sera are those in which the referring laboratories have obtained reactive screening tests for syphilis or in which the history, clinical signs, and serological test results conflicted.

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### Material and methods

A series of 793 problem sera was tested by the TPI, FTA-ABS, and TPHA methods. In all cases in which there was a discrepancy in test results, the tests were repeated to exclude technical error.

The TPHA test was carried out by a manual micro-method. The volumes of serum and reagent used were those recommended by the Venereal Disease Research Laboratory, Atlanta, in 'Automated Qualitative and Quantitative Micro-Hemagglutination Assay for *Treponema pallidum* Antibodies (AMHA-Tp), Provisional Technique, Modified October 20, 1970'.

The reagents used in the TPHA test were manufactured by the Fuji Zoki Pharmaceutical Company, Tokyo, Japan, and included absorbing diluent, not separate absorbent and test diluent.

Quantitative tests were carried out on 100 of the sera tested. The remaining sera were tested qualitatively.

Routine serological tests for syphilis were also carried out on each serum. These were a cardiolipin Wassermann reaction (CWR), a Venereal Disease Research Laboratory (VDRL) test, and a Reiter protein complement-fixation (RPCF) test.

### Results

Four sera were found to be unsuitable for testing in the haemagglutination test as they caused agglutination of both the sensitized and unsensitized sheep red blood cells. This report therefore concerns the 789 sera which were found to be suitable for testing.

The TPHA test results agreed with those of the TPI test in 758 sera (96·1 per cent.) and with those of the FTA-ABS test in 765 sera (97 per cent.). There was complete agreement between the results of the TPI, FTA-ABS, and TPHA tests in 746 sera

(94·6 per cent.) and in 43 there was some discrepancy between the results of these three tests.

The Table compares the results of the TPHA test with those of the FTA-ABS and TPI tests in the 789 problem sera tested. The 43 sera with discrepant results could be divided into five groups.

**(1)** Four sera gave a non-reactive TPHA test result and reactive FTA-ABS and TPI test results (Table, a). These comprised two from patients with penile sores (one positive for *T. pallidum* on darkground examination, and the other developing an early secondary rash), one from a patient with gout, and one from a blood donor.

**(2)** Eight sera gave reactive TPHA and non-reactive FTA-ABS and TPI test results (Table, b). Five of these sera were from patients who had been treated for syphilis; one was in the primary stage, and in the others the stage was not stated. Of the remaining three sera, one was from a man whose previous serological test results were non-reactive, but whose wife had been treated for syphilis, one from a patient with a penile sore, negative on darkground examination, whose serum gave a reactive RPCF test, and one from an antenatal patient whose serum was considered to show a biological false positive reaction in the CWR and VDRL tests. A quantitative TPHA test was carried out on these three sera, the titres being 1 in 80, 1 in 320, and 1 in 80 respectively.

TABLE *Results of TPHA, FTA-ABS, and TPI tests on* 789 *problem sera*

| *Test* | *Result* | *TPHA test results* | |
|---|---|---|---|
| | | *Reactive* | *Non-reactive* |
| FTA-ABS | Reactive | 325 | 4[a] |
| TPI | Reactive | | |
| FTA-ABS | Non-reactive | 8[b] | 421 |
| TPI | Non-reactive | | |
| FTA-ABS | Reactive | 19[c] | 9[d] |
| TPI | Non-reactive | | |
| FTA-ABS | Non-reactive | 3[e] | — |
| TPI | Reactive | | |
| Total | | 355 | 434 |

[a]Group 1
[b]Group 2
[c]Group 3
[d]Group 4
[e]Group 5

**(3)** Nineteen sera gave reactive TPHA and FTA-ABS test results and a non-reactive TPI test result (Table, c). Thirteen came from patients whose histories suggested primary syphilis—penile sore, *T. pallidum* found on darkground examination (4), treated primary syphilis (4), penile sore (4), and husband treated for primary syphilis (1). A further three sera were from patients who gave histories of treated congenital syphilis (1) and treated syphilis, stage not stated (2). The remaining three sera in this group came from patients who had no history or clinical signs of syphilis; each showed a 1+ fluorescence in the FTA-ABS test and a quantitative TPHA test on each gave a titre of 1 in 80. Two of these last three sera came from blood donors and each gave a reactive VDRL test result; the other serum was non-reactive in the routine tests.

**(4)** Nine sera gave non-reactive TPHA and TPI and reactive FTA-ABS test results (Table, d). Five were from patients with penile sores which were positive for *T. pallidum* on darkground examination. The other four showed a 1+ fluorescence in the FTA-ABS test and were non-reactive in the routine serological tests for syphilis; they came from patients whose histories were: penile sore darkground examination negative (1), myocardial infarct (1), and no history obtainable (2).

**(5)** Three sera gave reactive TPHA and TPI test results and a non-reactive FTA-ABS test result (Table, e). One serum came from an antenatal patient, one from a female with vulval warts, and one from a patient for whom no history was obtainable.

### BIOLOGICAL FALSE POSITIVE (BFP) REACTIONS

These were seen in 41 sera of the total group. Of these only one serum, discussed in Group 2, gave a reactive TPHA test result of low titre.

### CEREBROSPINAL FLUID (CSF)

In addition to the above sera, 38 samples of CSF were tested. The TPHA, FTA-ABS, and TPI tests were non-reactive in 36 and reactive in one. In the one remaining CSF sample, from a patient treated for neurosyphilis, the TPHA test was non-reactive and the FTA-ABS and TPI tests were reactive.

### QUANTITATIVE TPHA TESTS

These were carried out on the first 94 sera tested and on the six sera already discussed. Very little correlation was found between the CWR and TPHA titres and no standard could be decided on to establish what was a high TPHA titre. The quantitative testing of the sera was therefore discontinued in this survey.

## Discussion

It is difficult to compare a new test procedure with accepted tests as each presumably detects a different antibody and each has its known deficiencies: the TPI test fails to detect some 60 per cent. of cases of primary syphilis and sorbent in the FTA-ABS test will, in some sera, fail to remove all group antibody (Király and others, 1967). Problem sera, referred for specific tests for syphilis, rarely give straightforward classical serological test results. For this reason, and because of the practical application of the TPHA as a specific test for syphilis, this group of sera was chosen for our study.

The overall agreement between the TPI, FTA-ABS, and TPHA tests of 94·6 per cent., and the agreements between the TPI and TPHA tests of 96·1 per cent., between the FTA-ABS and TPHA tests of 97 per cent., and between the TPI and FTA-ABS tests of 95·7 per cent. can be considered to be satisfactory.

Our results showed that the overall specificity and sensitivity of the TPHA and FTA-ABS tests were equal. The specificity of the TPHA test was slightly less than that of the TPI test, while the sensitivity was slightly higher. These results are in general agreement with those of Tomizawa and others (1969), who found that the TPHA test was comparable to the TPI and FTA tests in sensitivity and specificity.

Among the 43 sera showing discrepancies, there were 21 from patients with primary syphilis. In these the FTA-ABS test was more sensitive than the TPHA test, and the TPI test was relatively lacking in sensitivity. A history of treated syphilis—1 congenital, 5 primary, and 6 stage not stated—was obtained from twelve patients in this group showing discrepancies. The TPHA test was reactive in all twelve of them, the FTA-ABS test in seven, and the TPI test in none. These numbers are too small for anything but speculation, but suggest that perhaps the TPHA test is more sensitive than either the FTA-ABS or TPI tests in detecting antibodies in cases of treated syphilis.

There was one reactive TPHA test result among the 41 sera classed as BFP reactions on the basis of non-reactive TPI and FTA-ABS tests and a complete absence of history or clinical signs of syphilis. This was probably a false positive TPHA test result. Two other sera gave reactive TPHA test results only. One of these may have been from a patient treated before his original testing in our laboratory as his wife was being treated for syphilis. The other serum in which the TPHA titre was 1 in 320 and the RPCF test only was also reactive, raises the question whether, in this particular serum, all the group antibody had been removed by the absorbing diluent.

No definite conclusions could be reached about the three sera in which the TPHA tests were reactive at a titre of 1 in 80 and the FTA-ABS tests showed 1+ fluorescence.

Approximately 1 in 200 of the group of sera tested showed agglutination of both the sensitized and unsensitized sheep red cells used in this test. This non-specific agglutination made these sera unsuitable for TPHA testing.

From the results obtained in our group of problem sera, it appears that the TPHA test has a place, much like that of the FTA-ABS test, among the specific tests for syphilis. There seems to be no reason to add to the specific tests already performed in a laboratory, because the more tests that are carried out, the more complex are the results and the more confusing the diagnosis.

The TPHA test is easy to perform, requiring neither highly skilled staff nor expensive equipment, and reliable reagents are available commercially. It therefore appears to be worth consideration in laboratories where it is wished to carry out a simple confirmatory specific test for syphilis. It must be remembered, however, as with the FTA-ABS test, that a certain number of sera will give results in the TPHA test the significance of which can only be determined by a TPI test result.

## Summary

The TPHA test for syphilis was carried out on 789 problem sera and the results were compared with those of the FTA-ABS and TPI tests. The agreement between the test results was: TPHA, FTA-ABS, and TPI 94·6 per cent.; TPHA and FTA-ABS 97 per cent.; TPHA and TPI 96·1 per cent.

The TPHA test was found to be equal in specificity and sensitivity to the FTA-ABS test. The specificity of the TPHA test in the group of problem sera was slightly less than and the sensitivity slightly greater than that of the TPI test.

The TPHA test appears to be an acceptable alternative to the FTA-ABS test in laboratories where a simple, not too expensive, confirmatory test for syphilis is required, but as with the FTA-ABS test, there will always remain a certain number of sera in which the final diagnosis of syphilis will depend on the TPI test.

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## Le test d'hémaglutination au *Treponema pallidum* pour la syphilis. Comparaison avec le TPI et l'épreuve FTA-ABS

SOMMAIRE

Le TPHA pour la syphilis fut pratiqué sur 789 sérums posant des problèmes et les résultats furent comparés avec les tests FTA-ABS et TPI. L'accord entre les résultats fut: TPHA, FTA-ABS et TPI 94,6 pour cent; TPHA et FTA-ABS 97 pour cent; TPHA et TPI 96,1 pour cent.

Le test TPHA fut trouvé égal en spécificité et sensibilité avec le test FTA-ABS. Dans le groupe des sérums posant des problèmes, et par rapport au TPI, le test TPHA fut légèrement moins spécifique et légèrement plus sensible.

Le test TPHA apparaît comme une alternative acceptable du test FTA-ABS dans les laboratoires qui recherchent, pour la confirmation de la syphilis, une épreuve simple et pas trop coûteuse mais, comme pour le test FTA-ABS, il restera toujours un certain nombre de sérums pour lesquels le diagnostic final dépendra du TPI.