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Treponemal haemagglutination test

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The introduction of the treponemal immobilization (TPI) test by Nelson and Mayer (1949) provided the first verification test for syphilis which proved fully acceptable to the clinician. After 20 years it is still accepted by a large body of opinion as the verification test of choice for the diagnosis of latent and late syphilis.

The test's characteristic of becoming positive towards the end of the primary or in the early secondary stage of the disease, after the classical tests and the Reiter protein complement-fixation (RPCF) test, requires that a further specimen be examined after a few weeks before the diagnosis of a biological false positive (BFP) reaction can be accepted.

The technical difficulties of the TPI test are numerous, many stemming from the need to use freshly-harvested living treponemes for each batch of tests. The introduction of the fluorescent treponemal antibody (FTA) tests removed this problem, but until the development of the FTA (Absorbed) test, these procedures lacked either sensitivity or specificity. While the FTA Absorbed (FTA-ABS) test is replacing the TPI test in many centres, the limiting factor in their application is that both are read microscopically.

The publication by Rathlev (1965, 1967) of a haemagglutination test utilizing pathogenic *Treponema pallidum* for the serology of syphilis, provided the first practical serological test using these organisms which could be read with the naked eye. It was decided to investigate this reaction, which was based on the agglutination of tanned formolized sheep erythocytes sensitized with a sonicate of washed Nichols strain *Treponema pallidum*. Experiments were carried out to define the conditions for the test and these confirmed Rathlev's findings, as did the work of Tomizawa and Kasamatsu (1966).

The results of the preliminary examination of the test were satisfactory, and it was decided to modify the test so that it could be applied to all specimens received by the laboratory. The need to absorb all specimens before testing was eliminated by replacing the sheep erythocytes with those from the fowl and adjusting the technique to allow integration with the routine of the laboratory. After analysis of the results obtained on about 8,000 specimens, the test was introduced into the routine of the laboratory in June, 1969, and reported from July, 1969.

This report is based on results obtained on 64,000 specimens received from July, 1969, to the end of March, 1970, supplemented by the experience of the test as a routine procedure on over 300,000 specimens. The direct comparison with the FTA-ABS test was carried out on 4,111 specimens selected from 84,000 consecutive requests received between October, 1971, and July, 1972.

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Material and methods

TREPONEMAL ANTIGEN

20 to 40 ml. quantities of azide saline were added to extraction flasks containing two or four orchitic testes of rabbits inoculated for the TPI test which had been obtained either after extraction of suspension for the TPI test or from any surplus animals. The flasks were placed on the Kahn shaking machine for 20 minutes and the fluid decanted and lightly centrifuged. The resulting supernatant was stored at 4°C. until a convenient volume was accumulated. The treponemes were deposited by centrifugation in an angle centrifuge at approximately 7,000 r.p.m. and the deposits re-suspended in azide saline. The suspension was again lightly centrifuged and the supernatant retained. The treponemes were further washed by centrifugation at 7,000 r.p.m. and re-suspended in fresh azide saline six times. The resulting buttons of treponemes were suspended in distilled water to give a density of Brown opacity standard 3, and disrupted in a water-jacketed vessel using a M.S.E. sonicator fitted with a 20 mm. diameter probe moving 7 μ at 20 kHs. for 10 minutes. Sodium azide was added to give a concentration of 1 in 1,000 and the resulting antigen stored at 4°C.

The titre was determined by sensitizing small quantities of formolized tanned cells with serial dilutions of antigen and setting them up against dilutions of positive and negative sera as a chess board titration. The working dilution of the antigen is that giving the maximum differentation between positive and negative sera and the correct titre of the positive serum. The majority of the batches have a working titre of about 1 in 40.

FORMOLIZED CELLS

Fresh citrated blood from adult fowls was washed in saline until the supernatant was clear and the fluffy white cell layer removed. The erythrocytes were then suspended as a 7 per cent. suspension in phosphate-buffered saline pH 7·2 (PBS 7·2). An equal volume of 7 per cent. w/v formaldehyde solution in PBS 7·2 saline was prepared, and one quarter of the volume was added to the cell suspension. The mixture was incubated with frequent shaking for 1 hr at 37°C. The remaining formaldehyde solution was then added and the suspension incubated for 24 hrs at 37°C., being shaken frequently during the first few hours. The cells were then washed ten times in saline and re-suspended as a 10 per cent. stock suspension in azide saline and stored at 4°C.

PRESERVED FOWL CELLS (prepared weekly)

The preservative used was made to the formula of Richardson (1941) as follows: Sodium tartrate 2·3 g. Formalin 36 per cent. v/v 0·3 ml. Normal saline to 100 ml. An equal volume of preservative was added to the packed washed fowl cells and the resulting suspension stored at 4°C. For use the cells are washed and re-suspended as a 5 per cent. suspension in saline.

STOCK TANNIC ACID SOLUTION

1 per cent. solution of B.P. grade tannic acid (Evans Pharmacuticals) in distilled water was diluted for use in saline to 0·005 per cent. (A batch of reagent grade tannic acid was found to be unsatisfactory).

POSITIVE CONTROL SERUM

This was pooled positive serum as used for the TPI test.

PREPARATION OF TEST AND CONTROL CELL SUSPENSIONS

Tanning

The required volume of 3·75 per cent. tanned cells was prepared by diluting the well mixed 10 per cent. stock suspension of formolized cells with the calculated volume of saline, adding an equal volume of 0·005 per cent. tannic acid, incubating for 15 minutes at 37°C. (shaking once during incubation), centrifuging, and washing twice, and re-suspending in pH 6·4 buffered saline to 3·75 per cent.

Test suspension

The tanned cells were sensitized by mixing 1 volume of cell suspension with 4 volumes of pH 6·4 buffered saline and 1 volume of antigen diluted to the required titre in pH 6·4 buffered saline, incubating for 30 minutes at 37°C. (shaking twice during incubation), centrifuging, and washing once in saline, and re-suspending in 4 volumes of rabbit serum saline (1·5 per cent. rabbit serum in saline).

Control suspension

The control cell suspension was prepared as above using pH 6·4 buffered saline in place of antigen dilution.

Screen test

As part of the procedure for preparing and distributing the serum dilutions for the cardiolipin Wassermann reaction (CWR) and the Reiter complement-fixation test using the multiple pipetting and diluting machine, 0·3 ml. volumes of a 1 in 30 dilution of serum in barbitone buffered saline (BBS) were placed in the wells of M.R.C. pattern plastic plates. At the same time that the antigens and complement dilutions were added to the complement-fixation tests, 0·2 ml. of the test suspension was added to the 0·3 ml. of the serum dilution, making a final serum concentration of 1 in 50. The plates were well shaken and placed in a single layer on the bench in the hot room (37°C.) and covered with a polythene sheet to reduce evaporation. The THA tests were read when the positive and negative controls showed maximum differentiation, that is after 30 to 45 minutes' incubation. It is important that the plates should not be disturbed during incubation. The THA test may also be carried out at room temperature, and at a total volume of 0·2 ml. by using equal volumes of 1 in 25 serum dilution and cell suspension adjusted to 0·75 per cent. in 1·2 per cent. rabbit serum saline. The test can also be carried out in microplates using commercially prepared reagents*.

Results were recorded as follows:

Negative	= A smooth ring or button of cells
Positive	= A thin granular deposit covering the entire bottom of the well
Weakly reactive	= A thin granular deposit on bottom of well surrounded by an irregular rough ring
Doubtful	= Intermediate appearance with an enlarged ring surrounded by a rough margin of cells. These specimens were re-tested quantitatively and reported as negative if the reactivity was less than 'Weakly reactive'.

Quantitative test

Sera selected for confirmatory testing and control sera were absorbed by adding 1·5 ml. 5 per cent. suspension of preserved fowl cells (or tanned fowl cells) to 0·05 ml. of serum, incubating for 1 hr at 37°C., and centrifuging the suspension.

0·7 ml. of supernatant was withdrawn from the tubes using the multiple pipetting and diluting machine and 0·3 ml. distributed into each of the first two rows of a M.R.C. pattern plate. The remaining 0·1 ml. was mixed with 0·3 ml. of BBS in the next row to prepare a 1 in 4 dilution of the supernatant and similarly in further rows to prepare 1 in 16 and 1 in 64 dilutions, the last 0·1 ml. being discarded. 0·2 ml. of control suspension was added to the first row and 0·2 ml. of the test suspension to the remaining rows. The plates were shaken and placed in the hot room as above.

During the latter part of this study, it was found that this absorption procedure was unnecessary, and a 1 in 30

*Wellcome Reagents Limited, Beckenham, Kent, BR3 3BS

dilution of serum in BBS was used in place of the supernatant.

Titres are expressed in terms of the dilution of serum used in the screen test, *i.e.* positive neat, 1 in 4, 1 in 16, or 1 in 64. If the control cells did not settle to a button, the test was reported as 'No valid result.'

SPECIMENS EXAMINED

Where technically possible, the CWR and the RPCF test using the Whitechapel technique, and the THA test were performed on all specimens received between July, 1 1969, and March 30, 1970. TPI tests were carried out on selected specimens where required to clarify or confirm the diagnosis and, in general, on demand.

For analysis, patients were classified by the department attended as General Hospital, Antenatal, Special Clinic, and Others when the specimens were received for routine testing, and as Reference Laboratory when they were submitted by other laboratories for confirmation of their results. Because of the small numbers of patients classified under 'Others' these have been excluded. The numbers of specimens in each group and the age distribution of the patients are shown in Table I.

TABLE I *Material examined classified by sex and department of origin*
Age distribution shown as percentages of each group

Decade of birth	*Special clinic*		*Ante-natal clinic*	*General hospital*		*Reference laboratory*	
	Male	*Female*		*Male*	*Female*	*Male*	*Female*
Before 1890				2·3	0·3	1·0	1·0
1890	0·3	0·5		6·8	2·2	5·8	3·6
1900	1·4	1·2		16·7	6·7	13·2	5·7
1910	4·0	2·0		18·8	10·2	16·2	6·5
1920	9·8	5·5	2·0	18·9	11·9	15·7	6·7
1930	24·6	12·7	20·3	14·3	13·4	17·8	15·4
1940	49·3	47·4	63·4	14·3	15·2	23·4	45·0
1950	9·7	28·6	14·0	4·2	29·0	4·1	13·9
1960	0·8	2·1	0·3	3·6	8·7	2·8	1·6
1970	0·1			0·1	2·4		0·6
Total requests	8,932	3,361	35,291	6,399	8,406	487	695
Sex not recorded	296			327		266	
Grand total	12,589		35,291	15,132		1,448	

DATA PROCESSING

The reports and records of the laboratory are prepared on International Computers Limited 40-column punch-card equipment.

When one or more tests gave a positive reaction or a TPI test was carried out, the test results and clinical findings were coded and punched on a summary card which is included in the record. The summary card generates an entry for the cumulative patient file which includes all patients diagnosed as cases of syphilis or yaws since June, 1963. The summary cards are analysed to provide the data required, while the number of requests in each group is provided by analysis of the patient detail cards which form the first line in each report.

Results and discussion

The sensitivity of the test was established by analysis of results found in Special Clinic patients where a diagnosis of syphilis was entered on the request form. In Table II these patients are classified by treatment status and stage of disease. Because of the frequent use of the term 'Early Syphilis' to denote the primary and secondary stages, patients are classified as 'Acquired infections diagnosed within one year of infection', shown as 'Acquired < 1 year'. Acquired infections diagnosed more than one year after the date of the infection are shown as 'Acquired > 1 year'. 'Congenital infections' are listed and cases of syphilis in which the stage of the disease was not indicated are shown as 'Stage Unspecified'. Excluded from the Tables are 58 patients in whom the treatment status was not indicated and 28 diagnosed as cases of yaws.

Among the untreated patients, it will be seen that the CWR was the most sensitive test in infections of less than 1 year's duration, and was as sensitive as the THA test in the 'Stage Unspecified' group. The THA test was the most sensitive test in the remaining groups, and in all groups of treated patients. The high sensitivity of the THA test in treated as compared with untreated early infections is remarkable, although foreshadowed in the preliminary surveys, and found by other workers when untreated and treated patients are classified separately, as in the reports of Logan and Cox (1970) and Cox, Logan, and Stout (1971), who used the technique of Tomizawa and Kasamatsu (TPHA test). Tringali (1970) found the TPHA test slightly less sensitive than the FTA-ABS test but more sensitive than the Kolmer 1/5 vol. test in untreated patients with primary syphilis. This is in contrast with our findings where the Whitechapel CWR was more sensitive than the FTA-ABS test in these patients. The overall sensitivities of the THA, CWR, and RPCF tests, are shown in Table III, in which the results for untreated and treated patients have been combined. The sensitivity is calculated by summing the number of positives and weakly reactive results and expressing

TABLE III *Sensitivity of routine tests in treated and untreated patients, showing percentage of groups with positive or weakly reactive results*

Diagnosis	*THA test*	*CWR*	*RPCF test*
Acquired syphilis			
< 1 year	83	47	55
> 1 year	98	72	83
Congenital syphilis	98	86	58
Stage unspecified	94	67	68
All patients	92	64	65

TABLE II *Test results in untreated and treated syphilis*

Test		Untreated					Treated				
		Acquired					Acquired				
		<1 year	>1 year	Congenital	Stage unspecified	Total	<1 year	>1 year	Congenital	Stage unspecified	Total
THA	+	12	6	4	8	30	73	59	35	150	317
	±	6	0	0	1	7	11	3	2	6	22
	−	7	0	0	2	9	12	3	1	9	25
CWR	+	20	4	2	7	33	29	43	31	99	202
	±	1	0	0	1	2	5	3	1	9	18
	−	4	2	2	3	11	62	19	6	57	144
RPCF	+	15	1	2	4	22	33	39	14	92	178
	±	4	5	0	2	11	15	15	9	21	60
	−	6	0	2	5	13	48	11	15	52	126
Totals		25	6	4	11	46	96	65	38	165	364

+ = Positive, ± = Weakly reactive, − = Negative

this as a percentage of the total number of patients in the group.

The specificity was assessed by analysis of results in antenatal patients. Infected patients were excluded from this group on the basis of information obtained from the clinician, departmental records, and TPI tests.

It will be seen from Table I that the age distribution of the Special Clinic patients used for the assessment of sensitivity of the test is similar to that of the antenatal patients. Table IV shows the positive and weakly reactive THA test results and those of the TPI test carried out on the antenatal group. On the basis of the TPI test results, the 71 patients with positive reactions and the fifteen with weak reactions may be considered as having had syphilis or yaws. Also there were fourteen patients with a positive THA test on whom a TPI test was not carried out, who were diagnosed as having had treponemal infections on clinical grounds or on the basis of the CWR and RPCF test results. Examination of the laboratory records allowed the elimination of eighteen of the 89 discrepancies, ten from duplication of specimens, and eight from records of previous positive TPI test results or a diagnosis of syphilis.

TABLE IV *THA test results in antenatal patients Negative agreements include patients with BFP reactions*

TPI test	THA test			Total
	+	±	−	
+	62	4	5	71
±	14	0	1	15
−	49	34	185	268
Not valid	1	1	1	3
Total	126	39	192	357

Four of the six patients with negative THA test results and reactive TPI tests were found to have had treated syphilis. The FTA-ABS test was subsequently carried out on fourteen of the remaining sera giving discrepant results and was positive in eight. If these patients are excluded, 26 positive and 33 weakly reactive THA tests remain unexplained out of a total of about 35,000 requests. This corresponds to a specificity of over 99·9 per cent. for positive reactions, and 99·8 per cent. for positive and weakly reactive reactions combined.

On the basis of the results presented, the THA test combines high sensitivity in treated and untreated syphilis (except in primary and early secondary stages) with high specificity. The characteristics of the test are therefore similar to the TPI test, both in relatively late appearance of antibody and persistence of positive reactions after treatment, but the latter to a considerably greater degree; it therefore may be expected to share many of the advantages and disadvantages of the TPI test.

The performance of the THA test on specimens from General Hospital Departments and those sent from other laboratories is shown in Table V (overleaf). TPI tests were carried out on 892 of the 15,132 specimens from General Hospital Departments. Of these, the TPI test was not carried out on 188 on which the THA test was positive or weakly reactive. This group included patients in whom the diagnosis of syphilis or yaws was considered to be established on serological or clinical grounds.

The distribution of titres between the positive, weakly reactive, and negative TPI test results suggest that the antibodies detected by the two tests are similar. The THA test has a sensitivity about two dilutions greater than that of the TPI test, and this is supported by the results obtained using the TPI

TABLE V *Comparison of THA and TPI test results of General Hospital and Reference Laboratory specimens*

	General hospital (TPI test)					*Reference laboratory (TPI test)*				
THA test titre	+	±	−	*O or N.V.*	*Total*	+	±	−	*O or N.V.*	*Total*
64	16	1	1	41	59	55	2	4	18	79
16	59	2	8	55	124	94	4	10	23	131
4	80	21	54	60	215	135	22	40	30	227
Neat	27	13	81	22	143	41	11	41	16	109
+ no titre	1	1	2	4	8	0	0	0	3	3
±	7	8	44	7	66	11	4	21	4	40
Negative	10	2	454	14,051	14,517	17	18	746	78	859
Totals	200	48	644	14,240	15,132	353	61	862	172	1,448

Note Disagreement was found in 190 and 116 patients when the TPI test was negative, and in 12 and 35 patients when the THA test was negative
O or N.V. = Test not carried out or result not valid

test positive control serum. It is therefore not ununexpected that discrepancies will occur where the TPI test is negative and the THA test is positive. However, the finding of patients with a negative THA test and positive TPI test indicates that the antibodies detected are not identical. Where reliable data was available, these patients were found to have fully treated syphilis or yaws.

The pattern of results found with the 1,448 Reference Laboratory specimens is similar to that of the General Hospital patients except that a very large proportion of the non-reacting specimens have been excluded by the selection process.

FTA-ABS tests were carried out on 257 sera during the latter part of the study. In general, these were selected because there were discrepancies between the results of the serological tests, or between the test results and clinical findings. The results of the THA, FTA-ABS, and TPI tests are shown in Table VI. The total column in the upper part of Table VI allows direct comparison between the TPI and FTA-ABS tests, while the total sections in the lower part of the table present the direct comparisons between these tests and the THA test. It will be seen that, while discrepancies of all types occur, agreement is greatest between the FTA-ABS and THA tests. The low level of agreement between the TPI and THA tests is at least in part due to the selection of sera for FTA testing because the former tests gave discrepant results. Where clinical information was available, discrepancies between the tests again appeared to occur in cases of adequately treated syphilis.

Table VII shows the results found in the 33 patients included in Table VI in whom a diagnosis of syphilis was entered on the request form. The high sensitivity of the THA test and the low sensitivity of the TPI test in this group is largely due to the inclusion of a large proportion of patients with treated early syphilis. The group contained one patient with untreated primary syphilis who was not detected by any of the three tests, but whose serum gave positive results with the CWR, PPR, and RPCF tests.

TABLE VI *Comparison of results of THA, FTA-ABS, and TPI tests*

			THA			
Test	*TPI*	*FTA*	+	±	−	*Total*
	+ ±	+ ±	65	3	13	81
	+ ±	−	8	5	4	17
	−	+ ±	68	7	12	87
	−	−	9	6	57	72
Total	TPI	+ ±	73	8	17	98
	TPI	−	77	13	69	159
Total	FTA	+ ±	133	10	25	168
	FTA	−	17	11	61	89
Grand totals			150	21	86	257

Note The use of the TPI test as the principal reference test excluded the majority of specimens in which the THA and TPI tests were in agreement.

TABLE VII *Sensitivity of TPI, FTA-ABS, and THA tests in patients included in Table VI in whom a diagnosis of syphilis was already established*

		Acquired				
Test		*<1 year*	*>1 year*	*Congenital*	*Stage unspecified*	*Total*
THA	+ ±	9	5	8	5	27
	−	2	1	1	2	6
FTA	+ ±	6	5	7	1	19
	−	5	1	2	6	14
TPI	+ ±	0	4	4	0	8
	−	11	2	5	7	25
Total		11	6	9	7	33

Table VIII shows the results of the THA and

TABLE VIII *Comparison of THA and FTA-ABS tests*

Results found when the THA test was carried out on about 84,000 *unselected specimens with the FTA-ABS test as the reference test*

	FTA test			
THA test	+	±	−	*Total*
+	1,002	364	105	1,471
±	146	155	116	417
−	131	156	1,936	2,223
Total	1,279	675	2,157	4,111

FTA-ABS tests when the latter was used as the principal reference test from October, 1971, to July, 1972. During this period, no absorption procedures were carried out for the THA test and invalid results were only encountered in two per thousand uncontaminated sera. The 4,111 specimens were selected from about 84,000 requests because reactions were found with one or more of the routine tests (CWR, RPCF, THA) or the clinical findings recorded on the request form suggested a diagnosis of syphilis, or in general on request by the clinician. The group included a proportion of treated patients. The tests agreed in 3,603 patients (88 per cent.). The 1,936 patients with negative THA and FTA-ABS tests included 1,898 in whom both the CWR and RPCF test gave valid results. In 693 the CWR was positive or weakly reactive, corresponding to a rate of 0·8 per cent. and in 174, the RPCF test was positive or weakly reactive, a rate of 0·2 per cent. In four patients, the CWR and RPCF test were both positive. These included two patients with untreated primary syphilis confirmed by dark-field examination. In all, twenty patients were found to have positive or weakly reactive CWRs and RPCF tests, a rate of about 1 per 4,000.

In the 221 and 287 patients in whom a discrepancy was found between the THA test and FTA-ABS test, a diagnosis of syphilis had been established before the examination of the specimens in 18 per cent. of both groups. The FTA-ABS test was more sensitive in untreated early acquired and treated congenital syphilis, detecting fourteen cases compared with one, and six cases compared with none respectively. It will also be noted that the specificity of the THA test in this unselected material is virtually dentical to that found in the antenatal group.

The 1,667 specimens with positive or weakly reactive FTA-ABS and THA tests included 424 (25 per cent.) in whom the CWR and RPCF tests were negative and would therefore not have been detected without the use of the THA test as a routine. The additional patients detected by the THA test included seventy who were said to have been treated and 113 who were recorded as untreated, although further investigation provided a history of treatment for syphilis or yaws, perhaps 20 or more years before, in a proportion of cases.

Conclusion

The general impression of the test system consisting of the THA, CWR, and RPCF tests has been of great sensitivity in the detection of treponemal disease, treated and untreated, with the reproducibility of the THA test equal or superior to that of the complement-fixation tests. The rate of non-specific reactions given by the THA screen test using unabsorbed sera was about two per thousand, but was considerably higher with plasma and infected specimens. Partial prozones were not uncommon but were invariably associated with strongly positive results of the complement-fixation tests. The CWR and RPCF tests were repeated and a quantitative THA test were set up on all specimens giving a positive or weakly reactive THA screen test, or where a reactive complement-fixation test was found which did not confirm the results on a previous specimen, and on selected specimens giving negative results with all tests where the clinical findings suggested syphilis. In all, over 300,000 specimens have now been examined.

The formal estimation of the specificity of the THA test as 99·8 or 99·9 per cent. may be regarded as an underestimate, as careful investigation of the remaining discrepant results generally, though sometimes with difficulty, revealed a history of treated syphilis, often a congenital infection treated in childhood, or of treated yaws.

The association of a positive CWR and RPCF test and a negative THA test was found with such regularity in patients with untreated primary and early secondary syphilis as to allow a presumptive diagnosis when this pattern of results was found, especially if supported by a positive FTA-ABS test. The late appearance of THA antibody may allow the demonstration of a rise in titre in some patients with early syphilis even if treatment has been given.

The finding of a positive THA test and negative CWR, RPCF, and TPI tests was frequently associated with a final diagnosis of treated treponemal disease, perhaps 20 or more years before. In the cases of men, this was often early syphilis treated in the Armed Forces during the second world war. In women it was not uncommon in multipari who, after a number of uneventful pregnancies, were discovered to have been treated for congenital syphilis or yaws in childhood. The THA test is therefore of particular value in the diagnosis of latent, late, and congenital

syphilis, and its persistence after treatment may be of value in epidemiological studies. The test is not suitable for the diagnosis of congenital syphilis in the infant, as positive reactions due to maternal antibody may persist for up to about 6 months after birth.

In general, the behaviour of the THA test is similar to that of the TPI test but with a higher sensitivity and even greater persistence of positive reactions after treatment. Its application differs from the TPI and FTA tests in that it is technically possible to carry out the THA test on all specimens received, although this is not necessarily desirable. The use of the CWR and RPCF tests with the TPI or FTA test for confirmatory purposes has proved highly successful in the detection of latent syphilis, and the last 10 years have seen a continuing fall in the incidence of late symptomatic syphilis. This has been reflected in the number of positive laboratory findings in specimens of cerebrospinal fluid submitted for examination, which has fallen by more than two-thirds. It is therefore only the exceptional patient with active syphilis who may be missed by the established methods; a far greater cause of error is perhaps the deviation of technical standards and human and mechanical errors. The addition of the THA test as a routine will reveal a large number of patients fully treated for treponemal disease, followed up for the recommended period, and discharged as cured, in addition to the defaulter and the occasional case which is missed by conventional test systems. This phenomenon was experienced when the cardiolipin antigens came into use and caused considerable unnecessary distress to patients through misinterpretation. Provided serological test results are regarded by the clinician as data and not as diagnosis and so long as the incidence of treponemal disease in the community is low, the routine use of the THA test may provide the clinician with important information about a patient or a patient's contact in circumstances which the laboratory cannot assess. The carrying out of the THA test on all specimens with reactive or discrepant standard tests and on specimens from patients presenting with clinical evidence suggesting late or congenital syphilis is strongly recommended, as it allows the immediate presumptive identification of biological false positive reactions and treponemal infections using very simple equipment with a precision not hitherto attained except by the use of the TPI or FTA tests.

Summary

A treponemal haemagglutination (THA) test for syphilis, using tanned formalised avian R.B.Cs sensitized with a sonicate of Nichols strain *Treponema pallidum*, is described. Except in cases of untreated early syphilis, the THA test was found to have a sensitivity of over 90 per cent. in untreated and treated syphilis when assessed in patients diagnosed at Special Clinics. The specificity of the THA test was found to be over 99·9 per cent. in 35,000 antenatal patients when those with a history of treponematosis or with reactive TPI or FTA-ABS tests were excluded.

The results of over 65,000 requests in which the THA test was carried out as a routine are summarized. These findings were confirmed by the results of over 4,000 FTA-ABS tests carried out on specimens selected from a further 84,000 patients on the basis of the THA test result, the clinical findings, or the results of the cardiolipin Wassermann reaction or Reiter protein complement-fixation test.

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Test d'agglutination tréponémique

SOMMAIRE

On décrit un test d'hémaglutination tréponémique (THA) pour la syphilis, utilisant des érythrocytes aviaires tannés par le formol et sensibilisés avec un ultrasonat de *Treponema pallidum* souche Nichols. Sauf dans les cas de syphilis récente non traités, le test THA s'est montré avoir une sensibilité supérieure à 90 pour cent dans la syphilis non traitée ou traitée si l'on se refère aux diagnostics des cliniques spécialisées. La spécificité du test THA a été trouvée supérieure à 99·9 pour cent chez 35.000 sujets soumis à un examen pré-natal, en excluant ceux qui avaient des antécédents de tréponématose ou ceux qui étaient positifs au TPI ou au FTA-ABS.

On résume les résultats sur plus de 65.000 examens demandés, dans lesquels on effectua le test THA à titre de routine. Ces résultats furent confirmés par ceux de plus de 4.000 FTA-ABS effectués sur des sérums choisis dans un lot supplémentaire de 84.000 malades, sur la base du résultat du test THA, des constatations cliniques ou des résultats de la réaction de Wassermann à la cardiolipine ou du test de fixation du complément à la protéïne de Reiter.