Use of *T. pertenue* in the fluorescent and immobilization tests

Investigation of sera from yaws areas found reactive only in the TPHA test

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In a previous study of the *Treponema pallidum* haemagglutination (TPHA) test on sera from patients in areas in which yaws is endemic, Garner, Backhouse, Daskalopoulos, and Walsh (1972a) found 110 sera which were reactive only in the TPHA test. These sera were non-reactive in the cardiolipin Wassermann reaction (CWR), Venereal Disease Research Laboratory (VDRL), Reiter protein complement-fixation (RPCF), fluorescent treponemal antibody absorption (FTA-ABS), and *Treponema pallidum* immobilization (TPI) tests. The FTA-ABS and TPI tests were carried out using *T. pallidum* as antigen. All the sera were from persons who had no history or clinical signs of syphilis or yaws.

It was found that, as the prevalence of yaws increased in the areas surveyed, the percentage of sera reactive only in the TPHA test tended to increase. No satisfactory explanation for this could be found. Various theories were considered—oversensitivity of the TPHA test; failure of the absorbing diluent used in the TPHA test to remove all group antibody from the test sera; some sera from adults being from old cases of yaws in which the FTA-ABS and TPI tests had become non-reactive; and the possibility that some subjects had developed low antibody levels because of previous contact with yaws, without ever showing clinical signs of the disease.

As *Treponema pertenue* is the causative organism of yaws and part of the problem was to determine whether these 110 subjects had ever had yaws, it was decided to test the sera using *T. pertenue* in place of *T. pallidum* in the FTA-ABS and immobilisation tests.

Material and methods

T. pertenue, Haiti B strain, was used. The treponemes had been placed in ampoules 4 years and 4 months previously and stored and transported in liquid nitrogen. When they

were thawed and examined there were twenty treponemes per high dry field; 56 per cent. were mobile though their movements were sluggish. Intratesticular inoculation of 0.5 ml. of the treponeme suspension into rabbits was followed after 11 weeks and four transfers by an increase in yield of *T. pertenue* to ninety per high dry field. These organisms were used instead of *T. pallidum* in the immobilization and FTA-ABS tests.

There was sufficient serum to test only 104 of the 110 sera which had previously been found reactive only in the TPHA test.

Four groups, each of twelve sera, were used as controls. These were tested in the FTA-ABS and immobilization tests using *T. pallidum* and *T. pertenue* as antigens. The sera were from known cases of syphilis and yaws, from persons who had not had yaws but lived in a yaws area, and from non-syphilitic patients.

Results

IMMOBILIZATION TEST

Using *T. pertenue* as antigen, all 104 sera reactive only in the TPHA test were non-reactive in the immobilization test (Table). In the control group, the twelve yaws sera and the twelve syphilitic sera were reactive, and the twelve non-yaws and twelve non-syphilitic sera were non-reactive, in the immobilization test.

The results for the 48 control sera, using *T. pallidum* as antigen, were the same as those with *T. pertenue*.

FTA-ABS TEST

Of the 104 sera tested, 89 were non-reactive in the FTA-ABS test when *T. pertenue* was used as antigen, and fifteen showed a 1+ fluorescence which held when repeated (Table). One serum showed a definite beading, but this was regarded as a non-reactive FTA-ABS test result.

Eleven of these fifteen sera came from the area which showed the highest percentage (21.3 per cent.) of sera reactive only in the TPHA test (Garner and

TABLE 104 sera reactive in TPHA test

Antigen	Immobilization test		Fluorescent test	
	Reactive	Non-reactive	Reactive	Non-reactive
<i>T. pallidum</i>	—	104	—	104
<i>T. pertenue</i>	—	104	15	89

All 104 sera were non-reactive in the immobilization test using *T. pertenue* as antigen; 89 sera were others, 1972a). In this area four of the sera were from males whose ages ranged from 30 to 48 years and seven were from females aged from 32 to 55 years. In the area which showed the next highest percentage (18.9 per cent.) of sera reactive only in the TPHA test, only two sera showed a 1+ fluorescence; these were both from females, aged 9 and 17 years. The remaining two of the group of fifteen sera came from areas with 9.6 and 2.7 per cent. of sera reactive only in the TPHA test. These were from a male aged 40 years and a female aged 60 years.

In the control group, the twelve yaws sera and the twelve syphilitic sera were reactive in the FTA-ABS test when tested against *T. pallidum* and *T. pertenue* as antigens. Non-reactive FTA-ABS test results were obtained using either treponeme as antigen when the sera from the twelve persons who did not have yaws and the twelve non-syphilitic sera were tested.

Discussion

There was no difference in the reactions of the control yaws and syphilitic sera when either *T. pallidum* or *T. pertenue* was used as the antigen in the fluorescent or immobilization tests. It therefore appears that there is at present no means of distinguishing yaws from syphilis serologically even when using the specific treponeme which causes each disease.

The problem of the diagnosis in the 104 sera reactive only in the TPHA test is a serological one, as these sera were all from people who had no clinical evidence of past or present yaws and from whom no reliable history was available.

All 104 sera were non-reactive in the immobilization test when either *T. pallidum* or *T. pertenue* was used as the antigen. If the immobilization test results were used as criteria of the presence or absence of treponemal disease, then the results of the TPHA test must be considered to be false positive.

Of the 104 test sera, 89 were non-reactive in both the immobilization and fluorescent tests using either *T. pallidum* or *T. pertenue* as antigen. The TPHA test has been shown to be slightly more sensitive than the TPI test in late syphilis (Johnston, 1972). This has not yet been demonstrated in yaws, although as the two diseases are indistinguishable sero-

logically, it would be reasonable to expect the same type of result to occur. It is therefore probable that some, at least, of the 89 sera may be in this group. Garner (1973) has found some discrepancy between test results using different batches of TPHA antigen. This must also be considered as a possible cause of false reactive TPHA test results. Thus no definite conclusion can be reached as to whether some or all of the 89 sera reactive only in the TPHA test were showing false positive results or not.

Of the 104 sera, fifteen were reactive in the TPHA test when *T. pallidum* was the antigen, and reactive in the FTA-ABS test (1+ fluorescence) with *T. pertenue* as antigen. As sera from yaws and syphilis cases reacted in the same way in the FTA-ABS test when either treponeme was used as antigen, it seems probable that these fifteen sera were showing false reactive FTA-ABS test results. It could be argued that the two younger members of the group, aged 9 and 17 years, had early yaws infections, but, if this were so, it would be reasonable to expect that the FTA-ABS test using *T. pallidum* as antigen would also have been reactive. It has been shown that the TPHA test is less sensitive than the FTA-ABS test in early syphilis (Johnston, 1972; Garner and others, 1972b). Assuming this also applies to early yaws infections it seems highly probable that the two young females did not have early yaws. The remaining thirteen sera in this group were from adults aged between 30 and 60 years. The same reasoning indicated earlier in the discussion could apply to the reactive TPHA test results shown by these sera.

No definite conclusions could be reached from our series of tests. It seems that irrespective of whether the antigen used is *T. pallidum* or *T. pertenue* and the disease syphilis or yaws, some discrepant results will occur if sufficient tests are performed. In areas where yaws has been prevalent, the interpretation of results on sera reactive only in the TPHA test must remain in some doubt unless there is a definite history or clinical signs of past or present yaws.

Summary

In a previous survey of yaws areas, 110 sera were found reactive only in the TPHA test. All non-treponemal tests as well as treponemal tests which used *T. pallidum* as antigen were non-reactive in these sera. The sera were from people who had no history or clinical signs of past or present yaws. As *T. pertenue* is the organism which causes yaws, 104 of these sera were tested using *T. pertenue* in place of *T. pallidum* in the fluorescent and immobilization tests.

non-reactive in the fluorescent test and fifteen sera showed a one plus fluorescence.

Possible explanations for the discrepancies in test results are discussed.

No definite conclusion was reached as to whether the reactive TPHA test results were false or not.

We should like to thank Dr. Paul Hardy, Johns Hopkins School of Medicine, Baltimore, for supplying us with *T. pertenue*. Without his generous assistance this study would not have been possible.

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 JOHNSTON, N. A. (1973) *Ibid.*, **48**, 474

Emploi de *T. pertenue* dans les tests de fluorescence et d'immobilisation. Examen, dans des zones pianiques, de sérums trouvés positifs uniquement à l'épreuve TPHA

SOMMAIRE

Au cours d'une étude antérieure dans des zones pianiques, 110 sérums se montrèrent positifs au seul TPHA. Tous les tests non tréponémiques, aussi bien que les tests tréponémiques utilisant *T. pallidum* comme antigène, étaient négatifs pour ces sérums. Ces sérums provenaient de sujets qui n'avaient pas d'antécédents ou de signes cliniques, passés ou présents, de pian. Comme *T. pertenue* est l'organisme responsable du pian, 104 de ces sérums furent éprouvés vis-à-vis des tests de fluorescence ou d'immobilisation, en utilisant *T. pertenue* à la place de *T. pallidum*.

Les 104 sérums furent négatifs au test d'immobilisation utilisant *T. pertenue* comme antigène: 89 sérums furent négatifs au test d'immuno-fluorescence et 15 montrèrent une fluorescence à une croix.

On discute les explications possibles de ces désaccords.

On n'est arrivé à aucune conclusion définitive quant à savoir si les résultats positifs au TPHA étaient faux ou non.

A series of 31 patients were treated with a single dose of 300 mg. doxycycline. Cures occurred in periods of 1 to 6 days after treatment in thirty of the 31 patients treated.

The remaining patient vomited the doxycycline at the time of administration. No immediate recurrence or apparent re-infections were noted in eight cases treated as out-patients or in any patients retained under observation.

Author's Summary

Variable Significance of Condylomata Acuminata

FITZGERALD, D. M., and HAMIT, H. F. (1974) *Ann. Surg.*, **179**, 328

Public health and social aspects

Adapting the Venereal Disease Clinic to Today's Problem

ATWATER, J. B. (1974) *Amer. J. publ. Hlth*, **64**, 433

Venereal Disease Campaign in Colorado—a Model for Community Action

TAYLOR, J., and GONRING, R. W. (1974) *Hlth Serv. Rep.*, **89**, 47

Contact Tracing

Brit. med. J., (1974), **3**, 75 (Leader)

Miscellaneous

Genital Infections in Developing Countries: Experience in a Family Planning Clinic

HOPCRAFT, M., VERHAGEN, A. R., NGIGI, S., and HAGA, A. C. A. (1973) *Bull. Wld Hlth Org.*, **48**, 581 19 refs

African women attending one urban and one rural family planning clinic in East Africa were investigated to detect the presence of gonorrhoea, candidiasis, and trichomoniasis. 200 women were examined at the urban clinic, 100 of whom were married and the other 100 unmarried. Fifty women in each of these groups were already practising contraception, while the remainder, attending for the first time, were not. Gonorrhoea and

candidiasis were diagnosed by conventional methods, but trichomoniasis from Papanicolaou smears only. Fifty women were seen at the rural clinic and tested for gonorrhoea and candidiasis only.

At the urban clinic gonorrhoea was found to be more prevalent among the unmarried (27 per cent.) than the married women (12 per cent.), but there was no significant difference in its prevalence between those practising contraception and the remainder. In the rural clinic the incidence of gonorrhoea was 14 per cent. *Candida albicans* was isolated from 49 women at the urban clinic, a higher incidence being noted in those using contraception and also in the unmarried women. *Candida* was detected in only four women attending the rural clinic. Trichomoniasis was found in a similarly high proportion in all four groups (20 to 26 per cent.).

Although many of the women were found to have symptoms on close questioning, these were not specific for a particular infection and were also often reported by women in whom none of the three infections was found. Women often incorrectly attribute symptoms of vaginal infection or venereal disease to the contraceptives they are using. The authors regard this as a major obstacle to family planning programmes. Diagnosing and treating these conditions in family planning clinics, which are ideally suited for this purpose, would not only help in correcting the erroneous view but also in controlling sexually-transmitted disease, since as many as 54 per cent. of the women investigated had one or more of the three infections (gonorrhoea, candidiasis, trichomoniasis).

C. S. Ratnatunga

A Rapid Method of Assaying Gentamicin and Kanamycin Concentrations in Serum

LOUVOIS, J. DE (1973) *J. med. Microbiol.*, **7**, 11

A simple method is described for the assay of gentamicin and kanamycin in human serum. It is a modification of the monosaccharide hydrolysis method of Faine and Knight (1968) and will estimate low concentrations of antibiotic accurately within 3 hours.

The method gives results similar to those obtained by a double-row tube-dilution growth inhibition technique, is unaffected by the presence of penicillins or cephalosporins, and requires only standard laboratory equipment. Because the method gives readily reproducible results and is easy to perform, it should commend itself to all laboratories dealing with patients receiving aminoglycoside therapy.

Author's summary

Cancer of the Cervix: A Sexually-Transmitted Infection?

SINGER, A. (1974) *Lancet*, **2**, 41 (Letter)

Improved Sampling Device for Cervical Cytology

WACHTEL, E., and GORDON, H. (1974) *Lancet*, **2**, 26

Scanning Electron Microscopy of Human Female Genital Tract

FERENCZY, A., and RICHART, R. M. (1974) *N.Y. St. J. Med.*, **74**, 794

Urinary Tract Infection: Problems in Diagnosis and Management, 1973

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Problems in Diagnosis of Bacterial Prostatitis: Gram-Negative, Gram-Positive, and Mixed Infections

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Trimethoprim/Sulfamethoxazole Therapy of Chronic Bacterial Prostatitis

DRACH, G. W. (1974) *J. Urol. (Baltimore)*, **111**, 637

Sexual Problems as seen by Proctologist

TURELL, R. (1974) *N.Y. St. J. Med.*, **74**, 697

CORRIGENDUM

In the August 1974 issue of the journal, in the article by Garner, Backhouse, Daskalopoulos, and Walsh, it is regretted that two lines were accidentally transposed from the top of col. 1, p.266 to the top of col. 1, p. 265