

REITER'S PROTEIN COMPLEMENT-FIXATION TEST*

REPORT OF A TRIAL IN 1,000 UNSELECTED CASES

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In recent years there has been a tendency to experiment with antigens derived from treponemata in the diagnostic tests for treponemal disease in the hope that they might prove more sensitive and more specific than the cardiolipin antigen which is now the standard preparation.

These antigens have been prepared either from a virulent strain of *Treponema pallidum* or from the avirulent Reiter strain, which is said to have been originally derived from a virulent *Treponema pallidum* (Ruge, 1956). The preparation of antigens from virulent *Treponema pallidum* involves the maintenance and handling of infected rabbits, a task which routine laboratories would prefer to avoid. The Reiter treponeme, however, grows readily *in vitro* and is convenient to handle. Tests using the Reiter strain have been of two types:

- (1) Those in which the whole treponeme has formed the antigen.
- (2) Those in which a protein extract of the treponeme has been used.

D'Alessandro and Dardanoni (1953) have shown that at least four antigenic fractions of the Reiter treponeme can be prepared:

- (1) A protein antigen,
- (2) A lipid antigen which has the same specificity as cardiolipin,
- (3) A further lipid antigen,
- (4) A polysaccharide antigen.

The protein antigen was more sensitive than the whole treponeme when titrated with the serum of experimentally-infected animals and showed no reaction with an antibody prepared against cardiolipin. Sera from syphilitic patients also reacted to a higher titre with the protein antigen than with cardiolipin. However, the antibody involved in the Reiter protein complement-fixation test (RPCFT)

does not appear to be the same as that responsible for the immobilization of the treponemata in the *Treponema pallidum* immobilization test (TPI) because an antibody prepared artificially against the Reiter protein antigen does not immobilize *Treponema pallidum*. De Bruijn (1957), in Holland, who published a series of cases using this type of protein antigen in parallel with the standard cardiolipin test and the TPI test, found that the protein antigen was both more sensitive and gave closer agreement with the TPI test than the cardiolipin antigen. The superior sensitivity and specificity of this antigen was confirmed in a larger series of cases reported by Rein, Kelcec, D'Alessandro, and De Bruijn (1957) in the U.S.A.

The protein antigen used by de Bruijn was prepared by the technique of D'Alessandro and others (1953), and it is available commercially.

This present report gives the results obtained using this test in parallel with the Wassermann reaction (WR) and Price's precipitation reaction (PPR) in 1,000 unselected cases attending the Venereal Disease Department at St. Thomas's Hospital.

Technique

The technique was modelled as closely as possible on the standard WR technique employed in this hospital. A single 1 in 5 dilution of the patient's serum and a single concentration of complement (2½ mhd) was incubated with an equal volume of antigen for one hour, with the subsequent addition of one volume sensitized sheep red cells (approximately 3 per cent. with 6 mhd amboceptor). For the RPCF test, the diluted protein antigen was simply substituted for the cardiolipin antigen (Standard WR antigen - V.D. Reference Laboratory). The only modification which was found necessary was that the antigen, serum, and complement had to be left in contact overnight in the refrigerator and then incubated at 37°C. for 15 minutes before the addition of the sensitized sheep cells. The antigen is supplied dry in sealed ampoules and

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is reconstituted by the addition of 2 ml. distilled water. This antigen keeps well at 4°C. and is used diluted 1 in 80 with saline in the test.

Results

The comparative findings for the RPCF test, WR, and PPR in 1,000 consecutive cases are shown in Table I.

TABLE I
RPCF, WR, AND PPR RESULTS IN 1,000 CONSECUTIVE CASES

Group	Standard Tests (WR and PPR)	RPCF Tests	No. of Cases
1	One or Other or Both Positive	Positive	178
2	One or Other or Both Positive	Negative	13
3	Both Negative	Positive	97
Total	288

The patients in Group 1 are regarded as definitely suffering from treponemal infection and the clinical findings did not disagree with this conclusion. Of the thirteen patients showing negative RPCF results in Group 2, eight were cases of treated syphilis, but in the other five no clinical evidence of treponemal disease could be elicited.

A further analysis of the 97 patients in Group 3 showing positive RPCF tests with negative standard tests is presented in Table II. Through the courtesy of Dr. Orpwood Price of the V.D. Reference Laboratory treponemal Wassermann reactions (TWR) were also carried out in 79 of these cases.

This analysis shows that the positive RPCF test results give a high degree of specificity, in that there is evidence of past or present treponemal infection in 82 cases. In no instance was the test positive in a white patient in whom there was no clinical evidence

TABLE II
TWR RESULTS IN 97 PATIENTS WITH NEGATIVE WR AND PPR BUT POSITIVE RPCF

TWR Results	Total Cases	White Patients	Coloured Patients		
		With Syphilis	With Syphilis	With a History of Yaws	Denying Yaws
Positive ..	51	9	24	13	5
Negative ..	28	6	3	13	6
Not Done	18	5	4	5	4
Total ..	97	20	31	31	15

of syphilis. Of the non-syphilitic coloured patients, the majority gave a history of having had yaws in childhood, and the fifteen who denied having had yaws may well have forgotten the original illness.

Conclusions

It is considered that the evidence presented here indicates that the RPCF test is more sensitive and more specific than the standard WR. The test is no more difficult to perform than the WR and the antigen is available commercially, and therefore the test is very suitable for routine use in the clinical laboratory.

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