SPECIFICITY AND SENSITIVITY OF THE REITER PROTEIN COMPLEMENT-FIXATION (RPCF) TEST*
REPORT ON 1,400 SELECTED SERA

BY
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The reliability of the sero-diagnosis of syphilis depends on the specificity and the sensitivity of the tests used. The specificity of a test is determined by the nature of the antigen; the sensitivity depends mainly on the technique by which the test is performed.

The most specific though not the most sensitive test at the moment is, without doubt, the T. pallidum immobilization (TPI) test of Nelson and Mayer (1949). This test, however, has the disadvantage of being complicated and expensive, and its application is restricted to special laboratories.

Simpler, and within the range of any routine serological laboratory, is the use of antigens obtained from the Reiter treponeme. The preliminary promising results presented by D’Alessandro and Dardanoni (1953) led us to study the significance of a complement-fixation test using as antigen the protein fraction of the Reiter treponeme (RPCF test) in the sero-diagnosis of syphilis (Bekker, de Bruijn, Coster, and Onvlee 1956; de Bruijn, 1957a; de Bruijn and Bekker, 1957; Bekker, 1958).

The purpose of the present study is to give a survey of the results obtained by the use of the RPCF test in comparison with the TPI test, and to evaluate the specificity and the sensitivity of the test in three series of sera.

Materials and Methods

Sera.—Three series of sera forwarded to our laboratory for the TPI test were investigated. The series differ only in the technique by which the RPCF tests were performed. The sera came from patients suffering from syphilitic infection, or, in some cases, from yaws, and from patients without clinical evidence of a treponemal disease. The sera were considered to be probably syphilitic, either because all tests were positive or on account of the clinical data, or presumably non-syphilitic because all tests were negative or because of the clinical data. The majority of the syphilitic sera came from patients with tertiary, neuro-, or latent syphilis, treated and untreated. The sera represent problem cases in which there was often a discrepancy between the clinical findings and the results of the standard tests performed elsewhere. Cases with insufficient clinical data or tests with doubtful or inconclusive results were excluded.

Antigen.—The protein antigen used in the RPCF tests was prepared by the method of de Bruijn (1957a), the optimum antigen dilution employed was 1:80.

Techniques.—The technique of the TPI test remained the same in all series of the study (Bekker and Onvlee, 1955).

First Series.—The technique of de Bruijn (1958a) was followed in the RPCF test.

Second Series.—The RPCF test was performed with the regular Kolner technique, using 2 full units of complement.

Third Series.—Kolmer’s technique, with 1½ exact units of complement as described by Portnoy and Magnuson (1956), was employed in the RPCF test. Both Kolmer techniques were slightly modified in that the serum dilutions 1:1, 1:2, 1:4, etc., were used.

The reproducibility of all the tests was ascertained by the use of positive control sera.

Results

First Series.—Table I shows the results obtained on 300 sera, 145 of which were considered to be syphilitic and 155 non-syphilitic.

<p>| TABLE I |
| COMPARISON OF SENSITIVITY AND SPECIFICITY OF THE RPCF AND TPI TESTS IN FIRST SERIES |</p>
<table>
<thead>
<tr>
<th>Sera &amp; Number of Sera</th>
<th>RPCF Test &amp; TPI Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>300 145 Syphilitic . .</td>
<td>+ 142 + 142 + 132 + 132 + 10 + 10</td>
</tr>
<tr>
<td>145 Non-syphilitic . .</td>
<td>+ 0 + 4 + 0 + 4 + 151 + 151</td>
</tr>
</tbody>
</table>

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1Technique of de Bruijn (1958a).
2Technique of Bekker and Onvlee (1955).
Of the 145 syphilitic sera, 142 were revealed by the RPCF test and 135 by the TPI test. The relative sensitivity of the RPCF test was 98·0 per cent. against 93·0 per cent. of the TPI test.

Of the 155 non-syphilitic sera, the RPCF test gave four positive reactions and the TPI test none.

This indicates a relative specificity of 97·4 per cent. for the RPCF test and 100 per cent. for the TPI test. There was a 94·3 per cent. agreement (positive and negative) between the RPCF and TPI tests.

Second Series.—Table II shows the results obtained in 745 sera, representing 368 syphilitic and 377 non-syphilitic sera.

Of the 368 syphilitic sera, 347 were positive in the RPCF test and 350 in the TPI test. This gives a relative sensitivity of the RPCF test of 94·3 per cent. against 95·1 per cent. of the TPI test.

Of the 377 non-syphilitic sera the RPCF test revealed eight and the TPI test two positive results.

The relative specificity of the RPCF test was 97·9 per cent. and of the TPI test 99·5 per cent., assuming that the two cases with positive TPI test were true non-treponemal reactions. There was a 93·4 per cent. agreement between the RPCF and the TPI tests.

In this series the sensitivity of the RPCF test was insufficient, as is shown by the 23 positive TPI tests found in 390 sera with negative RPCF.

<table>
<thead>
<tr>
<th>Number of Sera</th>
<th>RPCF Test</th>
<th>TPI Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>368 Syphilitic</td>
<td>+ 347</td>
<td>+ 329</td>
</tr>
<tr>
<td></td>
<td>- 21</td>
<td>+ 21</td>
</tr>
<tr>
<td>377 Non-syphilitic</td>
<td>+ 8</td>
<td>+ 0</td>
</tr>
<tr>
<td></td>
<td>- 369</td>
<td>+ 2</td>
</tr>
</tbody>
</table>

Third Series.—In Table III the results obtained in 355 sera are given.

In 187 syphilitic sera, 185 positive RPCF tests and 179 positive TPI tests were found. This means a relative sensitivity of the RPCF test of 98·9 per cent. against 95·7 per cent. for the TPI test.

Of the 168 non-syphilitic sera the RPCF test gave four and the TPI test no positive results. The relative specificity was 97·6 per cent. for the RPCF test and 100 per cent. for the TPI test.

Between RPCF and TPI test there was an agreement of 96·1 per cent.

Discussion

From the results of the comparative studies on the RPCF and TPI tests presented in this report (Table IV, opposite) the following conclusions may be drawn:

1. The specificity of the RPCF test is only slightly less than that of the TPI test. The RPCF test showed an overall specificity for all series of 97·7 per cent. against 99·7 per cent. for the TPI test.

2. The sensitivity of the RPCF test at least equals that of the TPI test, provided that an adequate technique of complement-fixation is used. The overall sensitivity of the RPCF test for all series was 96·3 per cent. against 92·9 per cent. for the TPI test.

3. The sensitivity of the RPCF test is greatly influenced by the technique by which it is performed. The lowest sensitivity was found with the regular Kolmer technique. The highest sensitivity was obtained by using Portnoy and Magnuson's modification of the Kolmer technique (with 1·5 exact units of complement).

4. There was a 95·3 per cent. agreement (positive and negative) between the RPCF and TPI tests.

Our results compare favourably with those obtained by Cannefax and Garson (1957), Rein, Kelcec, D'Alessandro, and de Bruijn (1957), Miller, Boak, and Carpenter (1958), De Groat and Miyao (1958), and Kostant and Kelcec (1958). The RPCF test
appears to be a dependable test in the sero-diagnosis of treponemal infections and in our opinion should be used routinely in conjunction with the cardiolipin tests. In differences between the results of the RPCF and the cardiolipin tests, a positive RPCF test, even in a low titre (1:1, 1:2), must be considered as highly indicative of a treponemal infection, while a negative RPCF test renders this diagnosis improbable.

### Table IV

<table>
<thead>
<tr>
<th>Number of Sera</th>
<th>RPCF Test</th>
<th>TPI Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>700 Syphilitic</td>
<td>+ 674</td>
<td>+ 638 36</td>
</tr>
<tr>
<td></td>
<td>- 26</td>
<td>+ 26 0</td>
</tr>
<tr>
<td>700 Non-syphilitic</td>
<td>+ 16</td>
<td>+ 0 16</td>
</tr>
<tr>
<td></td>
<td>- 684</td>
<td>+ 2 682</td>
</tr>
</tbody>
</table>

How can the specificity of the RPCF test be explained? According to D’Alessandro and Dardanoni (1953), four antigenic fractions can be prepared from the Reiter treponeme:

1. A lipid antigen with the same specificity as cardiolipin;
2. Another lipid antigen;
3. A polysaccharide antigen;

It was shown in our laboratory (de Bruijn 1958b, 1959) that the protein antigen prepared according to the directions given by D’Alessandro and Dardanoni is probably a lipopolysaccharide-protein complex. The lipid part of the complex is active only in low antigen dilutions and does not interfere with the use of the protein antigen in the RPCF test provided that the protein antigen is used in a sufficiently high dilution. The polysaccharide part of the complex does not interfere in the RPCF test because there is no corresponding antibody in syphilitic serum.

Cross-absorption experiments (de Bruijn, 1957b) show that the complement-fixing antibody to the protein antigen of the Reiter treponeme differs from the so-called Wassermann antibody (reagin). The antibody also differs from the complement-fixing antibody reactive in the TPCF test (Kelce, personal communication). Gelperin (1951) has shown that the antibody to the Reiter treponeme is distinct from the immobilizing antibody.

To explain the specificity of the RPCF test in the sero-diagnosis of treponemal infections one may assume with Dardanoni and Censuales (1957) the occurrence of common or related proteins in the different strains of treponemes. These protein antigens may seem to be species-specific while other antigens, e.g. polysaccharides, may prove to be type-specific.

### Summary

A survey is given of three comparative studies between the RPCF test, a complement-fixation test using a protein fraction prepared from the Reiter treponeme as an antigen, and the *T. pallidum* immobilization (TPI) test.

The study of 700 sera from probably syphilitic patients, treated and untreated, showed the RPCF test to be at least equal in sensitivity to the TPI test. The RPCF test gave 674 positive results and the TPI test 650, a sensitivity of 96·3 and 92·9 per cent. respectively.

The study of 700 sera from presumably non-syphilitic patients, showed the specificity of the RPCF test to be only slightly less than that of the TPI test. The RPCF test gave sixteen positive results and the TPI test two, a specificity of 97·7 and 99·7 per cent. respectively.

There was a 95·3 per cent. agreement (positive and negative) between the RPCF and TPI test.

The sensitivity of the RPCF test is greatly influenced by the technique of complement-fixation. The modification of Portnoy and Magnuson of the Kolmer technique, using 1·5 exact units of complement, is recommended for the RPCF test.

The antibody to the protein antigen of the Reiter treponeme is different from reagin and probably from some antibodies to the virulent *T. pallidum*.

The RPCF test is a reliable and simple test in the sero-diagnosis of the treponematoses, and can easily be used routinely in serological laboratories.

### References

Specificity and Sensitivity of the Reiter Protein Complement-Fixation (RPCF) Test: REPORT ON 1,400 SELECTED SERA

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