LIMITATIONS OF THE REITER PROTEIN COMPLEMENT-FIXATION (RPCF) TEST*

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In previous papers we have recommended the application of the Reiter protein complement-fixation (RPCF) test as a simple, reliable, and specific test for the sero-diagnosis of syphilis, provided that an adequate antigen and technique are used (De Bruijn and Bekker, 1957; Bekker, 1958, 1959). We emphasized the use of the RPCF test for confirmation of the cardiolipin tests and as a pre-selection procedure for the *T. pallidum* immobilization (TPI) test. The RPCF test cannot be used as a substitute for the TPI test, however, nor can the RPCF test replace the cardiolipin tests (Bekker, 1960).

No laboratory test for syphilis is perfect as regards specificity and sensitivity, and reactive results in non-syphilitic sera and non-reactive results in syphilitic sera do occur with all tests. For a correct interpretation of the results of the RPCF test its limitations must be recognized.

In the present study some of the causes of unsatisfactory results of the RPCF test are discussed.

Reactive RPCF Tests in Non-syphilitic Sera

The specificity of the RPCF test is determined by the nature of the antigen. In our experiments the Reiter protein antigen was prepared according to De Bruijn (1957, 1960) and employed in the optimal dilution. Many authors have stated that the specificity of the RPCF test is only slightly less than that of the TPI test. Exceptionally, however, a reactive RPCF test is found in sera which are non-reactive to the TPI test and come from patients without present or past treponemal infection. Although Reiter protein antigen is a lipo-polysaccharide-protein complex (De Bruijn, 1959), it was assumed

that only the protein component is responsible for the reactivity with syphilitic serum. Searching after the origin of the "non-specific" positive results of the RPCF test, De Bruijn (1961) was able to demonstrate an antibody against the polysaccharide fraction of the Reiter treponeme in some of the sera. As this antibody was found in three out of 597 non-syphilitic and in twelve out of 205 syphilitic sera, its presence seems not to be a consequence of the treponemal infection. The possibility exists that this antibody is produced as a response to a micro-organism sharing the polysaccharide antigen with Reiter treponeme.

We may conclude that in some cases a non-specific reactive RPCF test can be explained by the occurrence in the serum of an antibody against the polysaccharide fraction of the Reiter treponeme, but that in the majority of the cases the cause is still obscure.

Non-reactive RPCF Tests in Syphilitic Sera

The sensitivity of the RPCF test depends mainly on the technique by which it is performed. In our experiments Kolmer's technique was used with $1\frac{1}{2}$ exact units of complement (Portnoy and Magnuson, 1956), slightly modified in that the serum dilutions 1:1, 1:2, 1:4, etc., were used. (The saline contained 100 mg. magnesium sulphate per litre). With this technique the sensitivity of the RPCF test at least equals that of the TPI test (Bekker, 1959), and in treated syphilitic patients its sensitivity is even greater (Bekker, 1960; Bekker and Onvlee, 1959). Exceptionally, a non-reactive RPCF test is found in syphilitic sera which react in the TPI test. In nearly all of these cases we could demonstrate that the negative result was due to a prezone (Neisser-Wechsberg) or related phenomenon.

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In Table I the normal pattern of the titration of a syphilitic serum with protein antigen is given, showing the optimal antigen dilution as 1 : 80.

Table II gives a clear example of a prezone phenomenon in the same antigen dilution.

In some cases the "optimal" antigen dilution shows a weakly reactive or even a nonreactive result (Table III).

From these observations it can be concluded that to ensure optimal accuracy the RPCF test must be performed quantitatively. Where sera give non-reactive RPCF and reactive TPI tests, it is necessary to repeat the RPCF test with a lower antigen dilution (e.g. 1 : 20). The use of two antigen dilutions was recommended by D'Alessandro and Dardanoni (1953).

Reproducibility of the RPCF Test

The reproducibility of the RPCF test, like that of any other serological test, depends on a rigid

standardization of the technique and of the reagents involved. To ensure this standardization the use of positive control sera is essential (Bekker, 1951).

Miller, Carpenter, Boak, Peterson, Pait, Lawrence, Heidebreder, Linscott, and Fazzan (1961) and Bissett, Browne, Coffey, and Michelbacher (1961), probably using a *qualitative* RPCF test, questioned its reproducibility and recommended a repeat testing at least once on another day. In our experience with many thousands of problem and routine sera, the *quantitatively* performed RPCF test showed an excellent reproducibility under the conditions mentioned. Repeat testing of the same specimen, either low or high titre, on consecutive days hardly ever gave inconsistent results. The same applied to the testing of a different specimen from the same patient on different days. During the last 2 years, 121 problem sera were re-tested. The lapse of time between the investigations of the two samples varied from one week to several months.

TABLE I
TITRATION OF SYPHILITIC SERUM WITH REITER PROTEIN ANTIGEN (NORMAL PATTERN)

Serum Dilution	Antigen Dilution					
	1 : 5	1 : 10	1 : 20	1 : 40	1 : 80	1 : 160
1 : 1	++++	++++	++++	++++	++++	++++
1 : 2	++++	++++	++++	++++	++++	++++
1 : 4	++++	++++	++++	++++	++++	++++
1 : 8	++++	++++	++++	++++	++++	++++
1 : 16	—	—	—	—	—	—
1 : 32	—	—	—	—	—	—
1 : 64	—	—	—	—	—	—

TABLE II
TITRATION OF SYPHILITIC SERUM WITH REITER PROTEIN ANTIGEN (PREZONE PHENOMENON)

Serum Dilution	Antigen Dilution					
	1 : 5	1 : 10	1 : 20	1 : 40	1 : 80	1 : 160
1 : 1	++++	++++	++++	—	—	—
1 : 2	++++	++++	++++	—	—	—
1 : 4	++++	++++	++++	—	—	—
1 : 8	++++	++++	++++	—	—	—
1 : 16	++++	++++	++++	—	—	—
1 : 32	++++	—	—	—	—	—
1 : 64	—	—	—	—	—	—

TABLE III
TITRATION OF SYPHILITIC SERUM WITH REITER PROTEIN ANTIGEN (ABNORMAL PATTERN)

Serum Dilution	Antigen Dilution					
	1 : 5	1 : 10	1 : 20	1 : 40	1 : 80	1 : 160
1 : 1	+++	++++	++++	+++	—	—
1 : 2	—	++++	++++	—	—	—
1 : 4	—	—	—	—	—	—
1 : 8	—	—	—	—	—	—
1 : 16	—	—	—	—	—	—

Table IV shows that, of these 121 duplicate sera, only one gave a significant difference in titre. The titre of this serum dropped in 9 months from 1:32 to 1:1, but the patient had received treatment.

TABLE IV
RESULTS OF REPEAT TESTING OF SERA FROM
121 PROBLEM CASES
(Two different serum samples of the same patient were tested on
different days)

Agreement in titre in both samples	81
Difference in titre between samples	1 dilution .. 31
	2 dilutions .. 8
	More than 2 dilutions 1
Total	121

From this experience, it can be concluded that the quantitative RPCF test, when performed under controlled standardized conditions, has an excellent reproducibility.

Summary and Conclusions

Like other tests in the sero-diagnosis of syphilis, the RPCF test has its limitations as regards specificity and sensitivity, and apparently non-specific positive and negative results do occur. For a correct interpretation of the results of the RPCF test these limitations must be recognized.

From our study of the causes of unsatisfactory RPCF test results the following conclusions may be drawn:

- (1) Non-specific reactive RPCF tests can in some cases be explained by the occurrence of an antibody against the polysaccharide fraction of the Reiter treponeme.
- (2) Non-reactive RPCF tests in syphilitic sera can be caused by a prezone or related phenomenon. To ensure optimal accuracy the RPCF test must be performed quantitatively. In sera with non-reactive RPCF and reactive TPI tests it is necessary to repeat the RPCF test with a lower antigen dilution.
- (3) The quantitative RPCF test has an excellent reproducibility, provided that it is carried

out under controlled standardized conditions.

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Limitations de la réaction de fixation du complément contre la protéine de Reiter (RPCF)

RÉSUMÉ

Comme les autres tests sérologiques pour la syphilis, le RPCF est limité en sensibilité et en spécificité, et les résultats non-spécifiques, négatifs et positifs, se voient de temps en temps. Pour interpréter ces résultats correctement il faut connaître les limitations du test. Les conclusions suivantes ont été tirées d'une étude des résultats imparfaits du test RPCF:

- (1) Les résultats positifs non-spécifiques peuvent être expliqués parfois par la présence d'un anticorps contre la fraction polysaccharide du tréponème de Reiter.
- (2) Les résultats négatifs dans les sérums syphilitiques peuvent être dus à la présence d'un "prézone" ou d'un phénomène de cette nature. Pour obtenir la plus grande exactitude il faut faire le test quantitativement. Dans le sérum où le RPCF est négatif et le test TPI positif, il faut répéter le test RPCF avec une dilution d'antigène plus basse.
- (3) Le test RPCF quantitatif est bien reproductible, pourvu qu'il soit fait dans des conditions bien contrôlées.