This paper explores the application of the RPCF test in the serodiagnosis of syphilis in the public health laboratory. The test is evaluated and the limits of its use are indicated.

PROBLEMS IN THE USE OF THE RPCF IN A PUBLIC HEALTH LABORATORY

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CURRENT interest in the serodiagnosis of syphilis has been directed to the development of a test to replace the specific but expensive and time-consuming TPI (Treponema pallidum immobilization) test. The technic which has recently received the most attention in this connection is the complement fixation test using a protein extract of the Reiter strain of treponemes as the antigen (RPCF).

The chemical fractionation of the Reiter treponeme by D'Alessandro and his co-workers in Italy in the early 1950's[8,9] provided the basis for subsequent work with the Reiter protein antigen. Several investigators have contributed further to our knowledge of the antigens of this organism.[7,10,13] Clinical evaluations of the RPCF test have been reported by several authors[6,11,14,16,19,22,24,26] and a number of different technics for the performance of the test have been described. However, many questions regarding the test and its practical application still require answers. How may the test best be utilized in a public health laboratory? Is the test an adequate screening method for subsequent TPI testing? Is this test sufficiently specific and sensitive to replace the TPI? Are results of this test reproducible when the same serum is retested on a different testing day?

It was in an attempt to supply some of the answers to these questions that the Microbiology Laboratory of the California State Department of Public Health undertook the studies described in this paper. In general, these studies relate the performance and reactivity of the RPCF test to the TPI and VDRL tests on specimens from several categories of patients.

The VDRL slide test was performed as described in the 1959 Manual of Serologic Tests for Syphilis.[18] The RPCF technic used was the 1/5 volume Kolmer technic described in the same source, except that saline containing optimal amounts of calcium ion as well as magnesium ion was used.* The TPI technic used is that of Nelson as modified by Harris and associates.[15] The technics for each of the tests were the same for each study. One lot of commercial RPCF antigen was used throughout to eliminate possible variation due to antigen differences. In each of the studies the RPCF test was performed on the same serum on each of two different days in an effort to determine reproducibility of the

* 100 mg $MgCl_2$ and 40 mg $CaCl_2$ per liter of 0.85 per cent saline.

Table 1—Routine Study, Results of RPCF and VDRL Tests on 200 Specimens Received for Routine Serologic Testing

VDRL	RPCF*	Number	Per cent
Agree			
R†	R	7	3.5
N	N	187	93.5
Disagree			
R	N	4	2.0
N	R	0	0.0
R	AC	0	0.0
N	AC	2	1.0
Total		200	100.0

* RPCF results first testing day.
† R=Reactive and weakly reactive.
N=Nonreactive.
AC=Anticomplementary.

RPCF test on specimens derived from various categories of patients.

Study I—Routine Study

This study was designed to determine the RPCF reactivity level on specimens received in this laboratory for routine lipid serologic tests for syphilis (STS).

Two hundred specimens received for routine STS from physicians in a city of 110,000 were selected on the basis of adequate serum volume. VDRL and RPCF tests were performed. It is evident in Table 1 that test results on 97 per cent of the specimens are in agreement. For purposes of simplification in this table, weakly reactive (WR) and reactive test results are grouped as reactive. The specimens which show discrepant results between tests (4/200 or 2 per cent) are all in the reactive VDRL and nonreactive RPCF category. TPI tests were performed on these four specimens. One specimen was TPI nonreactive in agreement with the RPCF test. The other three were TPI reactive in agreement with VDRL results.

The reactivity rate of RPCF in this group of specimens is 3.5 per cent, while the rate of reactivity of the VDRL was 5.5 per cent. In this predominantly nonreactive group of specimens, reproducibility of the RPCF is good. Exact agreement of results between runs was obtained in 97 per cent of the specimens.

Study II—Reactive Group

This second study was designed to determine the agreement between VDRL and RPCF tests on specimens selected on the basis of VDRL reactivity.

Specimens were obtained from private physicians and local health departments in counties of the state for whom this laboratory was currently performing routine serologic tests for syphilis. Serum specimens showing any degree of reactivity in the VDRL test were frozen in aliquots for testing by the RPCF and TPI technics. Serums from all reactive VDRL specimens were tested by the RPCF test in each of two different test runs. Any specimen which showed a difference in result of WR or greater between RPCF test runs was tested a third time. A consensus result was then used for comparison with VDRL and TPI. Any specimen showing a discrepant result between the VDRL and RPCF was tested by the TPI technic.

Results of comparison of VDRL and RPCF are reported in Table 2 (A). Sixty-six per cent or 120 of 182 specimens showed agreement between the two tests if reactive and weakly reactive results are grouped. Sixty-two serums gave discrepant results between the VDRL and RPCF tests. TPI tests were performed on these 62 specimens and results are given in Table 2 (B). Forty-six of these 62 serums were reactive in both the VDRL and TPI tests but nonreactive in the RPCF test. The remaining 16 serums were negative in the RPCF and TPI tests but reactive in the VDRL.

Reproducibility of RPCF results in

Table 2—Reactive Study

(A) RPCF Test Results on 182 VDRL Reactive Specimens

Results	VDRL Reactive Specimens* (182)	
	Number	Per cent
RPCF R*	120	66
RPCF N	62	34
Total	182	100

* Includes WR.

(B) TPI Test Results on 62 Specimens with Discrepant Results Between RPCF and VDRL Tests

Results			Number	Per cent
VDRL	RPCF	TPI		
R	N	R	46	74
R	N	N	16	26
Total			62	100

this group of specimens was considered poor. Only 75 per cent of the specimens repeated in a second test run showed exact agreement of results. Possible reasons for this lack of reproducibility will be discussed later.

Study III—Prenatal Study

In an effort to answer questions regarding the use of RPCF tests in serologic testing of prenatal patients the following study was undertaken (see Table 3).

One hundred and forty-eight prenatal specimens received in this laboratory for TPI testing were also tested by the VDRL and RPCF technics. In considering results of this study it must be realized that these serums represent specimens from prenatal patients with reactions to some type of STS in some laboratory during the course of the current pregnancy, since this is the criterion upon which the TPI testing service is based.

In this group, the VDRL and RPCF tests are in agreement in 91 of the 148 specimens tested or 62 per cent. In three of these 91 specimens, the TPI disagreed with the VDRL and RPCF. This represents less than 2 per cent of the total specimens in this study. Of the remaining 57 specimens showing discrepant results between VDRL and RPCF, the TPI agreed with the RPCF in 43 and disagreed in 14. All 14 disagreements were in the category of nonreactive RPCF and reactive TPI.

Reproducibility of the RPCF test in the prenatal group was good. Ninety-one per cent of the specimens showed exact agreement of results between two test runs.

Study IV—BFP Study*

Study IV represents tests on a group of 227 specially selected cases. The specimens consisted of serums submitted for TPI testing where the physician considered that the reactive STS results obtained did not indicate syphilis. No clinical or historical evidence of syphilis was indicated, treatment directed toward syphilis had not been administered, and the patient had shown reactivity in one or more of the STS for at least three months prior to the submission of the current specimen.

Specimens selected for this study were tested by the VDRL, RPCF, and TPI technics. RPCF tests were performed on each serum on each of two testing days. A third test was performed if the first two were not in agreement. A consensus report was used for comparison with VDRL and TPI tests. The VDRL and the RPCF were in agreement in 117 or 52 per cent of the 227 specimens tested (see Table 3). In five of these 117 specimens, results of the VDRL

* Biologic false positive study.

and RPCF disagreed with the TPI. This represents 2 per cent of the total specimens in this study. Of the 110 specimens showing discrepant RPCF-VDRL results, the RPCF agreed with the TPI in 81 and disagreed with the TPI in 29. Of these 29 specimens, 27 were in the category of nonreactive RPCF and reactive TPI. The reproducibility of the RPCF tests on specimens in this category was 88 per cent.

Discussion

At the beginning of this paper, a series of questions was presented regarding the application of the RPCF. These questions will now be considered in view of the studies just described.

1. Is the RPCF Sufficiently Sensitive and Specific to Replace the TPI?

Because of the well known difficulties in making a definitive diagnosis in many cases of syphilis, the TPI, which possesses a high degree of sensitivity and specificity, has come to be generally accepted as the standard of reference against which new treponemal tests are evaluated.

In the two studies where the TPI was performed on all specimens (Prenatal and BFP Studies) the RPCF showed an over-all agreement with TPI of 87 per cent. In these groups the RPCF showed a high degree of agreement in nonreactive TPI specimens for a specificity of approximately 98 per cent. Here, we are defining specificity as per cent agreement with nonreactive TPI specimens and sensitivity as per cent agreement with reactive TPI specimens. The sensitivity of RPCF in the prenatal group was 64 per cent and in the BFP group 73 per cent. This lack of sensitivity is also indicated in the Reactive Study, Table 2 (B). Of the 62 serums which showed discrepant results between VDRL and RPCF and which were tested by TPI technic 46 (or 74 per cent) were nonreactive RPCF with reactive TPI.

Disagreements between RPCF and TPI tests may be attributed to the antibody status of the patient, since it is well known that the TPI is usually nonreactive in early syphilis and remains

Table 3—Results of VDRL, RPCF, and TPI Tests on Selected Specimens Submitted for TPI Testing

Test Results			Prenatal (Study III)		BFP (Study IV)	
VDRL	RPCF	TPI	Number	Per cent	Number	Per cent
VDRL and RPCF Agree						
R	R	R	26	18	77	34
N	N	N	62	42	35	16
			91	62	117	52
R	R	N	2	1	2	1
N	N	R	1	<1	3	1
VDRL and RPCF Disagree						
R	N	N	42	28	78	34
N	R	R	1	<1	3	1
			57		110	
R	N	R	14	10	27	12
N	R	N	0	0	2	1
Total			148	100	227	100

reactive longer than other tests in treated syphilis. However, early syphilis and treated syphilis can be reasonably excluded in the BFP group because of the basis of selection of the specimens. The specimens were from patients with no stated history of syphilis who had had reactive STS at least three months prior to the current specimen and who had received no treatment for syphilis. It is evident that under conditions of testing in this laboratory, the RPCF shows a lack of sensitivity which makes it an unsatisfactory replacement for the TPI test.

2. Is the RPCF an Adequate Screening Method for the TPI?

Investigators[16,22] have suggested use of the RPCF together with one or more lipid tests as a preselection procedure for the TPI. This procedure would presumably eliminate the necessity of performing TPI tests on specimens where RPCF and lipid tests agree.

Results of the studies reported in this paper indicate that use of the selection method employing a combination of the VDRL and the RPCF tests would eliminate the necessity of performing 66 per cent of the TPI's in the Reactive Study, 52 per cent in the BFP Study, and 62 per cent in the Prenatal Study. The possible limitations of such a screening procedure must be recognized. The TPI will disagree with the results of the screen in a small percentage of the specimens showing agreement between the VDRL and RPCF (about 2 per cent of those reported here). We hope over a period of time to be able to determine the significance of these discrepancies. At the present time we can only state that they exist.

3. How May the RPCF Test Be Best Utilized in a Public Health Laboratory?

A review of the results obtained in the Routine Study indicates that the RPCF would offer little additional information over routine lipid tests in unselected "routine" specimens received for serologic testing. On problem serums, the test does not possess sufficient sensitivity to replace the TPI. It has been shown that the test in conjunction with the VDRL may be of value as a screen for the TPI. We feel that its true value in this respect will not be determined until an adequate evaluation can be made of the relatively small number of discrepancies between the screen and the TPI.

4. Are Results of the RPCF Reproducible?

Reproducibility or precision is an important consideration in the evaluation of any serologic technic. In the case of the treponemal tests it assumes added importance because of the reliance placed on the test result in establishing a diagnosis of syphilis. The TPI has been shown to possess a satisfactory reproducibility.[2,4,17,20,23,25] In the studies reported here the reproducibility of the RPCF varies considerably depending upon the type of specimen tested (Table 4).

In the Routine Study RPCF reproducibility is high with 97 per cent of the serums giving the same result on repeat testing. This is not surprising since 96 per cent of the specimens in this group were nonreactive and would be expected to yield highly reproducible results. As the proportion of reactive specimens increases in the study groups, reproducibility decreases. The Prenatal group with 20 per cent reactive RPCF specimens gave a reproducibility of 91 per cent. The BFP group, where approximately one-third of the specimens showed some RPCF reactivity, had a reproducibility of 88 per cent. In the Reactive group, where the opportunity for disagreement is even greater with two-thirds of the specimens showing some RPCF reactivity, RPCF reproducibility drops to 75 per cent.

It is known that in any serologic test the most difficult results to reproduce are

intermediate reactions. The reproducibility of a test is closely associated with the number of such reactions obtained. Previous work in this laboratory[4] has shown that only a small number of specimens tested by the TPI technic fall into this category. It is of interest to note that the number of weak reactions in the TPI on the group of specimens reported here is less than 10 per cent. In the RPCF in our hands approximately two-thirds of the reactions fall into the intermediate category, i.e., reactions other than negative or four plus. This large number of intermediate reactors undoubtedly contributes heavily to the general lack of reproducibility of the RPCF. Improvements in the RPCF antigen or changes in test technic could conceivably improve test reproducibility. Until such improvements are made, our experience suggests that the accuracy of the RPCF report can be improved by testing specimens on each of two testing days. If discrepancies occur, a third test should be performed and the report based on the three test results.

We have been discussing some of the problems encountered in the use of the RPCF test dependent upon types of specimens tested. There are further problems which may influence the type of reproducibility and reactivity obtained. These problems are technical in nature and should be carefully assessed before this procedure gains wide acceptance.

At the present time, at least three different technics for use with Reiter protein antigen are employed in the United States—the 1/5 volume Kolmer (KRP) as described by Bossak,[3] the 1/5 volume technic using one and one half units of complement as described by Portnoy and Magnuson,[21] and the 50 per cent end point technic of De Bruijn.[12] Bekker[1] recently reported on a comparison of these methods and advocated use of the technic employing one and one half units of complement as the most satisfactory from the standpoint of sensitivity and specificity. However, brochures accompanying RPCF antigens from various commercial sources and the USPHS Manual of STS[18] recommend use of two units of complement in the 1/5 volume Kolmer method. Use of a single technic throughout the country is desirable, but little work has been published regarding determination of the most satisfactory one.

The differences in RPCF technics have another significant facet. It is axiomatic that complement fixation methods utiliz-

Table 4—Reproducibility of RPCF Test Results on Four Study Groups

Type of Test Agreement \ Study Group	Routine (Study I)		Prenatal (Study III)		BFP (Study IV)		Reactive (Study II)	
	No.	%	No.	%	No.	%	No.	%
Exact agreement R→R; WR→WR; N→N	192	97	133	91	205	88	149	75
Partial agreement R→WR; WR→R	4	2	6	4	10	4	14	7
Partial disagreement WR→N; N→WR	1	<1	0	0	11	5	21	11
Complete disagreement R→N; N→R	0	0	7	5	6	3	15	7
Total	197	100	146	100	232	100	199	100

ing different amounts of complement will give different degrees of sensitivity and specificity. One must bear this in mind in comparing the work of investigators when different technics are employed.

Another difficulty was experienced by this laboratory soon after work began with the RPCF test. Very rapid clearing and reading times were obtained using the recommended control serum pattern as the basis of determining secondary incubation time. This rapid clearing was usually associated with low-complement titers. Similar difficulties experienced previously with full volume Kolmer tests were corrected by the addition of optimal amounts of calcium ion to the Kolmer saline.[5] When this saline was utilized in RPCF tests, secondary incubation times were increased and "valid" test runs were obtained as defined in the 1959 Manual of STS[18] (i.e., the expected pattern of the control serum was reached at least ten minutes after hemolysin controls cleared).

Serums of the Prenatal and BFP Study groups were tested in parallel with saline containing magnesium ion and no added calcium ion and with saline containing optimal amounts of both magnesium and calcium ions. Little variation of results of tests on serums so treated was obtained. When a difference in reaction occurred, the results of tests performed with saline containing added calcium and magnesium ions were in closer agreement with the TPI result than were results obtained using saline with only added magnesium ion.

Differences in the reactivity of different batches of RPCF antigen may well present an added problem in any evaluation of the RPCF test. The results of parallel testing of a limited number of serums in this laboratory indicate that antigens from different sources may vary in reactivity. Added problems may be presented by the deterioration of antigens. These problems indicate areas for further investigation.

Summary

In this paper we have explored the application of the RPCF in the serodiagnosis of syphilis in the public health laboratory. Evaluation of RPCF test results in four diagnostic categories of patients is described. It is emphasized that the sensitivity and reproducibility of the test is dependent upon the choice of diagnostic categories selected for study. The value of the RPCF appears to be limited by a lack of sensitivity and reproducibility in certain groups of patients. Because of lack of test sensitivity the RPCF alone is not a satisfactory replacement for the TPI. Use of the RPCF test in conjunction with a standard lipid test as a screening procedure for the TPI has been suggested. Evaluation of the small percentage of cases where the lipid test and the RPCF test are in agreement with each other but disagree with the TPI is necessary before full acceptance of this application of the test can be made. It is possible that with certain technical improvements eliminating the difficulties suggested in this study, the RPCF test may have a wider application than is indicated under current conditions.

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This paper was presented before a Joint Session of the Conference of State and Provincial Public Health Laboratory Directors and the Laboratory Section of the American Public Health Association at the Eighty-Eighth Annual Meeting in San Francisco, Calif., November 2, 1960.