Evaluation of Rapid Plasma Reagin (Circle) Card Test

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A RECENTLY reported modification of the rapid plasma reagin (RPR) card test (*1*) suggests its use in large-scale testing operations and provides for quantitative as well as qualitative testing. This method differs from the original RPR card test (*2*) in that the test areas are circular rather than in the shape of a "teardrop"; the antigen and serum are mixed by rotation on a mechanical rotator; and quantitative test results may be obtained by preparing and testing dilutions on the card. This test is referred to as the RPR (circle) card test.

This report presents the results of a study conducted to evaluate the performance of the RPR (circle) card test in parallel with VDRL slide tests, both qualitatively and quantitatively, on serum samples from clinically defined donor groups. The reproducibility of results obtained within and between laboratories performing this test experimentally on serum specimens distributed during the first 5 months of the current Syphilis Serology Evaluation Study is also reviewed.

Materials and Methods

The serums used in the study conducted to evaluate the results of the RPR (circle) card test in parallel with VDRL slide tests included samples from the serum bank, previously established at the Venereal Disease Research Laboratory; freshly collected serum samples from clinically defined donor groups; and specimens collected in 1963 for the Tuskegee Study. The serums from the serum bank were unheated samples (from selected donors) that had been stored frozen for varying periods of time. The serums from the Tuskegee Study (*3*) were unheated samples that had, for the most part, been stored frozen for a limited time.

The donors of the serums tested were classified in the following clinical categories:

Presumed normal. Donors with no history of previous or present infection with syphilis.

Syphilis. Donors with primary and secondary syphilis, proved by darkfield examination, who had received no treatment. Donors with latent, late, and congenital syphilis who were untreated, inadequately treated, or adequately treated.

Biologic false positive (*BFP*). Donors with reactive nontreponemal tests with no clinical evidence of syphilis and who had no previous *Treponema pallidum* immobilization (TPI) test.

The serum specimens distributed in the Syphilis Serology Evaluation Study were prepared from pools of human serum that were either reactive or nonreactive in serologic tests for syphilis. Neither the presence nor absence of syphilis in the blood donors used for this study was determined. The serums used were selected solely on the basis of their reactivity in serologic tests, since the objective of this study was only to determine the relative efficiency of each

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laboratory's performance of a testing procedure. Twenty prepared serum specimens were distributed to each of the participating laboratories each month from July through November 1963. The specimens distributed under code number in the first, fourth, and fifth months represented duplicates of 30 prepared serums. The coded serums distributed during the second and third months represented prepared individual serums.

The RPR (circle) card test was performed in accordance with instructions received from the test author, Dr. Joseph Portnoy. The exact procedure, as used in this testing, has since been published (*1*).

The VDRL slide tests were performed according to the techniques described in the Manual of Serologic Tests for Syphilis, 1959 revision (*4*). VDRL slide quantitative test B was the method employed in this study.

Results

The comparison of the test results of the qualitative RPR (circle) card and VDRL slide tests on 784 serums from clinically defined donor groups is shown in table 1. Absolute agreement (100 percent) was obtained between the RPR (circle) card and VDRL slide tests on the 260 serums from presumed normal donors. Agreement between the two tests with the group of BFP serums was 90.6 percent and in the total syphilis group, 92.4 percent. In the several syphilis categories where there was disagreement between the two tests, the RPR (circle) card test was more reactive on 22 serums and the VDRL slide test was more reactive on 14 serums. The overall agreement on the total of 784 serums was 94.8 percent.

The reactivity rate of the RPR (circle) card test and the VDRL slide test with the three groups (presumed normal, BFP, and syphilis) and with the individual syphilis categories are shown in table 2. For this comparison, reactive and weakly reactive results with the VDRL slide test are considered reactive. Neither test was reactive with serums from the 260 presumed normal donors. The RPR (circle) card test was 84.9 percent reactive with the serums from the BFP group, while the VDRL slide test had 90.6 percent reactivity. Reactivity with the total group of syphilis patients was 88.1 percent with the RPR (circle) card test and 86.4 percent with the VDRL slide test.

Table 1. Comparative results of qualitative RPR (circle) card and VDRL slide tests on serums from clinically defined donors

Comparative results, RPR (circle) card and VDRL slide tests	Presumed normal	BFP [1]	Syphilis					Total
			Primary	Secondary	Latent	Late	Congenital	
Agreement:								
Both reactive	0	31	94	209	43	5	2	384
RPR reactive, VDRL weakly reactive	0	13	7	2	29	1	1	53
Both nonreactive	260	4	28	0	12	2	0	306
Total	260 (100.0)	48 (90.6)	129 (96.3)	211 (97.2)	84 (80.0)	8 (72.7)	3 (75.0)	743 (94.8)
Disagreement:								
RPR nonreactive, VDRL weakly reactive	0	2	4	[2] 2	2	0	0	10
RPR nonreactive, VDRL reactive	0	2	0	[2] 4	2	0	0	8
RPR reactive, VDRL nonreactive	0	1	1	0	17	3	1	23
Total	0	5 (9.4)	5 (3.7)	6 (2.7)	21 (20.0)	3 (27.3)	1 (25.0)	41 (5.2)
Total specimens	260	53	134	217	105	11	4	784

[1] Biologic false positive reactors. [2] Five of these specimens gave zonal reactions in the quantitative RPR (circle) card test.

NOTE: Figures in parentheses are percentages.

In the individual syphilis categories the two tests were similar in reactivity in the primary and secondary groups, but the RPR (circle) card test was more reactive in the latent, late, and congenital groups.

The comparison of quantitative test results obtained with the RPR (circle) card test and the VDRL slide test (method B) on 377 serums from BFP and syphilis groups is summarized in table 3. Within the limits of reproducibility of quantitative testing, agreement is usually considered to be identical titers or agreement within plus or minus one dilution. There was 75.9 percent agreement in titers according to this definition, compared with 83.0 percent agreement reported by Portnoy on a group of 123 specimens. On the serums showing a difference of two or more dilutions between the two tests, 86 gave higher titers in the RPR (circle) card test and only 5 had higher titers in the VDRL slide test. These findings are comparable to the results reported by Portnoy, who found that of 21 specimens showing a difference of two or more dilutions in his study, 18 represented higher titers in the RPR (circle) card test.

To evaluate the relative efficiency of the performance of the RPR (circle) card test in a number of laboratories, the data obtained on the performance of this test in the first 100 specimens of the 1964 Syphilis Serology Evaluation Study were analyzed. Including the control laboratory, 14 laboratories performed the qualitative test and 6 laboratories performed the quantitative test.

The 14 laboratories included the Venereal Disease Research Laboratory (control laboratory), the author's laboratory (Portnoy), the

Table 2. Reactivity of the RPR (circle) card and VDRL slide tests on serums from clinically defined donors

Clinical category	Number specimens	RPR (circle) card		VDRL slide	
		Number reactive	Percent reactive	Number reactive	Percent reactive
Presumed normal	260	0	0	0	0
Biologic false positive reactors	53	45	84.9	48	90.6
Syphilis:	471	415	88.1	407	86.4
Primary	134	102	76.1	105	78.4
Secondary	217	211	97.2	217	100.0
Latent	105	89	84.8	76	72.4
Late	11	9	81.8	6	54.5
Congenital	4	4	100.0	3	75.0

Table 3. Comparison of quantitative RPR (circle) card and VDRL slide tests results on serums from clinically defined donors

Comparison of titers	Non-syphilis	Syphilis					Total	
	BFP [1]	Primary	Secondary	Latent	Late	Congenital	Number	Percent
Absolute agreement	14	29	53	18	2	----	116	30.8
Agreement within ±1 dilution	27	27	65	48	3	----	170	45.1
RPR (circle) card, 2 or more dilutions higher	8	10	20	38	6	4	86	22.8
VDRL slide, 2 or more dilutions higher	----	2	2	1	----	----	5	1.3
Total	49	68	140	105	11	4	377	100.0

[1] Biologic false positive reactors.

commercial manufacturer producing the RPR (circle) card test kits, and 11 State health department laboratories. The analysis of test results is given in table 4. Agreement with the control laboratory (VDRL) on the qualitative RPR (circle) card test ranged from 81 to 96.3 percent and reproducibility on the duplicate specimens varied from 93.3 to 100 percent. Defining adequacy of test performance as at least 90 percent agreement with the control laboratory and 96 percent reproducibility on paired specimens, 8 of 13 participant laboratories achieved satisfactory results. Six laboratories performed the RPR (circle) card quantitative test. Considering 85 percent agreement with the control laboratory and 93 percent reproducibility on duplicate specimens as indicative of adequacy of test performance, none of the laboratories attained satisfactory results with the quantitative test. Although four of five laboratories had satisfactory reproducibility on duplicate specimens, agreement with the control laboratory ranged from 70 to a high of only 82.5 percent.

Discussion

The RPR (circle) card test, a modification of the RPR (teardrop) card test, is designed for use in the public health or clinic laboratory where testing is performed on a large scale. Portnoy considers this test a diagnostic rather than a screening procedure and includes a technique for quantitation. All the materials for performing the test (antigen suspension, capillary pipettes, and qualitative and quantitative test cards) are included in kits and are entirely disposable. The test is performed on unheated serum directly from the collection tube, and results are read macroscopically after an 8-minute rotation period at 100 rpm on a mechanical rotator.

The overall agreement between the qualitative RPR (circle) card and VDRL slide tests on 784 serums from presumed normal donors, from a group of biologic false positive reactors, and from syphilitic donors in 5 categories was 94.8 percent. The RPR (circle) card test was 2 percent more reactive in the syphilis group of 471 serums, and the VDRL slide test gave 6.7 percent more reactions with the 53 specimens in the BFP category. Six serums from donors with secondary syphilis were nonreactive with the qualitative RPR (circle) card test and five of these serums gave zonal reactions in the quantitative RPR (circle) card test.

The evidence of zoning was eliminated by a slight modification in the published testing procedure, suggested by Portnoy, when this problem was brought to his attention. When the unheated serum was spread to fill the entire circle on the card and the drop of antigen was added just before rotation and was not mixed with the serum, reactive qualitative results were

Table 4. Analysis of test performance of RPR (circle) card test in syphilis serology evaluation study (specimens 1–100), fiscal year 1964

Laboratory code No.	Percent agreement with control laboratory results		Total serums	Percent reproducibility on duplicate specimens		Total serums
	Qualitative	Quantitative		Qualitative	Quantitative	
VDRL	----------	----------	100	100. 0	100. 0	60
1	96. 3	----------	80	100. 0	----------	40
2	94. 0	70. 0	100	100. 0	100. 0	60
3	93. 0	74. 0	100	100. 0	90. 0	60
4	93. 0	----------	100	100. 0	----------	60
5	93. 0	80. 0	100	100. 0	100. 0	60
6	92. 0	----------	100	100. 0	----------	60
7	91. 0	81. 0	100	100. 0	100. 0	60
8	90. 0	----------	100	100. 0	----------	60
9	89. 0	----------	100	100. 0	----------	60
10	88. 0	----------	100	96. 7	----------	60
11	88. 0	82. 5	100	93. 3	93. 3	60
12	87. 0	----------	100	93. 3	----------	60
13	81. 0	----------	100	100. 0	----------	60

obtained on those serums that gave nonreactive results qualitatively and gave zonal reactions quantitatively when tested according to the published procedure. Among a number of RPR card test antigen suspensions, differences in eliciting zonal reactions were observed; however, when testing was performed as described above, no antigen suspension tested showed a zonal reaction.

Summary and Conclusions

A comparison of results obtained with the RPR (circle) card and the VDRL slide tests on serums from clinically defined categories indicates that the qualitative RPR (circle) card test is comparable in reactivity to the VDRL slide test. The agreement of qualitative RPR (circle) card test results performed in a number of laboratories with the results obtained by the control laboratory was relatively satisfactory for a new test procedure.

The comparison of the quantitative RPR (circle) card and the VDRL slide tests on 377 serums, considering agreement to be identical titers or within plus or minus one dilution, showed a 75.9 percent agreement between the two tests. Disagreement in titer occurred in 24.1 percent of these specimens and in the majority of instances where differences in titer occurred, the quantitative RPR (circle) card test was two or more dilutions higher.

None of the five laboratories performing the quantitative RPR (circle) card test on the first 100 specimens of the Syphilis Serology Evaluation Study had satisfactory test performance, based on the criteria to indicate adequacy of test performance used in evaluating the results of the fiscal year 1963 Syphilis Serology Evaluation Study (*5*). The desirability of an adequate test procedure employing a stable antigen suspension and inexpensive, expendable equipment is obvious. Possibly, more favorable results will be obtained in the Syphilis Serology Evaluation Study when participating laboratories have gained more experience with the RPR (circle) card test.

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