Rapid Plasma Reagin Card Test for Syphilis and Other Treponematoses

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IN May 1961 Dr. John H. Brewer demonstrated a test for syphilis that mixed the patient's serum with VDRL antigen containing carbon particles that had been dried on plastic-coated white cardboard cards. This test had the advantage of using disposable components and of being performed without laboratory apparatus except that used for the separation of serum. At Dr. Brewer's request the Public Health Service Venereal Disease Research Laboratory entered into a cooperative agreement with him to evaluate this procedure.

Although this test had many attributes of a screening test for syphilis that could be rapidly performed under field conditions, there was an apparent need for a rapid, simple means of collecting and separating blood without centrifugation, if an ideal field-testing procedure was to be developed. Early testing indicated that VDRL antigen when dried on the cards or in ampules did not possess the desired stability.

The rapid plasma reagin test for syphilis with unheated plasma (*1,2*) or serum (*3*) had some of the characteristics desired for the "card" test, such as stable antigen suspension, rapid performance, and high sensitivity level. During this cooperative study, the desirable attributes of both procedures, together with a slide for the rapid collection and separation of plasma from finger-stick blood, were incorporated into the testing procedure herein designated as the rapid plasma reagin (RPR) card test.

This report describes the technique for the RPR card test and presents the preliminary evaluation of this test compared with the VDRL slide test in selected patient categories.

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The antigen suspension and testing materials, including the Brewer plasma collection slide, used in the serologic evaluations were supplied by Hynson, Westcott & Dunning, Inc.

Materials and Methods

Equipment and Glassware

1. Brewer plasma collection slide (*A*).
2. Brewer diagnostic card (*B*).
3. Capillary tubes capable of measuring 0.03 ml. (*C*).
4. Rubber bulbs for use with capillary tubes.

Reagents

1. Antigen: VDRL slide flocculation test antigen (*4*).
2. Saline solution: 1 percent buffered saline solution (VDRL flocculation test buffered saline).
3. Phosphate (0.02 M); Merthiolate, 0.2 percent solution (*D*): Dissolve 1.42 gm. Na_2HPO_4, 1.36 gm. KH_2PO_4, and 1 gm. Merthiolate in distilled water to a final volume of 500 ml. The pH of this solution should be 6.9. Store in

dark at room temperature. May be used for a period of 3 months.

4. Choline chloride solution (40 percent):

(*a*) Dissolve 40 gm. choline chloride in distilled water to a final volume of 100 ml.

(*b*) Filter and store at room temperature. May be used for 1 year. Refilter if visible particles form.

5. EDTA (0.25 M): Dissolve 9.3 gm. ethylene dinitrilo tetra-acetic acid, disodium salt in approximately 90 ml. of distilled water. Adjust pH to 7.0 with NaOH and add distilled water to 100 ml.

6. Charcoal suspension (0.25 percent): Suspend 25 mg. charcoal (*E*) in 10 ml. distilled water.

7. Resuspending solution: This solution is freshly prepared each time antigen suspensions are made. To prepare 10 ml. of resuspending solution, combine the following:

	Milliliter
EDTA (0.25 M)	0.5
Choline chloride (40 percent)	2.5
Phosphate (0.02 M), Merthiolate (0.2 percent)	5.0
Distilled water	1.0
Charcoal suspension (0.25 percent)	1.0

Preparation of Antigen Suspension

1. Prepare antigen emulsion as for the VDRL flocculation tests (*5*, *6*).

2. Centrifuge measured aliquots of the antigen emulsion at room temperature for 15 minutes at 2,000 g.

3. Decant the supernatant fluid by inverting the tube, taking care not to disturb the sediment. While holding the tube in an inverted position, wipe the wall of the tube with cotton gauze without disturbing the sediment.

4. Resuspend the sediment with a volume of resuspending solution equal to that of the centrifuged antigen suspension. Blow the solution directly onto the sediment. Agitate the centrifuge tubes by hand to aid in resuspension.

5. If more than one centrifuge tube is used, combine all resuspended aliquots. This is the completed antigen suspension.

Preparation and Use of Controls

Controls are prepared by diluting reactive plasma in nonreactive plasma or by diluting reactive serum in nonreactive serum. Dilutions that produce the desired degree of reactivity are selected by trial testing and are maintained for daily use.

Antigen suspension is tested with controls of known reactivity prior to performing tests with unknown specimens, and only those suspensions that have given the designated reactions are used in testing unknowns.

Plasma Collection

The plasma collection kit contains only three items: a sterile lancet, a Brewer plasma collection slide, and a toothpick, which serves as a stirring device. The kit is enclosed in a metal plastic-sealed package. The Brewer plasma collection slide measures 2 inches by 5 inches and is made from plastic-coated board (fig. 1). It has a 1-inch keyhole-shaped depression in the center and a perforated line 1 inch from the

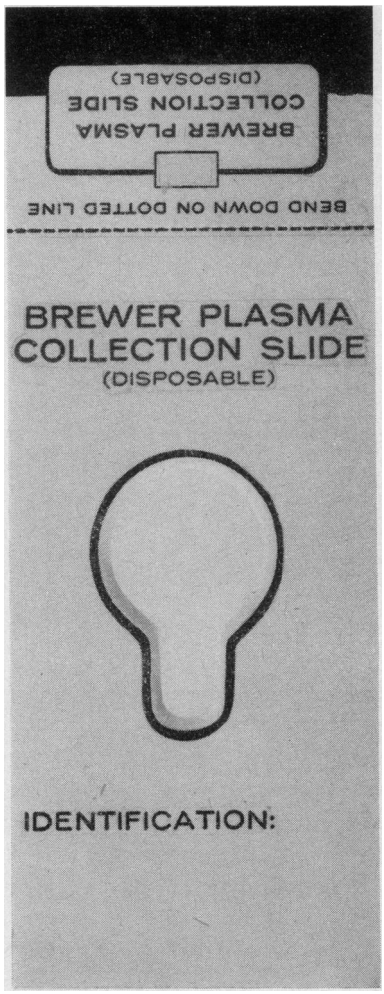

Figure 1

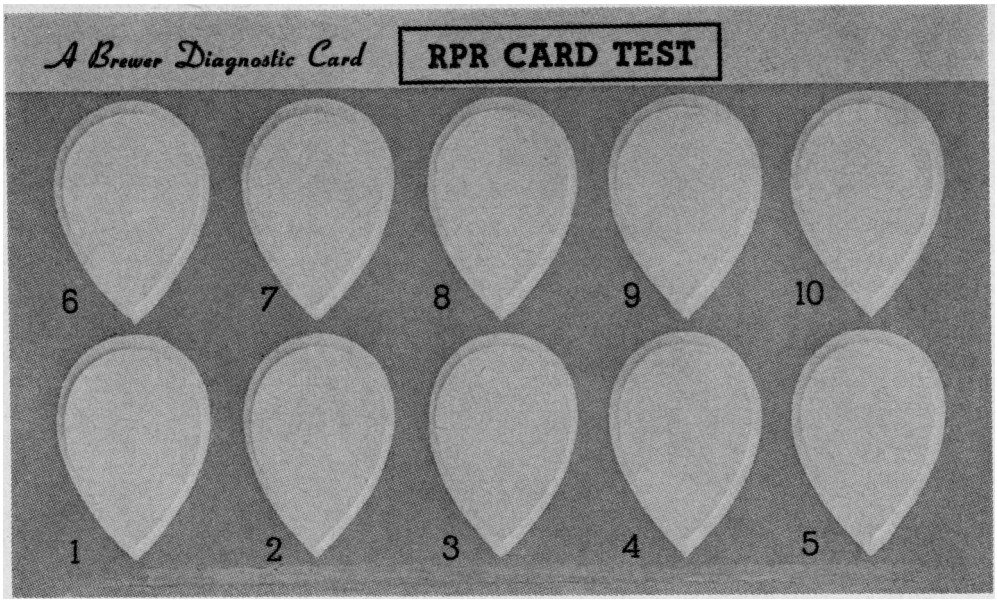

Figure 2

top. The top section can be bent down to form a support which will allow the plasma to drain down into the narrow part of the "keyhole." The depressed area of the slide is coated with an anticoagulant and a lectin, which are stable at room temperature. The lectin is of a type which will cause both red and white blood cells to agglutinate, leaving the plasma free to flow down into the collection slot.

The directions for use of the Brewer plasma collection slide are as follows:

1. Tear off end of package containing plasma collection kit and remove collection slide. Write patient's name or laboratory number at bottom of slide with a ballpoint pen or glass marking pencil. Cleanse area to be punctured—finger, toe, or ear—with antiseptic solution. Use sterile lancet to obtain blood.

2. Allow three drops of blood to fall freely on the circular portion of the depressed area of the slide, carefully avoiding entry of blood into narrow plasma collection slot.

3. Holding the toothpick in an almost horizontal position, spread the blood over the entire circular area, stirring gently for approximately 20–30 seconds. Avoid contact with the inked border which surrounds the depressed area of the slide.

4. Pick up slide and, using a tilting motion, cause the blood to rotate within the circle until a pronounced clumping of the blood cells and the coincident separation of the plasma are noted.

5. Place slide on table with the perforated line coinciding with the edge of the table and the short end of the slide extending over the edge. Fold down tab against table edge.

6. Place slide on flat surface to allow plasma to drain into the collection slot. This usually takes 1 or 2 minutes.

Plasma may then be removed for testing in the unheated state with the capillary tube.

NOTE: If considerable delay is encountered in testing the plasma and drying is anticipated, it is advisable to place the collection slide in a humidifying device.

SERUM COLLECTION

Blood is collected in clean, dry tubes not containing anticoagulant and is allowed to clot. Serum is separated in the usual manner and is tested in the unheated state.

PERFORMANCE OF TEST

1. Using capillary tube, remove 0.03 ml. of unheated plasma from the Brewer plasma collection slide or 0.03 ml. of unheated serum and place on one test area of the Brewer diagnostic card (fig. 2).

2. Add one drop (approximately 1/70 ml.) of antigen suspension, using needle and plastic

dispensing bottle, to each plasma or serum. Hold dispenser in vertical position.

3. Using a separate clean toothpick for each plasma or serum, mix antigen suspension with test specimen gently but completely, spreading the mixture so that it fills the entire test surface.

4. Shake by tilting test card to and fro for a maximum of 4 minutes, allowing time for the mixture to flow into the apex so that the par-

Table 1. Comparison of RPR card (plasma) and VDRL slide tests on 600 syphilis patients

Clinical category	RPR card test		VDRL slide test		
	Test result	Number patients	Reactive	Weakly reactive	Non-reactive
Primary and secondary syphilis:					
Untreated	Reactive	54	51	1	2
	Nonreactive	3	1	1	1
Treated	Reactive	94	75	7	12
	Nonreactive	85	6	6	73
Latent syphilis:					
Untreated	Reactive	24	23	0	1
	Nonreactive	2	2	0	0
Treated	Reactive	217	176	24	17
	Nonreactive	82	23	7	52
Late syphilis, treated	Reactive	9	8	0	1
	Nonreactive	0	0	0	0
Congenital syphilis, treated	Reactive	28	20	5	3
	Nonreactive	2	0	0	2
Totals	Reactive	426 (*71. 0*)	353 (*58. 8*)	37 (*6. 2*)	36 (*6. 0*)
	Nonreactive	174 (*29. 0*)	32 (*5. 4*)	14 (*2. 3*)	128 (*21. 3*)
Grand total		600 (*100. 0*)	385 (*64. 2*)	51 (*8. 5*)	164 (*27. 3*)

NOTE: In total lines, figures in parentheses are percentages.

Table 2. Comparison of RPR card (plasma) and VDRL slide tests on 1,802 patients without syphilis diagnosis

Clinical category	RPR card test		VDRL slide test		
	Test result	Number patients	Reactive	Weakly reactive	Nonreactive
Contact to infectious syphilis:					
Untreated	Reactive	27	16	4	7
	Nonreactive	218	0	2	216
Treated	Reactive	3	0	1	2
	Nonreactive	14	0	2	12
Cluster,[1] untreated	Reactive	11	2	1	8
	Nonreactive	63	0	2	61
No history of syphilis	Reactive	54	31	9	14
	Nonreactive	394	6	9	379
Premarital	Reactive	6	2	0	4
	Nonreactive	306	0	1	305
Gonorrhea, untreated	Reactive	16	2	0	14
	Nonreactive	690	0	3	687
Totals	Reactive	117 (*6. 5*)	53 (*3. 0*)	15 (*0. 8*)	49 (*2. 7*)
	Nonreactive	1, 685 (*93. 5*)	6 (*0. 3*)	19 (*1. 1*)	1, 660 (*92. 1*)
Grand total		1, 802 (*100. 0*)	59 (*3. 3*)	34 (*1. 9*)	1, 709 (*94. 8*)

[1] Contacts, associates, and suspects related to persons with infectious syphilis.

NOTE: In total lines, figures in parentheses are percentages.

ticles will be in close proximity and then to spread out as the particles flow away from the apex.

5. Read macroscopically and report as "reactive" specimens showing characteristic clumping; report as "nonreactive" specimens showing no clumping at the end of the 4-minute shaking period.

NOTE: Clumping is characterized by the appearance of a front of agglutinated particles which move out from the apex of the teardrop test area. As the particles reach the outer limit of the teardrop they tend to deposit at the periphery. When this clumping is observed in less than 4 minutes it is not necessary to continue the shaking. However, the full 4-minute shaking period must be used for specimens not considered reactive.

Evaluation

The RPR card test was performed on plasma from approximately 2,400 randomly selected patients of the Fulton County (Ga.) Health Department venereal disease clinic and the social hygiene clinic of the Houston (Tex.) City Health Department who were routinely subjected to venipuncture for VDRL slide testing. Technologists from the Venereal Disease Research Laboratory obtained the finger-stick blood and performed the RPR card test on these patients. Results of the VDRL slide test and diagnoses were later obtained from clinic records for comparative purposes. The VDRL tests discussed in this section of the report were performed by the Georgia Department of Public Health and the Houston City Health Department. Results of these tests, divided into diagnostic categories, are shown in tables 1 and 2.

The RPR card and VDRL slide tests were also performed at the Venereal Disease Research Laboratory in Chamblee, Ga., on serums from venipuncture blood stored in a serum bank that is maintained for comparative evaluation of new or modified testing procedures. The 248 serums used for this comparison were from several diagnostic categories, including three of the treponematoses—syphilis, yaws, and pinta—and were from presumed nonsyphilitic donors with or without other diseases. Results of this serum testing are shown in tables 3 and 4.

Discussion

The RPR card test is designed for use as a field test or an office procedure. It does not require any of the usual laboratory equipment. Centrifuges, water baths, rotating machines, microscopes, or other expensive apparatus usually associated with serologic testing are eliminated. All materials employed are inexpensive and disposable. All supplies and apparatus for performing 100 tests can be included in a kit which occupies less than 1 square foot of desk space. This includes the material for drawing the blood and separating the plasma. Individual tests, including collection of blood and separation of plasma, can be run in 7 or 8 minutes.

In principle, the RPR card test makes use of the RPR test antigen suspension (*2*) to which

Table 3. Comparison of results of RPR card and VDRL slide tests for treponematoses on serum bank specimens

Clinical category	RPR card test		VDRL slide test		
	Test result	Number patients	Reactive	Weakly reactive	Non-reactive
Primary and secondary syphilis, untreated	Reactive	18	16	2	0
	Nonreactive	7	0	0	7
Syphilis, treated [1]	Reactive	19	13	5	1
	Nonreactive	21	0	0	21
Yaws, treated	Reactive	29	29	0	0
	Nonreactive	0	0	0	0
Pinta, treated	Reactive	37	33	3	1
	Nonreactive	5	3	1	1

[1] Primary, secondary, latent, and late.

Table 4. Comparison of results of RPR card and VDRL slide tests on serum bank specimens from presumed nonsyphilitic patients

Clinical category	RPR card test		VDRL slide test		
	Test result	Number patients	Reactive	Weakly reactive	Non-reactive
Presumed normal	Reactive	0	0	0	0
	Nonreactive	20	0	0	20
Diseases other than syphilis	Reactive	0	0	0	0
	Nonreactive	20	0	0	20
Biologic false positive reactors	Reactive	27	20	7	0
	Nonreactive	18	2	0	16
Leprosy	Reactive	0	0	0	0
	Nonreactive	27	3	2	22

has been added a small amount of specially prepared charcoal, to act as a visualization agent. The particle size of the charcoal is such that the nonreactive specimens appear to have an even light-gray color. When flocculation occurs, there is a conglutination that is readily visible without the aid of a microscope. The test is performed on plastic-coated cards which, if allowed to dry properly, may be filed for future reference.

The effectiveness of the RPR card test in providing serologic support for the diagnosis of syphilis is apparent from the results given in table 1, which also demonstrates the generally good agreement between results of the card test and the VDRL slide test. In untreated syphilis the card test had essentially the same capacity to detect antibody (reagin) as the VDRL slide test, and a high level of agreement between the two tests was apparent. On the basis that a reactive RPR card test accompanied by a reactive or weakly reactive VDRL slide test constituted agreement, as did nonreactive results with both tests, 7 out of 83 cases of untreated syphilis, or approximately 8 percent, gave disagreeing results. By contrast, in the treated syphilis group, approximately 15 percent of 517 cases showed disagreement. There was little difference in the reactivity level of the two tests; 71.0 percent of the patients with a clinical diagnosis of syphilis were reactive in the RPR card test and 72.7 percent in the VDRL slide test (64.2 percent reactive, 8.5 percent weakly reactive).

The potential value of the RPR card test as a screening procedure and as an additional arouser of suspicion of syphilis is suggested from table 2, which presents a comparison of the RPR card test and the VDRL slide test on 1,802 patients without definite diagnoses of syphilis. In 245 untreated patients named as known contacts to infectious syphilis, 27, or approximately 11 percent, were reactive in the card test; approximately 7 percent were reactive and 2 percent were weakly reactive in the VDRL test. The group of patients identified as "cluster, untreated" represents persons asked to report to the clinics through the use of the cluster procedure (7), a technique for increasing the detection of syphilis by blood-testing contacts, associates, and suspects related to persons with known infectious syphilis. In this category approximately twice the number of reactors was obtained with the RPR card test as with the VDRL slide test. This disparity in number of reactors was likewise seen in the premarital and untreated gonorrhea categories. Although the percentage of reactive results in the RPR card test was low, approximately 2 percent in each of these categories, the VDRL test gave 1 percent or less reactors. It was only in the category "no history of syphilis," which included patients with genitourinary complaints or conditions, chancroid, or diagnoses of biologic false positive, that the number of reactors in the two tests was essentially the same.

Since the RPR card test was significantly more reactive than the VDRL slide test in patients without a diagnosis of syphilis, it might be inferred that the card test was more nonspecific than the VDRL test. However, since

patients attending the clinics are venereal disease prone, the possibility exists that syphilis, past or present, was being detected by the card test. In the absence of other data, such as results of treponemal tests, in these cases it is difficult to arrive at a better understanding of the reactivity pattern. If the card test were more nonspecific than the VDRL slide test, it might be expected that this would also be noted with serums of presumed nonsyphilitic patients. However, table 4 shows that the RPR card test was more specific than the VDRL test. It is possible, of course, that qualitative differences between plasma and serum may be responsible for this peculiar behavior. It should be realized that the reactions are compatible with the concept that the RPR card test may be used as a screening procedure and suspicion arouser.

Although the RPR card test was conceived for use as a field or office procedure employing plasma, it was of interest to examine the behavior of serum in an identical test. The results presented in tables 3 and 4 clearly indicate that the RPR card test had a satisfactory reactivity level with unheated serum.

Similar findings were obtained with the RPR card test and the VDRL slide test in syphilis, yaws, and pinta (table 3). In the presumed nonsyphilitic group the RPR card test was apparently more specific than the VDRL slide test (table 4). Its behavior in leprosy was of particular interest since all 27 patients were nonreactive, whereas 5 of these patients showed some reactivity in the VDRL test.

The results obtained in this study point to the potential usefulness of the RPR card test in syphilis and other treponematoses. The results obtained with leprosy patients suggest that it might be more specific than the VDRL slide test in detecting treponematoses in areas where leprosy is endemic.

There has been an urgent need for a more efficient field-testing procedure for the treponematoses. The RPR card test performed with plasma obtained with the Brewer plasma collection slide has the necessary components for an effective field test: (*a*) a rapid, simple method for obtaining plasma from finger-stick blood, requiring no centrifuge; (*b*) a stable antigen suspension; (*c*) rapid performance; and (*d*) adequate sensitivity and specificity.

This evaluation of the RPR card test is limited and preliminary, but results appear to justify an early report of technique and methodology so that more extensive testing and evaluation may be accomplished by other investigators. A broader field evaluation of the RPR card test now being conducted by the Venereal Disease Branch, Communicable Disease Center, will be the subject of a later report.

Summary and Conclusion

The RPR card test for syphilis and other treponematoses has the elements required for an ideal field test.

The chief characteristics of the test which make for rapidity and ease of performance are (*a*) application of a device for obtaining plasma from finger-stick blood in a rapid and simple manner; (*b*) a stable antigen suspension containing charcoal; and (*c*) use of a plastic-coated card surface to perform the test.

Results obtained with the RPR card test have been compared with clinical diagnoses and with results of the VDRL slide test. Preliminary findings contained in this report indicate that the RPR card test has adequate sensitivity and specificity.

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(*7*) Brown, W. J.: Cluster testing—a new develop-

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EQUIPMENT REFERENCES

(*A*) Brewer plasma collection slide (patent applied for), Becton-Dickinson & Co., Rutherford, N.J.
(*B*) Brewer diagnostic card (patent applied for), Hynson, Westcott & Dunning, Inc., Baltimore, Md.
(*C*) Capillary tubes, Hynson, Westcott & Dunning, Inc., Baltimore, Md.
(*D*) Merthiolate, Eli Lilly and Co., Indianapolis, Ind.
(*E*) Specially prepared charcoal, small-particle size, Hynson, Westcott & Dunning, Inc., Baltimore, Md.

Communications at Scientific Meetings

The National Association of Science Writers has published "A Handbook for Press Arrangements at Scientific Meetings."

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• Newsmen need and welcome assistance in performing their work, but do not expect to be and should not be coddled.

• A pressroom is not and should not be a barroom.

• It can be most helpful if, as a matter of course, all members and speakers list their local addresses when registering. . . . One press officer writes that "A paging or message system to find scientists and bring them to the pressroom can be the most important service that the individual in charge of a pressroom can perform. Plans for this must be made in advance."

• The pressroom should be large enough to accommodate the anticipated number of reporters and photographers.

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