A Preliminary Report On RPR Test for Syphilis Using Unheated Serum

JOSEPH PORTNOY, PH.D.
WARFIELD GARSON, M.D., M.P.H.

THE RAPID plasma reagin (RPR) test was developed chiefly to permit rapid and economical screening of large numbers of persons so that reactors could be given immediate specific and prophylactic treatment (*1*). As reported recently (*2*), the objectives of the RPR test were realized when it was used at the reception center in El Centro, Calif., where large numbers of Mexican farm laborers were processed rapidly and economically by a small staff in an improvised laboratory at the site of operations. The success of the El Centro operation led to the establishment of four additional RPR testing stations on the border between Mexico and the United States.

Observations made during the development of the RPR test had suggested that the RPR antigen suspension could be applied to tests with unheated serum. It was found that the RPR antigen gave a satisfactory level of sensitivity and specificity in the SERA study (*3*) when 0.05 ml. of unheated serum was tested with 1/45 ml. of RPR antigen suspension.

As an extension of these studies, the testing of unheated serum using the RPR antigen and the same technique as for testing unheated plasma was undertaken. The results of testing two groups of specimens are reported here.

Materials and Methods

One group of 149 specimens was obtained during evaluation studies of anticoagulants for the RPR test on unheated plasma. These specimens were duplicate samples drawn to produce the conventional clotted-blood specimen.

Most (1,740) of the specimens studied were collected in North Carolina during a serologic survey conducted by personnel of the venereal disease control section, North Carolina State Board of Health, under the direction of Dr. B. J. Rosenblum.

The technique used for testing with unheated serum was as follows:

Preparation of antigen suspension. The RPR antigen suspension was prepared by the method originally described for use with the RPR test on unheated plasma (*1*).

Preparation of specimens. The blood specimens were centrifuged at room temperature at 1,500–2,000 rpm for 4 minutes. The serum was allowed to remain in the original collection tube. Specimens were then tested without heating.

Performance of test. Procedure was the same as originally described for performance of the RPR test (*1*), except that three drops of serum instead of three drops of plasma were used with one drop of antigen suspension. Both serum and antigen suspension were at room temperature at the time of testing.

In addition to the RPR test on unheated plasma (performed as described (*1*) on the first group of specimens only), the other serologic tests used were the VDRL slide (*4*) and the tpcf 50 (*5*). These two tests were performed on serum separated from the clot after completion of the RPR test with unheated serum. For both tests the serum was heated at 56° C. for 30 minutes.

Results

Samples of all 149 of the first group of specimens were subjected, over a period of several months, to the four serologic tests. Comparative serologic findings for this group are shown in table 1.

The reactivity rates (the sum of reactive plus weakly reactive results expressed as percentages of the total results) of the four tests were quite similar for this group of specimens. The RPR test on unheated serum and the VDRL slide test were 45.0 percent reactive, the tpcf 50 test was 47.0 percent, and the RPR test on unheated plasma was 49.0 percent reactive.

Dr. Portnoy and Dr. Garson are with the Venereal Disease Experimental Laboratory, Communicable Disease Center, Public Health Service, at the University of North Carolina's School of Public Health, Chapel Hill. Dr. Portnoy is an immuno-serologist and Dr. Garson is laboratory director. *Technical assistance was provided by Carl Adams.*

Table 1. Comparison of results of RPR test (unheated serum) with results of 3 other serologic tests on 149 specimens

Type of reaction	RPR test with unheated serum	RPR test with unheated plasma			VDRL slide test			tpcf 50 test	
		Reactive	Weakly reactive	Nonreactive	Reactive	Weakly reactive	Nonreactive	Reactive	Nonreactive
Reactive	56	55	1	0	50	4	2	53	3
Weakly reactive	11	9	2	0	6	2	3	11	0
Nonreactive	82	3	3	76	3	2	77	7	75
Total	149	67	6	76	59	8	82	71	78

Defining agreement as a reactive or weakly reactive result to one test accompanied by a reactive or weakly reactive result to the other test, results to the RPR test on unheated serum and to the RPR test on unheated plasma agreed in 96.0 percent of the cases. Results to RPR on unheated serum and to both VDRL slide and tpcf 50 tests agreed in 93.3 percent of the cases.

The group of 1,740 specimens obtained from the serologic survey were given the RPR test with unheated serum in the Venereal Disease Experimental Laboratory, with the following results:

	Number of specimens	*Percent of specimens*
Reactive	156	9.0
Weekly reactive	48	2.8
Nonreactive	1,536	88.3
Total	1,740	100.1

On the basis of results, 417 specimens were selected for comparative testing with the VDRL slide and tpcf 50 tests. The specimens, when sufficient serum was available, included all those which had tested reactive, weakly reactive, or "negative rough," as well as an approximate 10 percent sample of the nonreactives (showing no clumping). Specimens in the "negative rough" category showed some clumping, but less than those categorized as weakly reactive and were reported nonreactive.

The VDRL slide and tpcf 50 tests were performed on these 417 specimens 6 to 7 days after the RPR test with unheated serum (table 2). The RPR test on unheated serum showed the lowest reactivity rate—45.6 percent. VDRL slide test results were 49.9 percent reactive, while the tpcf 50 test, with results 54.5 percent reactive, showed the highest reactivity rate. RPR test (unheated serum) results agreed with VDRL slide test results in 86.8 percent of the cases, with tpcf 50 test results in 82.7 percent of the cases.

To determine the effect on the RPR test of

Table 2. Comparison of results of RPR test (unheated serum) with results of 2 other serologic tests on 417 specimens

Type of reaction	RPR test with unheated serum	VDRL slide test			tpcf 50 test	
		Reactive	Weakly reactive	Nonreactive	Reactive	Nonreactive
Reactive	150	102	38	10	143	7
Weakly reactive	41	4	28	9	30	11
Negative rough	53	4	30	19	34	19
Nonreactive	173	0	2	171	20	153
Total	417	110	98	209	227	190

Table 3. Comparison of results of original RPR test with unheated serum and retest 1 week later of 68 specimens

Type of reaction	Original RPR test with unheated serum	Repeat RPR test with unheated serum stored approximately 1 week		
		Reactive	Weakly reactive	Nonreactive
Reactive	16	14	2	0
Weakly reactive	2	0	2	0
Nonreactive	50	0	1	49
Total	68	14	5	49

storage of serum separated from the clot, repeat tests with unheated serum were performed on 68 specimens about 1 week after the first test (table 3). Of 16 originally reactive specimens, 14 were reactive and 2 weakly reactive on the retest. No change occurred for the 2 weakly reactive specimens. Of the 50 originally nonreactive specimens, 1 was weakly reactive on the retest.

Discussion

The results of testing the group of 149 specimens (table 1) indicated a close correlation between the RPR test on unheated serum and the RPR test on unheated plasma, as well as between RPR (unheated serum) and the VDRL slide and tpcf 50 tests. Agreement between test results on the group of 417 specimens (table 2) was somewhat lower.

Most of the disagreement observed for the group of 417 specimens was confined to the 94 specimens which tested either weakly reactive or negative rough on the RPR test with unheated serum (table 2). Since these 94 represented almost 25 percent of the total group of 417 specimens, a greater effect on the degree of agreement for that group could be expected than for the group of 149 specimens, where the 11 weakly reactive specimens comprised only 7.3 percent of total specimens. The percentage of agreement would be higher for the RPR test and both the VDRL and tpcf 50 tests if the specimens giving negative rough reactions in the RPR test with unheated serum and a reactive or weakly reactive result in the other serologic tests (table 2) were considered to be in agreement.

The two groups of specimens also may have undergone dissimilar losses of reactivity due to different lengths of time between the taking and testing of the samples. This is another possible explanation of the lesser agreement of the 417 samples (table 2). The 149 specimens were examined within 24 to 48 hours after withdrawal, but a longer period separated the withdrawal and the testing of the 417 specimens. Although there could thus have been a change in reactivity to the RPR test with unheated serum between the time when these 417 samples were drawn and the time they were first tested, no significant changes in reactivity after the first test were observed in the 68 samples retested about 1 week later (table 3).

Although the RPR test with unheated serum appears to be less reactive than the RPR test on unheated plasma, its reactivity compares favorably with that of the VDRL slide test. The demonstration that RPR antigen can be used in tests with unheated serum widens the field and program usefulness of this antigen, since specimens collected in anticoagulants or in tubes without anticoagulant can be examined rapidly and economically with the same equipment, reagents, and personnel. However, if the objective is to obtain the maximum screening efficiency of RPR antigen, the test with plasma should be employed.

Conclusion

RPR antigen suspension may be used to test unheated serum using the same procedure as for unheated plasma. This broadens the usefulness of RPR antigen in syphilis control screening programs, though its maximum screening efficiency is realized in its use with the plasma test.

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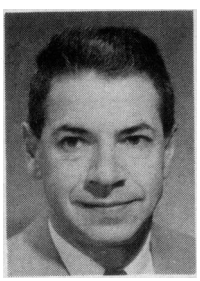

Roger W. Howell, M.D., is currently an associate professor of public health practice in the School of Public Health at the University of Michigan. He has held positions on the faculties of the medical schools of the University of Michigan and the University of Minnesota, as well as in the School of Public Health of the University of North Carolina.

Dr. Howell has worked in public health programs since 1951. He has served on several committees in the American Psychiatric Association and is a member of the Committee on Preventive Psychiatry of the Group for Advancement of Psychiatry. He also serves as a consultant for the Public Health Service in the field of mental health.

Most of Dr. Howell's psychiatric training was obtained at the University of Michigan, where he also received his basic medical training.

Albert L. Chapman, M.D., has been Assistant Surgeon General and chief, Division of Special Health Services, Public Health Service, since 1956. He was regional medical director of Region II during 1954–56, and of Region III in the period 1951–54, after serving 2 years as assistant chief of the Chronic Disease Branch.

After he was graduated from the Long Island College of Medicine in 1937, he received the master of public health degree from Johns Hopkins University in 1941. Dr. Chapman held the post of regional medical director of the New Jersey State Health Department at Trenton for 2 years. During the following 3 years, he held successive posts with public health agencies at town, county, and State levels and with the Public Health Service in Region II.

Dr. Chapman is a fellow of the American Public Health Association and the Royal Sanitary Institute of England. In addition to membership in numerous professional organizations, he is prominent in activities of the National Safety Council and the American Public Health Association.

Helen M. Wallace, M.D., M.P.H., has been professor of maternal and child health at the University of Minnesota School of Public Health since 1956. Previously she was professor and head of the department of preventive medicine and public health in New York Medical College, 1955–56. During the preceding 13 years, she served in the New York City Health Department as chief of the maternity and newborn division and director of the bureau for handicapped children. Dr. Wallace received the degree of master of public health, cum laude, from the Harvard School of Public Health after earning a doctorate in medicine at the Columbia University College of Physicians and Surgeons.

Besides serving as the current national health chairman of the National Congress of Parents and Teachers, Dr. Wallace is secretary of the maternal and child health section and a member of the committee on child health of the American Public Health Association. She is, in addition, assistant editor of the *Journal of the American Women's Medical Association,* as well as a diplomate of both the American Board of Pediatrics and the American Board of Preventive Medicine.