Rapid Plasma Reagin Test for Syphilis

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THE RAPID movement or concentration of relatively large numbers of persons into and within the United States has presented certain difficulties in the control of venereal disease. Examples of such movement and concentration of population can be seen among workers in industrial defense plants, migrant farm and marine laborers, immigrants, potential civil defense mass evacuation groups, and similar population concentrations.

Analysis of the problem as it relates to syphilis has suggested that more effective control might be realized by use of a serologic test which would permit rapid and economical screening supplemented by immediate on-the-spot specific and prophylactic treatment of reactors at the times and locations where they are assembled for processing and assignment.

To meet the requirements of a more rapid serologic test for syphilis, a substitute for the conventional serum specimen was needed since considerable time and labor are involved in the processing of blood to serum.

A review of the literature on the use of plasma in serologic tests for syphilis suggested that this type of sample might serve as the needed substitute for serum. Burdon (*1*, *2*) noted that citrated plasma gave more sensitive results in the Kline and Kahn tests than did serum. The greater sensitivity of plasma was attributed to the presence of "more syphilitic reagin" in plasma than in serum. Fresh unheated plasma gave insensitive findings, but exposure at 56° C. water bath for 10 minutes was sufficient to raise the reactivity level to that obtained by heating for 30 minutes. It was necessary to centrifuge the plasma after heating to remove the precipitate which formed during the heating process.

Burdon noted that considerable time was saved by the substitution of plasma for the conventional serum specimen. Barnard and Rein (*3*) found certain objections, such as increased anticomplementary findings and turbidity of specimen, to the use of citrated plasma and utilized the procedure of recalcification with dicalcium phosphate to obtain a serum specimen from the parent citrated plasma. Listed as advantages of this type of specimen were greater resistance to hemolysis of the citrated specimen, ease of separation of the plasma without resort to centrifugation, and greater stability of reagin in stored citrated plasma.

The results on the recalcified plasma specimen had the same validity as those obtained with serum. However, the process of recalcification was rather laborious and time-consuming.

Barnard and Van Hala (*4*) substituted gypsum for dicalcium phosphate for recalcifying citrated plasma. The inactivation of the specimen was accomplished in the presence of the clot and an excess of gypsum. In reports

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of more recent studies of the use of plasma specimens in serologic tests for syphilis, which have included the employment of cardiolipin-lecithin antigens, Rein, Schwartz, and Kelcec (*5*) described a procedure for the conversion of heparinized plasma to serum by use of protamine sulfate. Converted plasma and conventional serum samples gave similar findings. Although the heparinized plasma could also be used in serologic tests, the formation of a precipitate upon heating made such a specimen less desirable for both flocculation and complement fixation tests.

Coleman and Appleman (*6*) reported on the study of plasma specimens obtained with four different anticoagulants. All plasma specimens were heated at 56° C. for 30 minutes and centrifuged to remove precipitated fibrinogen before performing tests. The results indicated that oxalated plasma used in flocculation and complement fixation tests gave reactions similar to those observed with serum; disodium sequestrene plasma yielded reactions similar to those obtained with serum in flocculation tests but gave a very high percentage of anticomplementary tests; treburon plasma produced variable results in flocculation tests and gave anticomplementary results with all specimens; finally, citrated plasma showed slightly more sensitive results in flocculation tests, and essentially similar reactions in complement fixation.

From the foregoing review it was evident that plasma would fit into a procedure for a rapid serologic test for syphilis, provided the plasma could be used directly without heating or subjecting it to time-consuming chemical procedures or cycles of centrifugation. The chief disadvantage to the use of unheated plasma or serum appeared to be the reduced sensitivity of the results obtained (*2, 7*). The possible association of this reduced level of reactivity with the presence of a labile factor, such as complement, suggested the use of a simple and rapid chemical method for inactivating this labile factor.

Unpublished experiments by the senior author using choline chloride indicated it was anticomplementary. The behavior of an antigen suspension containing choline chloride to differentiate the positive reactions occurring in leprosy from those occurring in syphilis (*8*) suggested trial of this antigen suspension with unheated specimens. The results obtained with unheated serums were highly encouraging. It was then found that such an antigen suspension could be used in a test with unheated plasma.

This preliminary report describes the rapid plasma reagin (RPR) test, presents some of the test findings, and discusses some of the potential applications of the procedure.

Collection of Blood Samples

Various anticoagulants were used in obtaining the plasma specimen, including heparin, potassium oxalate, and potassium sequestrene (*4*).

Duplicate samples, to obtain serum, were taken by venereal disease prevention and control centers and venereal disease investigators in all instances. Depending on the availability of particular blood collecting kits, these split samples were obtained by dual collection in B–D Vacutainer tubes, by the piggyback technique (*9, 10*), or by needle and syringe, dividing the sample.

Rapid Test and Treatment Tried

The rapid plasma reagin test for syphilis was used in California this year as part of the program of examining Mexican farm workers entering the United States. At the reception center in El Centro, 47,579 workers were tested from April 16 through June 28. Blood specimens were examined and differential diagnoses completed on the spot. Workers with syphilis were treated and contact information was obtained before they left the center.

Of the 47,579 tests, 3,913, or 8.2 percent, were reactive, and an additional 685 were weakly reactive. Among the reactors diagnosed as syphilitic were 31 primary and secondary cases, 985 early latent cases, and 2,712 latent. The results of the RPR test compared favorably with those of other nontreponemal and treponemal tests done on a sample of about 1,600 specimens.

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The serums were examined by the VDRL slide technique (*11*) in either the North Carolina State Board of Health Laboratory of Hygiene or a North Carolina county health department laboratory. The plasma specimens were either mailed or brought by messenger to the authors' laboratory.

Test Technique

Preparation of Antigen Suspension. VDRL slide test antigen emulsion (*B*) was prepared (*11*). Measured aliquots of the suspension were then centrifuged in stainless steel tubes in a Servall SS–1 angle centrifuge at room temperature at a relative centrifugal force approximately 2,000×gravity for 15 minutes. The supernatant was decanted and, while the stainless steel centrifuge tube was held in an inverted position, the wall of the tube was wiped with cotton gauze without disturbing the sediment.

A solution containing 10 percent choline chloride solution in 0.85 percent sodium chloride was used in resuspending the sedimented antigen. The volume of the resuspending solution was equal to the volume of the antigen suspension which was centrifuged and was added by blowing it directly onto the sediment.

Agitation of the centrifuge tube by hand was then performed to resuspend the antigen adequately. In early studies antigen suspension was prepared daily. Later it was found that the addition of merthiolate in a final concentration of 0.01 percent maintained the reactivity of the antigen for at least a week when stored at room temperature. The merthiolated antigen suspension was then used in the plasma test over a period of 1 week.

Preparation of Specimens. The blood specimens were centrifuged at room temperature at a force sufficient to separate the plasma from the cellular elements, usually 1,500–2,000 r.p.m. for 4 minutes. The plasma was allowed to remain in the original collecting tube. The specimens were then tested without heating.

Performance of Test. Boerner (*C*) concavity slides were used. Plasma and antigen suspension were measured with disposable capillary pipettes (*D*). For each specimen three drops of plasma were placed on one concavity. One drop of antigen suspension was then added to each plasma specimen. The mixtures were rotated on a mechanical rotator for 4 minutes at 180 r.p.m. The rotator circumscribed a circle three-fourths of an inch in diameter. Tests were read microscopically immediately after rotation, using 100×magnification, and recorded as reactive—large clumps; weakly reactive—medium clumps; nonreactive—small clumps or less.

Results

The results obtained with the RPR test in comparison with the standard VDRL slide test are given in table 1. Regardless of the type of

Table 1. Comparison of results of RPR test and VDRL slide test with a blood specimen

Number cases	Anticoagulant	Results				Percent reactive [1]		Percent agreement [1]
		RPR test	VDRL slide test					
			Reactive	Weakly reactive	Nonreactive	RPR	VDRL	
277	Oxalate	Reactive	11	11	7	15. 2	9. 7	94. 6
		Weakly reactive	0	5	8			
		Nonreactive	0	0	235			
114	Heparin	Reactive	3	1	1	6. 1	3. 5	98. 2
		Weakly reactive	0	1	1			
		Nonreactive	0	0	107			
1, 218	Potassium sequestrene	Reactive	25	43	14	9. 1	6. 3	97. 2
		Weakly reactive	0	9	20			
		Nonreactive	0	0	1, 107			

[1] Reactive plus weakly reactive.

Table 2. Clinical histories on cases with disagreement between RPR and VDRL slide tests

Anticoagulant	Serologic pattern	Number cases	Number with history of syphilis
Oxalate	RPR reactive, VDRL nonreactive	7	[1] 6
	RPR weakly reactive, VDRL nonreactive	8	6
Heparin	RPR reactive, VDRL nonreactive	1	1
	RPR weakly reactive, VDRL nonreactive	1	0
Potassium sequestrene	RPR reactive, VDRL nonreactive	14	14
	RPR weakly reactive, VDRL nonreactive	20	[2] 18
Total		51	45

[1] 1 case could not be located.
[2] 1 case received numerous treatments for gonorrhea.

anticoagulant used, the RPR test was consistently more reactive than the VDRL test on serum. Highly significant differences between the two tests were noted for the 1,609 cases tested without regard to the type of anticoagulant used. In no instance was a reactive or weakly reactive result obtained in the VDRL test in the face of a nonreactive RPR test.

The differences in the percentage of combined reactive and weakly reactive findings obtained with the three anticoagulants should not be attributed to the anticoagulant used inasmuch as the samples collected were from different areas in North Carolina. However, the ratio of reactive RPR to reactive VDRL tests in the groups representing the three anticoagulants was fairly constant at about 1.5 : 1.

Clinical documentation on those cases giving discordant findings between RPR and VDRL tests is presented table 2). In 51 cases of disagreement, 45 or 88 percent gave a history of treated syphilis; 1 case could not be located.

Discussion

Several factors can be considered to account for the greater sensitivity of the RPR test as compared with the VDRL slide test with serum. An obvious factor is the reactivity level of the VDRL slide test, which is set at a lower level than some of the other serologic tests for syphilis. Somewhat greater agreement in sensitivity would be expected if the more sensitive procedures were compared with the RPR test. However, these data are lacking at present.

Another factor is the enhancing effect of choline chloride on the reactivity level of the antigen. This effect has been shown to occur in tests with heated serums (*8*). In 112 syphilitic serums, all TPI reactive, 87.5 percent reactive results were obtained with the VDRL slide test, whereas 97.3 percent reactive results were obtained with antigen containing choline chloride. Still another factor may be the greater reagin content of unheated plasma as compared with the reagin content of heated serum. Barnard and Rein (*3*) have referred to the greater stability of reagin in stored citrated plasma than in the conventional serum sample obtained from whole clotted blood. The destruction of reagin due to heating of serum has been reported and review by Rein and Hazay (*7*).

The results thus far obtained do not indicate any lower degree of specificity of the RPR test as compared with the VDRL slide test. As

was shown in table 2, nearly all of the cases wherein discrepancies between the RPR test and VDRL slide test were encountered represented treated cases of syphilis. In view of the prior notation of the greater specificity of the antigen suspension containing choline chloride when used in conjunction with heated leprosy serums of nonsyphilitic origin, these preliminary findings on unheated plasma are perhaps not unexpected.

In considering the potential applications of the RPR test, the cost of the test is of interest. It has been estimated that the cost of the RPR antigen per test dose is a fraction of 1 cent more than the cost of the conventional VDRL antigen test dose. However, if early experiences with stored antigen suspensions are substantiated, the cost of the RPR test might conceivably be lower than the cost of the VDRL test since it would be possible to utilize antigen suspensions more completely.

The most significant economies with the RPR test will probably be associated with time and personnel. The time and labor involved in bringing the serum specimen to the testing stage is considerable. These are markedly reduced by use of plasma directly from the blood collection tube. The individual specimen is ready for testing immediately after centrifugation.

Exclusive of the glassware employed in the preparation of the antigen suspension and the concavity slides, all other items are disposable, thereby eliminating washing and glass breakage. The present cost of the disposable pipette is a fraction more than 2 cents. This cost may be lowered with greater demand and perhaps with the development of a cheaper substitute. It is hoped, too, that a disposable plastic slide will be developed.

The RPR test might be of value in mass blood testing programs since it would require fewer man-hours to handle a given load than conventional serologic methods. The rapidity of the RPR test would make it valuable in screening large numbers of migrant laborers, immigrants, and industrial groups—operations in which speed is or may be most desirable, if not critical.

Another potential application of the RPR test is its use by venereal disease investigators or other authorized personnel in conducting on-the-spot serologic testing. Present practice involves the collection and shipment of blood specimens to a distant laboratory. Upon receiving notice of the serologic findings the investigator must locate the individuals for further study and treatment. It is not always possible to find these patients. In a recent survey for syphilis among migrant farm laborers, 10 percent of nonwhite persons found to have positive serologic tests could not be located for treatment (*12*). By operating out of a base laboratory, which would supply prepared antigen suspension and other materials, and with a modicum of training, it would be possible for field personnel to collect and test blood at the source and take immediate action in those individuals giving reactive results.

Finally, the RPR test might also prove to be valuable (*a*) as an adjunct in planning for syphilis control among civil defense and major disaster evacuee groups, (*b*) in processing sudden mass immigration groups, (*c*) in hospital and clinical laboratories, and (*d*) in public health programs of a multiphasic nature, particularly in those situations where hematological or other studies require blood collected in anticoagulant.

Summary

A review of the literature on the use of blood plasma in serologic tests for syphilis suggested that plasma would fit into a procedure for a rapid serologic test, provided that the plasma could be used without heating or time-consuming chemical procedures or cycles of centrifugation.

Plasma and serum specimens from 1,609 syphilitic patients were tested. Sedimented VDRL slide test antigen emulsion was resuspended in 10 percent choline chloride and used in the plasma test. Blood specimens were centrifuged at room temperature, and the plasma was tested without heating. Mixtures of plasma and antigen were rotated on a mechanical rotator for 4 minutes at 180 r.p.m., and tests were read microscopically immediately after rotation.

The rapid plasma reagin (RPR) test on plasma was consistently more reactive than the VDRL slide test on serum, regardless of the

anticoagulant used. In no instance was a reactive or weakly reactive result obtained in the VDRL test when the RPR test was nonreactive. In 51 of the 1,609 cases, the RPR test was reactive to some degree when the VDRL test was nonreactive. Of these 51 patients, 45 (88 percent) gave a history of treated syphilis; 1 patient could not be located.

The RPR test for syphilis may prove to be valuable for several reasons. It is more economical than the VDRL slide test. It requires fewer man-hours to examine a given number of specimens. It could be used for on-the-spot serologic testing by venereal disease investigators instead of sending blood specimens to distant laboratories. It might also prove to be valuable in planning syphilis control among civil defense and major disaster evacuee groups, in processing sudden mass immigration groups, in hospital and clinical laboratories, and in multiphasic public health programs.

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EQUIPMENT REFERENCES

(*A*) The anticoagulant containing tubes were supplied by Tom Starling of the Becton, Dickinson & Co., Rutherford, N. J.

(*B*) VDRL slide test antigen was supplied by Sol Rosenberg, Sylvania Chemical Co., Orange, N. J.

(*C*) No. 66550 Micro Slide, Boerner. American Hospital Supply Co. Scientific Products Division, Evanston, Ill.

(*D*) No. 67770 diSPO-pette. American Hospital Supply Co. Scientific Products Division, Evanston, Ill.

Symposiums on Industrial Health Problems

The effects of habituating drugs on industrial workers will be discussed in the morning of a full-day session on industrial health problems on November 14, 1957, during the annual meeting of the American Public Health Association in Cleveland, Ohio. For the afternoon meeting, the scheduled topic is the status of the pneumoconioses. The session will be co-sponsored by the occupational health section of the American Public Health Association, the Industrial Medical Association, and the American Industrial Hygiene Association.