RAPID REAGIN TEST WITH UNHEATED SERUM AND NEW IMPROVED ANTIGEN SUSPENSION

Joseph Portnoy, Ph.D., Hilfred N. Bossak, B.S., Virginia H. Falcone, B.S., Ad Harris

THE rapid plasma reagin (RPR) test for syphilis (*1*) 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. Experience has indicated the value of the test for examining migratory workers (*2*). It has also been found useful in testing inmates at the Cook County Jail in Chicago and clinic patients in Memphis and Shelby County, Tenn. (personal communications).

During the development of the RPR test, the possibility of employing the same antigen suspension used in testing unheated plasma in a test with unheated serum was considered. It seemed likely that a test with unheated serum might have potential usefulness in a public health laboratory as a screening mechanism provided that a satisfactory level of sensitivity and specificity could be obtained.

An opportunity to evaluate the RPR suspension in a test with unheated serum was provided by the Serology Evaluation and Research Assembly study (*3*) conducted by the Public Health Service in cooperation with the authors of the tests. This study indicated that a test with unheated serum, designated as the USR test, had a satisfactory level of sensitivity and specificity. This technique used 0.05 ml. of unheated serum and 1/45 ml. of RPR antigen suspension on a Boerner concavity slide.

Later reports (*4,5*) showed that unheated serum could be tested exactly as described for unheated plasma in the RPR test, using three drops of unheated serum and one drop of antigen suspension delivered from disposable pipettes onto Boerner concavity slides.

The first study to determine the usefulness of a screening test with unheated serum in a public health laboratory was undertaken by the New York City Health Department (*6*). Meanwhile, other research efforts led to the development of a new improved antigen suspension which showed much greater stability than the original RPR suspension (*7*). Cooperative studies of the Venereal Disease Research Laboratory and various governmental agencies have indicated excellent agreement between the results of tests using unheated plasma and both old and new improved antigen suspensions.

However, the behavior of the new antigen suspension with unheated serum has received only limited study. Because of the increasing interest in rapid reagin testing in public health laboratories, it seemed appropriate to evaluate its sensitivity and specificity. In view of the many variations of technique used with unheated serum, it was of interest to make such comparisons as would permit a recommendation for a single technique.

Methods

The serums used were from known clinical categories and had been kept in the frozen state, previously unheated.

The authors are with the Venereal Disease Research Laboratory, Communicable Disease Center, Public Health Service, Atlanta, Ga. Dr. Portnoy is assistant director of the laboratory, Mr. Bossak and Mrs. Falcone are bacteriologists, and Mr. Harris is the director.

The VDRL slide test (*8*) results were those obtained on these serums in an earlier study. Whenever a difference in reactivity was noted among any of the tests, repeat testing with both VDRL slide and USR tests was accomplished simultaneously.

Using paraffin-ringed slides, as recommended for VDRL slide tests, comparative testing was accomplished with the following amounts of unheated serum and the new improved RPR antigen suspension:

Test A: 0.05 ml. serum and 1/60 ml. antigen suspension (delivered with 20-gauge needle)

Test B: 0.05 ml. serum and 1/45 ml. antigen suspension (delivered with 18-gauge needle)

Test C: 0.06 ml. serum and 1/50 ml. antigen suspension (delivered with 19-gauge needle)

These tests were carried out simultaneously.

Results and Discussion

The reactions obtained with the three variations of the USR test in comparison with results of the VDRL slide test are presented in table 1. The total number of reactive plus weakly reactive results was the same with methods A, B, and C. However, test B, employing 0.05 ml. of serum and 1/45 ml. of antigen suspension, gave the greatest number of reactive (not weakly reactive) results and would for this reason be more advantageous than the other two methods. A slightly lower reactivity level was observed with all three unheated serum tests as compared with reactivity of the VDRL slide test in the present study. On the other hand, the SERA study (*3*) indicated that the USR test was consistently more reactive than the VDRL slide test. It is probable that this difference between results of the two studies is related partially to the type of slide used in performing the tests and to the different methods of preparing RPR antigen suspensions. In the SERA study, a Boerner slide was used, whereas, in this study, a flat paraffin-ringed slide was employed. A small number of tests conducted simultaneously have indicated that more reactive findings are encountered with the Boerner slide.

In the New York City study 100,000 blood specimens were tested from a random population group. Essentially similar degrees of

Table 1. Reactions obtained with unheated serum tests and VDRL slide test, according to clinical category

Clinical category	Number of specimens	Test											
		A[1]			B[2]			C[3]			VDRL slide		
		R	WR	N	R	WR	N	R	WR	N	R	WR	N
Primary:													
Untreated	48	24	1	23	24	1	23	24	2	22	25	4	19
Treated	25	0	0	25	0	0	25	0	0	25	0	0	25
Secondary, untreated	25	22	2	1	23	1	1	21	3	1	25	0	0
Latent:													
Untreated	6	5	1	0	6	0	0	5	1	0	6	0	0
Treated	19	9	0	10	9	0	10	9	0	10	9	2	8
Late, treated	72	52	6	14	56	2	14	50	7	15	54	6	12
Presumed nonsyphilitic	225	0	0	225	0	0	225	0	0	225	0	0	225
Diseases other than syphilis	25	1	0	24	1	0	24	1	0	24	2	0	23
Biologic false positive:													
With nonreactive TPI	25	2	3	20	2	3	20	3	2	20	4	2	19
No previous TPI	22	4	2	16	4	2	16	3	3	16	6	2	14
Total	492	119	15	358	125	9	358	116	18	358	131	16	345

[1] Test A: 0.05 ml. serum and 1/60 ml. antigen suspension.
[2] Test B: 0.05 ml. serum and 1/45 ml. antigen suspension.
[3] Test C: 0.06 ml. serum and 1/50 ml. antigen suspension.

NOTE: R—reactive; WR—weakly reactive; N—negative.

Table 2. Agreement between VDRL slide test and unheated serum tests

VDRL slide test	Number of specimens	Unheated serum tests								
		A[1]			B[2]			C[3]		
		R	WR	N	R	WR	N	R	WR	N
Reactive	131	114	8	9	120	2	9	112	9	10
Weakly reactive	16	5	7	4	5	7	4	4	9	3
Nonreactive	345	0	0	345	0	0	345	0	0	345
Percent agreement[4] with VDRL slide test		97.4			97.4			97.4		

[1] Test A: 0.05 ml. serum and 1/60 ml. antigen suspension.
[2] Test B: 0.05 ml. serum and 1/45 ml. antigen suspension.
[3] Test C: 0.06 ml. serum and 1/50 ml. antigen suspension.
[4] Complete and partial.

NOTE: R—reactive; WR—weakly reactive; N—negative.

reactivity were obtained with unheated serum, using 0.06 ml. of serum and 0.02 ml. of antigen suspension on a paraffin-ringed slide, and the VDRL slide test. Satisfactory results with this procedure were reported by Widelock and co-workers (*6*).

In table 2, the agreement between the VDRL slide test and the unheated serum test is presented. The same high percentage of agreement was noted for all three tests.

On the basis of the findings obtained in this study, it appears advisable to recommend, in the interest of uniformity, the technique using 0.05 ml. of serum and 1/45 ml. of new improved antigen suspension on a 14 mm. paraffin-ringed slide for the performance of the unheated serum reagin test. This represents the same proportion of serum and antigen previously employed in the SERA study.

Summary and Conclusions

Three methods of performing the unheated serum reagin test were compared, and no significant difference in reactivity between these methods was observed.

It is recommended, in the interest of uniformity, that the unheated serum reagin test be performed on a 14 mm. paraffin-ringed slide using 0.05 ml. of serum and 1/45 ml. of new improved antigen suspension.

REFERENCES

(*1*) Portnoy, J., Garson, W., and Smith, C. A.: Rapid plasma reagin test for syphilis. Pub. Health Rep. 72: 761–766, September 1957.

(*2*) Simpson, W. G., Matthis, A. W., Harris, A., and Price, E. V.: Evaluation of the rapid plasma reagin test in field operation. Pub. Health Rep. 74: 473–477, June 1959.

(*3*) U.S. Public Health Service: Serology evaluation and research assembly (SERA), 1956–1957. PHS Publication No. 650. U.S. Government Printing Office, Washington, D.C., 1959.

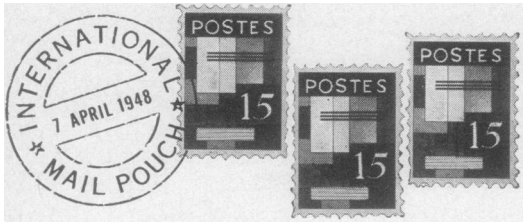
(*4*) Portnoy, J., and Garson, W.: A preliminary report on RPR test for syphilis using unheated serum. Pub. Health Rep. 74: 965–968, November 1959.

(*5*) Bossak, H. N., Duncan, W. P., Harris, A., and Falcone, V. H.: Assay of tests for syphilis on unheated serum. Pub. Health Rep. 75: 196–198, March 1960.

(*6*) Widelock, D., et al.: The USR test as a screening test in a public health laboratory. Pub. Health Rep. 76: 447–452, May 1961.

(*7*) Portnoy, J., and Garson, W.: New and improved antigen suspension for rapid reagin tests for syphilis. Pub. Health Rep. 75: 985–988, November 1960.

(*8*) U.S. Public Health Service: Manual of serologic tests for syphilis. PHS Publication No. 411. U.S. Government Printing Office, Washington, D.C., 1959.



Health Week in Ghana

Improved nutrition was emphasized during National Health Week, October 23–29, 1960, in Ghana. Pregnant women, infants, and weanlings received special attention. Schools, religious organizations, radio stations, and newspapers combined to help publicize the week's theme, "Good Food for Good Health."

—JEAN M. PINDER, *health education adviser, U.S. Operations Mission, Ghana.*

New Medical School

The Medical School of the University of Costa Rica began its first class in March 1961. The basic medical science courses will be taught in a new building on the university campus. Clinical study will be conducted chiefly at the San Juan de Dios Hospital in San José.

The Health and Sanitation Division of the U.S. Operations Mission assisted the school during the years of planning. The ICA has a contract with Louisiana State University whereby faculty members of the L.S.U. Medical School will be assigned to work for varying periods with Costa Rican educators who are being trained in the latest teaching methods and laboratory techniques.

—DR. ANTHONY DONOVAN, *chief, health and sanitation division, U.S. Operations Mission, Costa Rica.*

Eradication Preparation

To control the malaria epidemic and prepare for an eradication campaign, a control project was initiated in Haiti in November 1960. Reduction of malaria incidence is the primary object of the project, but the collection of data and the organization of an administrative unit will lay the groundwork for an eradication campaign which was suspended in 1958 and will be resumed in 1961.

The project, proposed by the Minister of Health, is being carried out by the Haitian American Development Organization in cooperation with the Servicio Cooperativo Inter-americano de Salud Pública.

Local staffs of the Ministry's hospitals, health centers, and rural dispensaries administer antimalarial drugs and take blood specimens from each person treated. The specimens are analyzed, and the results are tabulated.

—EDWARD E. MINTY, *acting chief, public health division, U.S. Operations Mission, Haiti.*

Milestone

Brazil's first director of the Division of Occupational Health of the SESP-Foundation (Fundação Serviço Especial de Saúde Pública) is Engineer Pedro Monteiro Gondim, appointed December 1, 1960.

SESP began occupational health activities in 1949 by conducting a survey to determine the health status of Brazilian workers and by sponsoring a 3-month course in industrial hygiene for physicians, engineers, and other public health workers. Since that time, the training of personnel has remained a major activity. Over a period of 11 years the occupational health project has provided a total of 13 training grants.

—DR. VERNON J. FORNEY, *chief, public health division, U.S. Operations Mission, Brazil.*

Diet Deficiencies

The high death rate among Guatemalan children is attributed to inadequate diet, chiefly in protein-containing foods, by Dr. Nevin S. Scrimshaw, quoted in the Washington *Star*. Dr. Scrimshaw, director of the Institute of Nutrition of Central America and Panama, said that investigations in four Guatemalan villages revealed that 40 percent of the deaths of children 1 to 4 years old were caused by kwashiorkor, a nutritional disease. Of the remainder, nearly all were caused by complications of common childhood infections not normally fatal to well-nourished children.

Dr. Scrimshaw also stressed that there is increasing evidence that deficiencies which retard physical growth are associated in some way with retardation of intellectual development. He said that in Mexico a direct correlation had been found between intelligence test scores and weight deficiencies due to poor diets.