Assay of Tests for Syphilis on Unheated Serum

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A METHOD for testing unheated serum in the same manner as plasma in the rapid plasma reagin (RPR) test (*1*) has recently been reported in which the comparative results obtained with this method and several other procedures on specimens from a serologic survey conducted in North Carolina, are described (*2*). An earlier method employing measured amounts of unheated serum and RPR antigen suspension, and referred to as the unheated serum reagin (USR) test, was included among the nontreponemal tests performed in the SERA study (*3*).

The establishment of a serum bank of clinically categorized donors at this laboratory has been previously described (*4*). Its availability has made possible the rapid assay of newly published procedures, and it has again been used in this study.

This report presents the comparative results obtained with the two procedures performed on unheated serum and the VDRL slide test on serums from medically defined patient groups.

Materials and Methods

The rapid plasma reagin test on unheated serum (RPR–US) was performed in exactly the same manner as the RPR test (*1*) except that serum was substituted for plasma.

The unheated serum reagin test was performed according to the technique described in the SERA study (*3*).

The VDRL slide test was performed in accordance with the technique described in the Manual of Serologic Tests for Syphilis, 1959 revision.

All tests were performed at the Venereal Disease Research Laboratory, Chamblee, Ga. VDRL slide test results on those serums that were included in both the SERA study and the serum bank were taken from the SERA study report. The two tests on unheated serum were run on all the specimens at a later date. VDRL slide tests were performed at the same time on any specimens added to the bank which had not been included in the SERA study.

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

Presumed Nonsyphilitic

- Apparently healthy donors presumably with no history of previous or present infection with syphilis.

Syphilis

- Donors with primary syphilis proved by darkfield examination, who had not received treatment.
- Patients having had primary syphilis proved by darkfield examination and who had adequate treatment with 2,400,000 units or more of penicillin not less than 2 nor more than 4 years prior to time blood was taken.
- Donors with secondary syphilis proved by darkfield examination, who had not been treated.

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• Patients with secondary syphilis proved by darkfield examination who had adequate treatment with 2,400,000 units or more of penicillin not less than 2 nor more than 4 years prior to time blood was taken.

• Donors with either latent or late syphilis, adequately treated with 4,800,000 units or more of penicillin.

WITH CONDITIONS OTHER THAN SYPHILIS

• Hospital patients with a variety of diseases or conditions, not receiving antibiotics and having no history of clinical evidence of syphilis; patients 12 years of age or younger with yaws; patients with pinta, below age at which associated syphilis might be expected; and leprosy patients not thought to have associated syphilis.

BIOLOGIC FALSE POSITIVES (BFP)

• Patients with reactive nontreponemal tests, at least one nonreactive (TPI) *Treponema pallidum* immobilization test, and no clinical evidence of syphilis.

• Patients with reactive nontreponemal tests, no previous TPI test, and no clinical evidence of syphilis.

Results

The results obtained with the three procedures on 592 serums from presumed nonsyphilitic persons are shown in table 1. Of the total number of serums tested, 329 were residuals of SERA study specimens which had not been previously heated or tested and had been stored in the frozen state in tightly sealed containers since the original date of collection and separation of the serum. The remaining 263 specimens were obtained from other sources and were added to the serum bank at later dates. Regardless of the origin of the serums, however, absolute agreement was obtained with the unheated serum reagin and the rapid plasma reagin test on unheated serum. Percentage reactivity was identical with all three tests (0.34 percent), although the two serums which reacted in the tests with unheated serum were not the same serums that were reactive in the VDRL slide test. The *Treponema pallidum* immobilization test, performed in this laboratory on these four specimens, was reactive in each instance, suggesting a treponemal infection, past or present.

Table 2 lists the results obtained in six clini-

Table 1. Results with 592 serums from presumed nonsyphilitic persons

Test	Nonreactive		Reactive [1]		Total	
	Number	Percent	Number	Percent	Number	Percent
Unheated serum reagin	590	99. 66	2	0. 34	592	100
Rapid plasma reagin on unheated serum	590	99. 66	2	. 34	592	100
VDRL slide	590	99. 66	2	. 34	592	100

[1] Reactive plus weakly reactive.

Table 2. Reactivity rate [1] in six clinically defined categories of syphilis

Category	Number of specimens	USR test		RPR-US test		VDRL slide test	
		Number	Percent	Number	Percent	Number	Percent
Primary, untreated	119	85	71. 4	86	72. 3	79	66. 4
Primary, treated	29	4	13. 8	4	13. 8	0	0
Secondary, untreated	98	98	100. 0	98	100. 0	98	100. 0
Secondary, treated	18	8	44. 4	8	44. 4	5	27. 8
Latent, treated	25	23	92. 0	23	92. 0	17	68. 0
Late, treated	203	190	93. 6	191	94. 1	178	87. 7

[1] Reactive plus weakly reactive.

Table 3. Reactivity rate [1] in conditions or diseases other than syphilis

Category	Number of specimens	USR test		RPR-US test		VDRL slide test	
		Number	Percent	Number	Percent	Number	Percent
Conditions other than syphilis	73	7	9. 6	7	9. 6	4	5. 5
Biologic false positive, nonreactive TPI test	111	53	47. 7	53	47. 7	54	48. 6
Biologic false positive, no TPI test	109	55	50. 5	55	50. 5	48	44. 0

[1] Reactive plus weakly reactive.

cally defined syphilis categories. Little difference was noted in the reactivity rate of the two tests on unheated serum although the RPR test appeared to be slightly more reactive than the procedure using measured amounts of reagents. Both tests were more reactive than the VDRL slide test in all categories except untreated secondary syphilis. In adequately treated primary, secondary, and latent syphilis, the lower reactivity rate of the VDRL slide test was most apparent, suggesting a more rapid reversion to nonreactivity after treatment.

Findings with the three procedures in conditions or diseases other than syphilis are summarized in table 3. With conditions other than syphilis the incidence of reactivity with the two tests on unheated serum was approximately twice that of the VDRL slide test. Results obtained with all three tests were approximately the same in one biologic false positive category, composed of serums from persons who had been screened with at least one nonreactive TPI test. None of the patients in the second BFP category had been previously tested with the TPI. In this group, the VDRL slide test was less reactive than the two procedures performed on unheated serum.

Summary and Conclusions

The comparative results obtained with the unheated serum reagin test, the rapid plasma reagin test on unheated serum, and the VDRL slide test on serums from medically defined patient categories are presented.

1. No significant differences were observed between the two tests on unheated serum.

2. Percentage reactivity was the same with all three procedures in the group of healthy, presumedly nonsyphilitic persons.

3. In serums from donors with syphilis, the two procedures with unheated serum were more reactive than the VDRL slide test in all categories except secondary, untreated, including those patient groups who had received adequate treatment.

4. The unheated serum reagin test and the rapid plasma reagin test on unheated serum were almost twice as reactive as the VDRL slide test in the category of patients with diseases other than syphilis.

5. The results obtained with the three procedures were quite similar in the two groups of patients classified as biologic false positive reactors.

REFERENCES

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