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Comparison of Serum and Plasma Specimens for Syphilis Serology Using the Reagin Screen Test

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One serum and three plasma samples were obtained from each of 125 normal individuals and from 140 patients with treated or untreated syphilis. Serum samples were tested by the Venereal Disease Research Laboratory (VDRL) test and by the Reagin Screen Test (RST). Plasma specimens were tested only with the RST. When tested within 24 h after collection, all specimens from normal individuals were nonreactive. Plasma specimens from normal individuals stored for up to 72 h after collection continued to yield a clearly nonreactive result in 423 of the 426 samples tested by the RST. Serum and plasma samples from syphilis patients tested within 72 h after collection by the RST yielded qualitative and quantitative results almost identical to results of serum tested by the VDRL test.

Many institutions, including blood banks and laboratories performing syphilis screening on small children, are currently using plasma specimens for testing with the Rapid Plasma Reagin card test. However, no test for use with plasma samples has been approved by the Center for Disease Control. Standard procedures for the Venereal Disease Research Laboratory (VDRL), Rapid Plasma Reagin 18-mm card, and Fluorescent Treponemal Antibody Absorption tests state that serum should be tested (2). No comparison of VDRL results on serum specimens versus results of a test which uses plasma, using a defined population, has been published.

We recently have evaluated the Reagin Screen Test (RST; Fisher Diagnostics, Orangeburg, N.Y.) using over 1,000 clinically defined patients (1). Only a few plasma specimens from normal individuals were included in that study. In the present investigation, the reactivity of plasma tested by the RST was compared to the reactivity of serum tested by the VDRL and RST procedures. The aim of this study was to provide data on the reliability of plasma samples for use with the RST.

MATERIALS AND METHODS

Samples. Serum and plasma samples from 125 normal individuals were obtained from laboratory personnel. Samples from 140 patients with syphilis were obtained from people presenting to the Houston Health Department Venereal Disease Clinic and from patients at the Ben Taub Hospital. The criteria for defining stages of syphilis were described previously (1). All samples were initially tested within 24 h after collection. Samples from normal individuals also were stored at room temperature or at 4°C for up to 96 h to determine the effects of storage on the reactivity of normal serum and plasma. Additional specimens from syphilitic individuals were sequentially studied at 24-h intervals up to 72 h after collection to determine storage effects on results from reactive samples. The plasma samples were drawn into 7-ml Vacutainer tubes containing 0.5 ml of 3.8% sodium citrate, 9 mg of ethylenediaminetetraacetic acid, or sodium heparin as anticoagulants. The serum samples were obtained in 10-ml Vacutainer tubes without anticoagulant. Four samples were obtained from each individual in the study. The samples were centrifuged to eliminate cellular components before testing.

Serological tests. Serum was heat inactivated at 56°C for 30 min and then tested with the VDRL test using standard, approved methods (2). Reagents for the VDRL test were provided by the Texas Department of Health Resources, Austin, Texas. Sera and plasma tested by the RST were not heated and were tested as described previously (1).

RESULTS

Results of qualitative tests performed on samples from normal and syphilitic individuals are presented in Table 1. These data are based on test results obtained within 24 h after the specimens were taken. All normal individuals were nonreactive by VDRL tests on serum and by RST tests of serum and plasma.

Specimens from 63 patients with primary syphilis yielded similar results for all specimens tested by both tests. Specimens from one patient yielded a weakly reactive VDRL on serum and a nonreactive RST on all samples. Specimens from two patients were reactive by RST, whereas the serum was nonreactive by the VDRL. Plasma samples yielded a reactive RST on one primary syphilis patient while the serum was nonreactive with the RST and VDRL tests. Samples from one patient were all reactive with

TABLE 1. *Reactivity of sera and plasma from normal and syphilitic individuals*

Individuals	VDRL reactions			RST reactions with anticoagulant:							
				None		EDTA[a]		Citrate		Heparin	
	N[b]	W[c]	R[d]	N	R	N	R	N	R	N	R
Normal (125)[e]	125	0	0	125	0	125	0	125	0	125	0
Primary (63)	15	10	38	14	49	13	50	13	50	14	49
Secondary (23)	0	0	23	0	23	0	23	0	23	0	23
Latent (5)	0	1	4	0	5	0	5	0	5	0	5
Treated (49)	2	9	38	1	48	1	48	2	47	2	47

[a] EDTA, Ethylenediaminetetraacetic acid.
[b] N, Nonreactive.
[c] W, Weakly reactive.
[d] R, Reactive.
[e] Number of individuals in group.

the exception of the plasma sample with heparin used as anticoagulant.

Specimens from the 23 patients with secondary syphilis were all reactive. Specimens from five patients with latent syphilis were all weakly reactive or reactive. Specimens from 49 patients who had been treated for syphilis in the past yielded similar results for all samples in the two tests.

When a specimen yielded a reactive qualitative test, a titer was obtained for that specimen. A comparison of the titers of the 103 sera and plasma tested by the RST versus the titer of serum tested by the VDRL is shown in Table 2. The titer obtained by the RST was usually equal to or one dilution higher than the titer of serum tested by the VDRL test. The agreement within one dilution was 87.3% for VDRL-RST serum, 84.5% for VDRL-RST ethylenediaminetetraacetic acid, 87.3% for VDRL-RST citrated blood, and 83.5% for the VDRL-RST heparinized blood.

Since our laboratory and others have noted atypical agglutination patterns with the Rapid Plasma Reagin card test when testing plasma specimens stored for two or more days before testing, we also examined the effects of storage on normal plasma specimens tested by the RST. One of 138 samples tested at 48 h, 2 of 288 samples tested at 72 h, and 6 of 18 samples tested at 96 h yielded an atypical agglutination that could be easily distinguished from true reactivity. This reaction was evident in samples containing ethylenediaminetetraacetic acid or heparin, but not in citrated samples. The storage temperature (22 or 4°C) had no effect on the appearance of this atypical reaction. Separation of plasma from the erythrocyte layer before storage also had no effect.

We also examined the effects of storage on reactive samples for up to 72 h after collection. Plasma samples from 12 patients whose serum was weakly reactive by the VDRL test, from 11 patients with a reactive undiluted VDRL, and from 16 patients whose VDRL titers were 1:2 to 1:32, were selected for this purpose. All samples were tested 24, 48, and 72 h after collection. No loss in reactivity in plasma specimens tested by RST was noted, even in specimens which were minimally reactive on initial testing.

TABLE 2. *Comparison of quantitative results*

Test	−1 dil[a]	equal	+1 dil	+2 dil
RST (serum) titer vs. VDRL	1	27	62	13
RST (EDTA)[b] titer vs. VDRL	1	23	63	16
RST (citrate) titer vs. VDRL	1	34	55	13
RST (heparin) titer vs. VDRL	1	24	61	17

[a] dil, Dilution.
[b] EDTA, Ethylenediaminetetraacetic acid.

DISCUSSION

Plasma samples from normal individuals yielded nonreactive RST results when tested within 24 h after collection. When normal plasma specimens were stored for up to 72 h, few specimens yielded atypical agglutination patterns. One normal specimen out of 138 tested at 48 h and 2 of 288 samples tested at 72 h yielded atypical threadlike agglutinations which could easily be distinguished from the usual agglutination pattern produced by reactive sera. Also, storage for up to 72 h did not affect the reactivity of specimens. If plasma is used for testing with the RST, all specimens probably should be tested within 48 h, and care should be taken to remove all cellular material before a test is performed. If an atypical agglutination is noted, a second specimen should be requested and tested. Citrated blood seems to be the best sample to use to prevent atypical agglutination in normal

specimens. If such precautions are taken, the specificity of the results of the RST with plasma specimens should be equal to that of the VDRL as indicated by the results of this investigation.

Plasma specimens tested by the RST demonstrated a sensitivity equal to that of serum samples tested by the RST or VDRL tests. This sensitivity was evident in all groups of syphilis patients. A previous evaluation indicated that the RST was slightly less sensitive than the VDRL, especially when samples were obtained from patients who had been treated for syphilis (1). Although the number of samples tested in the present study was small, the results obtained suggest that the RST antigen is equal to the VDRL antigen in sensitivity and specificity.

The results of this study suggest that plasma specimens that are processed and tested promptly by the RST yield results equal to those from serum tested by the RST and VDRL tests. However, laboratories that use plasma samples for screening should be warned that these samples cannot be sent to a reference lab for quantitative VDRL testing or confirmation by the Fluorescent Treponemal Antibody Absorption test. Since heating of samples is mandatory for the performance of these latter tests, only serum samples should be tested.

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