NOTES

Defibrination of Blood Plasma for Use in Serological Tests for Syphilis

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Received 30 April 2002/Returned for modification 22 July 2002/Accepted 22 August 2002

Syphilitic plasma can be salvaged from discarded blood donations and converted to serum by defibrination. Sixty-nine units of plasma were treated with a stock solution of 100 U of thrombin per ml in 1 M calcium chloride and then with a 10% (wt/vol) solution of kaolin. Fibrinogen concentrations detected in initial plasma samples ranged from 94 to 4,970 mg/liter (mean, 2,532 mg/liter) for samples that were reactive by the rapid plasma reagin circle card test (RPR) and from 314 to 2,742 mg/liter (mean, 1,528 mg/liter) for samples that were not reactive by the RPR. The treated samples showed no measurable fibrinogen remaining after the defibrination process. In the nontreponemal RPR for syphilis, 86% of the treated plasma samples retained the same endpoint titer as that of the initial plasma sample. When the Treponema pallidum passive-particle-agglutination test was used, 98% retained the same reactivity. In the Captia Syphilis-G enzyme immunoassay, 89% of the treated samples demonstrated no change in reactivity index, and in the fluorescent treponemal antibody absorption test, 96% showed no reduction in fluorescence. Human sera containing antibodies to syphilis are used at the Centers for Disease Control and Prevention for the preparation of reference controls or as samples for proficiency testing. Finding reactive sera is becoming more difficult due to the general decline of syphilis cases in the United States. The decreasing availability of these sera can be alleviated by salvaging plasma and converting it to serum.

In the American Public Health Association publication A Manual of Tests for Syphilis (3), the Centers for Disease Control and Prevention recommends that serum be used in the Venereal Disease Research Laboratory (VDRL) test. Serum should be heated at 56°C for 30 min prior to testing to inactivate complement (2), since the presence of complement in freshly drawn serum makes it less reactive. The heating of plasma at the same temperature and for the same period of time enhances fibrin formation (7), rendering plasma unsuitable for this test. At the Centers for Disease Control and Prevention, human sera that are seropositive for syphilis are used in the preparation of serology reference controls or as samples for proficiency testing. However, it is becoming more difficult to find reactive sera because of the general decline in the number of syphilis cases in this country (4). About 12.6 million units of whole blood are donated in the United States each year. After the blood is drawn, it is tested for ABO group and Rh type. Screening tests are also performed for evidence of donor infection with hepatitis viruses B and C, human immunodeficiency virus types 1 and 2, human T-lymophotropic virus types I and II, and syphilis (6). Whole blood for transfusion is collected into a bag that contains an anticoagulant-preservative solution designed to prevent clotting and to maintain cell viability during storage. Commonly used anticoagulants are citrate-phosphate-dextrose, citrate-phosphate-dextrose-dextrose, and citrate-phosphate-dextrose-adenine.

Citrate prevents activation of the clotting cascade by chelating calcium, thus inhibiting the several calcium-dependent steps in coagulation. Blood stored at 1 to 6°C for 21 days retains 15 to 30% of heat-labile coagulation factors V and VIII and most of the stable factors II, VII, IX, and X (6). Plasma derived from blood donations found to be reactive for infectious diseases is discharged according to the Food and Drug Administration’s Good Manufacturing Practices regulations. However, units of plasma that are reactive for syphilis but nonreactive for other diseases can potentially be used for manufacturing syphilis reference reagents or proficiency test samples. Plasma can be converted to serum by the method of defibrination. Coagulation factors present in plasma can be activated to form fibrin, with the addition of calcium chloride and thrombin (1). Thrombin cleaves fibrinogen to form fibrin monomers, which polymerize, creating a stable clot. Fibrinogen is a symmetrical dimer with three pairs of polypeptide chains (a, b, and g) linked by disulfide bonds. Soluble fibrinogen becomes fibrin when thrombin cleaves arginine-glycine bonds at the aminoterminal ends of the a and b chains, removing negatively charged fibropeptides A and B. The remaining fibrin monomers are linked by hydrogen bonds to form insoluble polymers (5). The purpose of this study was to demonstrate that syphilitic plasma derived from whole-blood donations can be salvaged and then converted to serum for the purpose of making reference controls and proficiency test samples.

Thrombin stock solution. A working stock solution of thrombin (Sigma, St. Louis, Mo.) at 100 U/ml in 1 M calcium chloride (Fisher Scientific, Suwanee, Ga.) was prepared, dispensed in 5-ml aliquots, and stored at −20°C until used.
TABLE 1. Effect of treatment on 48 reactive and 21 nonreactive plasma samples in the nontreponemal RPR for syphilis

| STS reactivity | Before treatment | After treatment |
|----------------|------------------|-----------------|
| Reactive       | R1–R64 (R32.5)   | R1–R32 (R16.5)  |
| Nonreactive    | NA               | NA              |

a STS, serologic test for syphilis. Forty-one of the reactive treated plasma samples had endpoint titers equal to those before treatment, and seven had endpoint titers less than those before treatment. Twenty-one treated plasma samples were nonreactive (their reactions were the same as those before treatment).

Plasma sample treatment. Sixty-nine units of plasma from different donors were obtained from the New York Blood Center (New York, N.Y.), Millennium Biotech, Inc. (Ft. Lauderdale, Fla.), and New York Biologicals (Southampton, N.Y.). These units were prescreened and found to be reactive or nonreactive in the serologic tests for syphilis. Ten milliliters of each plasma sample was placed into a 15-ml round-bottom centrifuge tube (Nalgene Nunc International, Rochester, N.Y.), and tubes were incubated in a water bath at 37°C for 30 min. One hundred microliters of thrombin stock solution was added to each sample; samples were incubated for an additional 10 min, and tubes were incubated in a water bath at 37°C for 2 h. The samples were then thawed at room temperature, and 1 g of kaolin, which had been washed with distilled water and then dried, was added to each sample; afterward, the suspension was mixed continuously for 4 h with the aid of magnetic bars.

Sample testing. Each of the 69 treated plasma samples and its corresponding untreated sample were tested for fibrinogen content by the radial immunodiffusion test (The Binding Site, Birmingham, United Kingdom), the rapid plasma reagin circle card test (RPR), the fluorescent treponemal antibody absorption double-staining test (FTA-ABS DS), the Treponema pallidum passive-particle-agglutination assay (TP-PA) (Fujirebio America, Fairfield, N.J.) (3), and the Captia Syphilis-G immunosassay (EIA) (Trinity Biotech, Bray, Ireland). Treated plasma samples were tested by the VDRL test.

The effectiveness of plasma conversion was measured by comparing the reductions in fibrinogen concentrations in the treated plasma samples to those in the untreated samples. In the untreated samples, fibrinogen concentrations ranged from 94 to 4,970 mg/liter (mean, 2,532 mg/liter) for the RPR-reactive samples and from 314 to 2,742 mg/liter (mean, 1,528 mg/liter) for the RPR-nonreactive samples. The treated samples showed no measurable amount of fibrinogen remaining after the defibrination process. There was no significant loss of reactivity when treated plasma samples were compared with the plasma baseline control samples by either the nontreponemal RPR or the treponemal tests. Of the 48 plasma samples that were reactive by RPR, 41 (85%) retained the same quantitative endpoint titer after treatment while 7 (15%) showed a reduction of one doubling dilution (Table 1). In the TP-PA analysis, 55 of 56 treated samples (98%) retained the same reactivity after treatment and only 1 sample (1.7%) showed a reduction in reactivity from 1+ to negative. Of 56 plasma samples found to be reactive by the EIA, 50 (89%) demonstrated no change in reactivity while 6 (11%) showed a reduction in the antibody index that reflected a change from reactivity to nonreactivity. Among the 54 samples that were reactive by the FTA-ABS DS, 52 (96%) showed no reduction in fluorescence, while 2 (4%) showed a reduction in fluorescence from 1+ to negative (Table 2). Treated plasma samples were also heat inactivated at 56°C for 30 min and subjected to the VDRL test. Of the 69 samples, 46 were reactive and 23 were nonreactive. There was no evidence of false-positive samples resulting from the heat treatment.

Concluding remarks. In this study, we have demonstrated that plasma can be successfully converted to serum by the addition of 1 ml of thrombin (100 U/ml) in 1 M calcium chloride to 100 ml of plasma, followed by a 10% (wt/vol) kaolin treatment. The converted plasma thus obtained is free of fibrinogen formation even after prolonged storage at −20 or 2 to 8°C. Converted plasma specimens could be used in the preparation of proficiency testing samples and as syphilis serology reference controls. Whole blood collected with anticoagulant has an approximate dilution ratio of 2.7 ml of plasma to 1 ml of anticoagulant; this dilution ratio, in addition to the defibrination procedure, is responsible for some loss in the reactivity of the converted plasma. The loss of reactivity is meaningful only

TABLE 2. Effect of treatment on reactive and nonreactive plasma samples in the treponemal test for syphilis

| STS reactivity | TP-PA | EIA | FTA-ABS DS |
|----------------|-------|-----|------------|
|                | Untreated plasma | Treated plasma | Untreated plasma | Treated plasma | Untreated plasma | Treated plasma |
| Reactive       | 56    | 56  | 54         |
| Reaction equal to that before treatment | 55 (98) | 50 (89) | 52 (96) |
| Reaction less than that before treatment | 1 (2) | 6 (11) | 2 (4) |
| Nonreactive    | 13    | 13  | 15         |
| Reaction equal to that before treatment | 13 (100) | 13 (100) | 15 (100) |

a STS, serologic test for syphilis.
if the antibody content of the sample is reduced to a point which affects the desired antibody titer. With the 69 treated plasma samples, no reduction in antibody titer was observed in 85% of samples in the nontreponemal RPR and no change in reactivity was observed for 98% of the samples in the TP-PA, 89% of those in the EIA, and 96% of those in the FTA-ABS DS. None of the nonreactive treated plasma samples became reactive as a result of the defibrination treatment.

Use of plasma is not recommended in the slide VDRL test for syphilis because heat inactivation at 56°C for 30 min enhances fibrin formation and fibrin strands entrap the liposomes present in the VDRL antigen emulsion. Because the slide VDRL test requires that the sample with the added emulsion be rotated at 180 rpm for 4 min, even minute amounts of fibrin aggregate, yielding false-positive results for nonreactive samples. In these studies, the thrombin, calcium chloride, and kaolin treatment removed fibrin from the system to such a degree that no false-positive reactions were obtained.

The syphilis elimination effort has resulted in a general decline in the number of syphilis cases in the United States, from 134,255 in 1990 to 31,575 in 2000. It has therefore become more difficult to obtain moderate- to high-titer sera from persons with syphilis for the purpose of manufacturing reference controls and proficiency test samples used for quality assurance in syphilis testing. The decreasing availability of these sera can be alleviated by salvaging plasma from blood donations that would normally be discarded because of syphilis seroreactivity and then converting the plasma to serum to be used in the treponemal and nontreponemal tests for syphilis.

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