A Monoclonal-antibody-Based Radioimmunoassay for Measurement of Protein C in Plasma

P. R. Howard,1 E. G. Bovill,2 K. G. Mann,2 and R. P. Tracy2-4

A monoclonal-antibody-based competitive radioimmunoassay for measuring human protein C is reported. With use of a purified protein C standard, the solid-phase assay was sensitive to less than 80 μg of protein C per liter. Intra-assay CVs ranged from 5% to 8%; the inter-assay CV was 5.4%. Analytical recovery averaged 104% for purified protein C added to 10 samples of normal plasmas. The assay antibody could deplete plasma of all protein C, as determined by immunoaffinity chromatography followed by polyclonal Western blot analysis. Liquid-chromatographic gel permeation of plasma indicated a single immunoreactive species that had an appropriate molecular mass for monomeric protein C. Studies of monoclonal-antibody specificity showed no significant interferences by other vitamin K-dependent proteins. The mean protein C antigen concentration in plasma of 54 healthy subjects was 3.21 (SD 0.56) mg/L and was 1.51 (SD 0.52) mg/L for 22 patients on long-term warfarin therapy. Results of the monoclonal RIA correlated well with those by a polyclonal RIA also developed in our laboratory (r = 0.93) and an amidolytic functional assay (r = 0.88) for both normal plasma and plasma from patients on long-term warfarin therapy.

Additional Keyphrases: vitamin K-dependent proteins · warfarin · coagulation · reference interval

Protein C, a vitamin K-dependent glycoprotein, is a regulator of blood coagulation, possessing anticoagulant properties when activated to its enzymatic form. Human protein C consists of a heavy chain (Mr 41,000) and a light chain (Mr 21,000), linked by a disulfide bond. The heavy chain possesses the active serine protease portion of the molecule. The amino terminal portion of the light chain contains 10 gamma-carboxyglutamic acid residues (1).

Circulating in plasma as an inactive zymogen, protein C is activated by thrombin, which results in the release of a small peptide (12 residues) from the amino-terminal region of the heavy chain (1). The in vivo activation of protein C is greatly accelerated by the presence of an endothelial cell surface cofactor, thrombomodulin (2-4). In the presence of calcium ions, thrombomodulin binds thrombin and increases the rate of generation of activated protein C by 30 000-fold (5). Activated protein C functions as an anticoagulant by inactivating Factors Va and VIIa (6), and it also promotes in vivo fibrinolysis. The in vivo administration of physiological amounts of bovine activated protein C into anesthetized dogs caused a dramatic increase in the rate of whole-blood clot lysis. The fibrinolytic response was correlated with increased concentrations of circulating plasminogen activator (7).

Measurement of protein C in plasma has become clinically important. The association between venous thromboembolic complications and congenital protein C deficiencies is well documented and appears to be transmitted as an autosomal dominant trait (8). Heterozygous protein C-deficient individuals are subject to severe, recurrent, deep venous thrombosis and pulmonary embolism at a young age; homozygous individuals succumb to neonatal purpura fulminans shortly after birth if not treated with protein C concentrates. Acquired protein C deficiency has been described in liver disease (9), disseminated intravascular coagulation (9), the postoperative period (10), and during treatment with anticoagulants (11). Warfarin-induced skin necrosis, a rare syndrome of transient paradoxical hypercoagulability resulting from the rapid decrease in protein C antigen concentrations, has been reported in patients with hereditary protein C deficiency at the initiation of warfarin therapy (12).

The recognition of the importance of protein C as a regulator of blood coagulation has initiated the development of immunological and functional assays for measurement of protein C deficiency. Initially, the availability of monoclonal protein C antisera allowed the measurement of protein C antigen by the Laurell "rocket" technique (10, 11, 13, 14). The sensitivity and reproducibility of protein C antigen determination have improved with the development of assays based on radioimmunoassay and enzyme-linked immunosorbent techniques (15-18).

The concentration of protein C antigen in normal plasma, as reported in both immunological and functional assays, has a wide range, with values from 2.5 to 4.8 mg/L (15, 16, 18, 19). Analytical variations in the measurements of normal antigen concentration could affect clinical interpretations. Unlike other coagulation factors, mild deficiencies of protein C antigen are associated with clinical thrombosis. An individual could be considered "normal" in one type of assay but "deficient" in another.

Here we report the development of a solid-phase radioimmunoassay for the quantification of protein C antigen with use of a monoclonal antibody. Except for that reported by Suzuki et al. (18), other protein C immunoassays have involved polyclonal antibodies. In addition, we report a second assay, based on a high-titer (1:40 000) equine polyclonal antiserum that can be used to measure human protein C. In both assays, a purified protein C preparation is used as standard instead of a diluted pool of normal plasma. Our emphasis has been on the monoclonal assay, with use of the polyclonal assay for validation. We have assessed antibody specificity with other vitamin K-dependent coagulation factors, assay reactivity with activated protein C, effects of interfering substances within the assays, reproducibility, and recovery. We have compared our values for protein C with those by other immunoassays and by a functional assay of protein C.

Materials and Methods

Materials

Reagents. All chemicals used were reagent grade and obtained from either Sigma Chemical Co., St. Louis, MO
Proteins. Protein C was isolated two ways. In the conventional preparation, barium citrate precipitates of human plasma were used. After elution with 0.2 mol/L EDTA solution and fractionation by precipitation with ammonium sulfate, the protein was chromatographed on diethylaminoethyl (DEAE)-Sephadex and dextran sulfate agarose (1). For further purification we used preparative gel electrophoresis (20). Protein C purity was assessed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli (21).

We also used immunoaffinity chromatography for protein purification (22). Monoclonal antibody was covalently coupled to CNBr-activated Sepharose 4B at a concentration of 4 mg/mL. "Fresh frozen" plasma, obtained from the Red Cross Blood Bank, Burlington, VT, or "PTC Concentrates" (Alpha Therapeutics, City of Industry, CA 91780) were passed over the affinity resin at 4 °C. Protein C was eluted from the column with 3 mol/L sodium thiocyanate solution and dialyzed in 20 mmol/L Tris-buffered isotonic saline (pH 7.4).

Protein C standard concentrations were determined on the basis of M, 62,000, with E1% cm. 280 sm = 14.5 (1). Protein C was diluted in an equivalent mixture of glycerol and water and stored at −20 °C.

Other vitamin K-dependent proteins used in cross-reactivity studies were prepared in our laboratory as described previously (22). Bone Gla protein (BGP) was prepared by a modification of the method of Price et al. (23).

125I-labeled protein C was prepared by the Chloramine T method (24). We diluted a 10-μg aliquot of protein to 25 μL with phosphate buffer (0.5 mol/L, pH 7.5), then added approximately 500 μCi of 125I (carrier free; Amersham, Arlington Heights, IL 60005), followed by 40 μg of Chloramine T diluted in 10 μL of the 0.5 mol/L phosphate buffer. After 30 s of hand mixing, we stopped the reaction by adding 100 μg of sodium metabisulfite in 10 μL of the phosphate buffer. After adding elution buffer (PBS; per liter, 10 mmol of monobasic sodium phosphate and 0.15 mol of sodium chloride, pH 7.4) to the reaction tube, we separated bound and free iodine by passing the mixture (total volume 10 mL) over a G25-150 Sephadex column. By this procedure, 35% to 65% of the radioactivity was incorporated into the protein C. The resulting radiolabeled protein was >90% precipitated by trichloroacetic acid, and its specific activity was approximately 20 Ci/g. We established its purity by SDS-PAGE and autoradiography. Stored in an equivalent mixture of glycerol and PBS at −20 °C, the labeled protein could be used for as long as three months.

Antisera. Polyclonal antisera to human protein C was produced in a horse by the intramuscular injection of 100 μg of purified protein C in 0.5 mL of PBS, mixed with an equal volume of complete Freund's adjuvant. All subsequent 100-μg injections were given weekly in incomplete Freund's adjuvant. Blood was collected six to eight weeks after each injection and antisera were prepared. The titer of the second antisera collected was 1:40,000 as assayed by RIA. Purified IgG was prepared by precipitation with ammonium sulfate followed by chromatography on DEAE-cellulose, and stored in an equivalent volume of glycerol and water at −20 °C.

A murine monoclonal antibody (HPC-2) to human protein C was produced by injecting 50 μg of purified protein, prepared in complete Freund's adjuvant as before. Another injection, containing 50 μg in incomplete Freund's adjuvant, was given on day 14, followed by a third injection on day 28. Fusion with NS-1 myeloma cells was performed on day 31 (25). We purified the monoclonal antibody from murine ascites fluid by chromatography on a 2.5 × 100 cm column of ACA 34 Ultrogel, followed by chromatography on DEAE-cellulose (26). The antibody was determined to be immunoglobulin isotype IgGκ. The Western blotting technique demonstrated that it recognized an epitope on the heavy chain of protein C. Based on solid-phase immunoassay data, the "apparent" Kd of the antibody was 5.2 × 10−8 mol/L.

Before its use in the RIA, we prepared a 1 mg/mL concentration of the monoclonal antibody in 15 mmol/L bicarbonate buffer (pH 9.5), then lowered the pH of the solution to 2.0. After a 1-h incubation at room temperature, the pH of the solution was returned to 9.5. The antibody was then diluted to the appropriate concentration for the RIA.

Plasma specimens. Normal plasma and plasma from warfarin-treated individuals were obtained from the Medical Center Hospital of Vermont, Burlington, VT. Specimens were anticoagulated with one part of 0.1 mol/L sodium citrate (pH 6.0) to nine parts of whole blood. We removed platelets from the plasma specimens by centrifugation at 17,000 g for 15 min at 4 °C, then quick-froze the samples in a bath of methanol/solid CO2, and stored them at −70 °C. For the assays, we thawed the samples at 37 °C, then placed them on ice. All patients being treated with warfarin were stable on their anticoagulant therapy for at least one month.

Preparation of plasma depleted of protein C. Plasma was depleted of protein C by passage over an affinity column, prepared by CNBr-activation and coupling with monoclonal HPC-2, 4 mg/mL (22). We collected the eluate and assayed it by the monoclonal and polyclonal competitive immunoassays and by Western blotting with the polyclonal antibody.

Functional protein C assay. For the functional protein C assay, based on the procedure of Sala et al. (27), plasma that had been depleted of platelets was adsorbed onto barium chloride. The vitamin K-dependent proteins were eluted with 0.2 mol/L EDTA solution, after which protein C was activated with a thrombin–thrombomodulin complex. After this activation, we added antithrombin III and heparin to the system to remove the thrombin activity. Antithrombin III was purified as previously described (28). Activated protein C was measured spectrophotometrically in microtiter wells after a synthetic substrate, S-2266, was added.

Radioimmunoassay. For the competitive solid-phase RIA we used Nunc-Immuno Plate I microtiter wells (USA Scientific Plastics, Waltham, MA 02154). Optional conditions for antibody coating were as follows: monoclonal antibody diluted to 5 mg/mL in 25 mmol/L bicarbonate buffer (pH 9.5), and polyclonal antibody diluted to 1 mg/L in bicarbonate buffer containing 10 mg of bovine serum albumin (BSA) per liter. We added 0.2 mL of antibody solution per well, then incubated the microtiter plates at 4 °C overnight. After 3 h at room temperature, we washed the wells twice with PBS buffer containing 10 g of BSA per liter, pH 7.4 (PBS/BSA buffer), we added 0.3 mL of blocking buffer (PBS containing 50 g of BSA per liter, pH 7.4) to each well, incubated for 1 h at room temperature, and washed twice with PBS/BSA. The
plates were now ready for assay components. Microtiter wells that had been coated with polyclonal antibody, followed by blocking buffer, could be prepared in batches. Stored at -70 °C, they were stable for approximately one month. Wells containing monoclonal antibody were freshly prepared for each assay run.

The protein C standard curve was prepared in an assay buffer containing 30 g of BSA and 1 mL of Tween 20 per liter of PBS. We assayed, in duplicate, serial twofold dilutions of a stock solution of protein C (initial concentration 3.20 mg/L). Control and patients' plasma specimens were also diluted in assay buffer (64-fold for the polyclonal antibody assay and 16-fold for the monoclonal antibody assay) and assayed in duplicate. Radiolabeled protein C was diluted in assay buffer to approximately 400,000 counts/min per milliliter. For the assay, we added 100 μL of standard, control, or patient's plasma to a microtiter well, followed by 100 μL of radiolabeled protein C, and incubated overnight at 4 °C (both polyclonal and monoclonal assays). After washing the wells twice to remove any unbound radioactivity, we eluted the bound radioactivity by adding 250 μL of 0.1 mol/L NaOH per well and incubating for 1.5 h at room temperature. We then transferred 200 μL of this to tubes for counting radioactivity with a Gamma 5500 counter (Beckman Instruments, Irvine, CA 92713).

The maximum binding value of the radiolabeled protein C (B0) was determined. The duplicate values for the standards, controls, and unknown plasmas were averaged and used to calculate a B/B0 value. A sample's value was excluded if the difference between duplicates exceeded the average by >10%. We plotted the standard curve on four-cycle semilogarithmic paper as protein C concentrations vs B/B0 values.

Gel-permeation liquid chromatography. Plasma was centrifuged briefly at 13,000 × g and 50 μL of the supernatant liquid was applied to a GPC-100 gel-permeation column (SynChrom, Lafayette, IN 47902), in 0.1 mol/L sodium phosphate buffer, pH 7.4. We developed the column at 1.0 mL/min, using a Model 2350 "high-performance" liquid chromatograph equipped with a gradient programmer and a Model V4 detector (all from ISCO, Lincoln, NB 68504). We collected 0.25-mL fractions and assayed 100-μL samples of these with the monoclonal-antibody-based assay.

Results

Assay validation. Figure 1 shows a representative dose–response curve for the monoclonal competitive RIA. The working range of the protein C assay extended to <80 μg/L, allowing quantification of protein C concentrations as low as 3% of the normal concentration. The standard curve was reproducible for several lots of purified standard and radiolabeled protein C. We monitored the interassay variability of standard curves by using the 50% B/B0 and B/T (total counts) values. The range of B/T in the monoclonal assay was 20–29%.

For parallelism studies we prepared serial twofold dilutions of several pools of normal plasmas and assayed them along with standards for a standard curve. In all cases, values for the pools paralleled those of the standard curve (Figure 1).

We performed competition studies with other vitamin K-dependent proteins in the monoclonal assay to assess antibody cross reactivity. In these studies, dilutions of Factors II, VII, IX, X, protein S, and BGP were assayed along with protein C; as a negative control we included antithrombin III. The ratio (counts per minute bound/total counts per minute) was plotted vs the log of the protein concentrations (mg/L). We saw no significant competition from Factors II, VII, IX, BGP, or antithrombin III in the monoclonal competitive assay (Figure 2). Slight cross reactivity was seen at a concentration of 100 mg/L for protein S and Factor X, but not at 10 mg/L. Because the concentrations of these proteins in normal plasma are low and because of the 16-fold dilution used in the assay, they caused no interference.

We also evaluated the measurement of activated protein C by the monoclonal competitive RIA. Results for equimolar concentrations of protein C standard and activated protein C correlated well (r = 0.99), indicating equivalent recognition of protein C and activated protein C.

![Fig. 1. Dose–response curves of protein C standard (○) and a serially diluted pooled normal plasma (★)](image)

![Fig. 2. Competitive inhibition studies of vitamin K-dependent coagulation proteins Factors II, VII, IX, X; bone Gla protein, antithrombin III, and proteins S and C in the monoclonal RIA](image)
To assess the complete removal of protein C from plasma passed through the monoclonal-antibody-containing affinity column, we assayed the depleted plasma by Western blotting with the polyclonal antibody, and also with the polyclonal-based RIA. Normal plasma and plasma depleted of protein C were precipitated with 1 mol/L barium chloride. The vitamin K-dependent proteins obtained were then dissolved in 0.2 mol/L EDTA and dialyzed. Western blotting of purified protein C yielded a dose-response curve sensitive to 8.75 ng of protein C. A sample of normal plasma containing the equivalent of 40 ng of protein C and the plasma sample depleted of protein C were subjected to electrophoretic analysis at comparable concentrations of total protein. In Western blotting of both samples with 2-mercaptoethanol present, normal plasma contained a component at Mr 41 000, whereas plasma depleted of protein C did not. Figure 3 is representative of several experiments. Immunoglobulin heavy chains account for the nonspecific binding in the blot. As shown by Western blotting, the plasma was >90% depleted of protein C and did not compete with radiolabeled protein C in the monoclonal assay at dilutions of 1:16, 1:8, or 1:1. No antigen could be measured in the depleted plasma by the polyclonal RIA at dilutions routinely used in this assay.

Because protein C is a vitamin K-dependent protein that binds Ca++, we examined the effect of EDTA on the assay. The presence of 2 mmol of EDTA per liter in the monoclonal RIA assay buffer caused the standard curve to shift slightly to the right, and the Bg/T increased. Normal plasma specimens diluted 16-fold gave a value for protein C of 2.8 mg/L in assay diluting buffer and 3.2 mg/L in the presence of 2 mmol/L EDTA. The antibody appears to depend only slightly on calcium.

For analytical-recovery studies we added purified protein C antigen, 1.32 mg/L, to 10 normal plasma samples. We also added 1 mg of antigen per liter to a plasma sample depleted of protein C. The average recovery for these samples was 104% (Table 1).

We evaluated assay reproducibility by using a 16-fold diluted pool of normal plasma specimens in the monoclonal assay. In 12 runs, the mean value for protein C antigen was 2.85 (SD 0.15) mg/L. The intra-assay CV ranged from 5% to 8%; the interassay CV was 5.4%. A low-value control was made by adding purified protein C (1 mg/L) to plasma depleted of protein C. For five runs, the mean was 1.15 (SD 0.12) mg/L, the interassay CV 10.3%.

We assessed the interference of lipemic, icteric, and hemolytic plasma on the measured concentrations of protein C antigen by comparing results for a plasma sample from one fasting individual with those for hemolytic, icteric, and lipemic plasma samples contrived from the fasting sample. The contrived samples contained added bilirubin (final concentration 25 g/L), Intralipid I.V. Fat Emulsion (200 mL/L suspension; KabiVitrum Inc., Alameda, CA 94501; 1 mL/L final concentration), or hemoglobin from mechanically disrupted erythrocytes (final concentration 725 mg/L). There were no significant effects on the measured concentrations of protein C antigen. Also, when we assayed five lipemic serum samples before and after ultracentrifugation to further assess lipid interferences, we found no significant differences (data not shown).

We used gel permeation liquid chromatography to see whether protein C exhibited any significant binding to other plasma proteins, as happens with protein S. After the chromatography, we assayed the fractions with the monoclonal RIA, and found a single peak of reactivity of Mr 60 000 (Figure 4). There was no evidence for a high-Mr binding protein, such as the C4b-binding protein that binds protein S.

Concentrations of protein C in plasma. We assayed protein C antigen concentration in plasma from healthy volunteers and patients being treated with warfarin. The mean value for protein C antigen from a healthy population of 28 men and 26 women (ages 21 to 62 years) was 3.21 (SD 0.56) mg/L. The mean concentration in 22 patients on long-term warfarin therapy was 1.51 (SD 0.52) mg/L.

Table 1. Recovery Studies of Protein C by RIA

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<th>Protein C concn, mg/L</th>
<th>Before addition</th>
<th>After addition*</th>
<th>Expected</th>
<th>% recovery</th>
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Mean 104
SD 7

*Added protein C, 1.32 mg/L final concentration.
^Protein C-depleted plasma (1 mg/L added).

Fig. 3. Western blot of normal plasma and protein C-depleted plasma
* indicates nonspecific binding (IgG, see text)

Fig. 4. Gel-permeation chromatography of normal plasma. Fractions were assayed for protein C by the monoclonal RIA
Comparison studies monoclonal competitive RIA vs polyclonal assay. We compared the monoclonal competitive RIA with a polyclonal competitive RIA also developed in our laboratory. Figure 5 illustrates the correlation for values from 24 healthy subjects and 12 patients on warfarin therapy. By an unweighted regression analysis, the slope of the regression line was 1.24 and the y-intercept was –0.40 mg/L.

Monoclonal competitive RIA vs protein C functional assay. We also assayed plasma specimens from 12 healthy subjects and 10 patients on warfarin therapy for antigen concentrations and for the concentrations of functional protein C. The functional assay we used was a modification of the amidolytic assay of Sala et al. (27). The correlation between these two different methods was good \((r = 0.88; \text{Figure } 6)\).

Other results reported in the literature. A wide range of immunoassay-determined reference values have been reported for protein C antigen (Table 2). The mean protein C concentration we found for the reference population in our study, 3.21 mg/L, agrees well with previous studies, especially that of Epstein et al. (15).

Discussion

During development of the present assay for protein C, several considerations were raised besides those of parallelism, recovery, and interference:

- Does our monoclonal antibody recognize all "forms" of protein C in plasma?
- Does our monoclonal antibody exhibit significant cross reactivity with any other vitamin K-dependent proteins?
- Does protein C exhibit significant binding to other plasma proteins, such as is seen for protein S, which might complicate the assay?
- Because protein C is an enzyme, do antigen concentration values correlate with enzyme activity?
- Because relatively small decreases in protein C plasma concentration can represent deficiency states, can we produce an assay of sufficient reproducibility to be useful for these borderline cases?

Studies of parallelism did not reveal any significant differences between buffer and plasma matrix. Recovery studies confirmed that the plasma matrix does not in any way "mask" added protein C. Interference studies showed that bilirubin, lipid, and hemoglobin had no significant effect.

The question concerning the completeness of antibody reactivity with protein C in plasma was a legitimate one. We had already identified a monoclonal antibody, during the initial screening of hybridomas, that reacted with purified protein C, but not protein C in plasma (unpublished observation). Western blotting studies of plasma from healthy subjects and a plasma depleted of protein C by monoclonal HPC-2 immunoaffinity chromatography showed that the assay antibody (HPC-2) reacted with all the protein C present in plasma, as evidenced by the finding of complete immunodepletion.

In addition, there was a good correlation \((r = 0.93)\) between values produced with our monoclonal assay and those produced with a polyclonal competitive solid-phase RIA also developed in our laboratory for plasma specimens from normal subjects and patients receiving warfarin therapy. Because the specimens from warfarin patients contain protein C that cannot express gamma-carboxyglutamyl acid-dependent binding (owing to the lack of gamma-carboxyglutamyl acid residues), one would expect the presence or absence of calcium binding to have no effect on the assay. However, we did observe a small effect on the assay when EDTA was added to the assay buffer.

Assay specificity is potentially a major problem, given the sequence homology between protein C and other vitamin K-dependent proteins (29). In fact, another monoclonal antibody we have produced demonstrates a shared epitope on human protein C, Factor X, Factor VII, and prothrombin (30). However, studies of cross reactivity for the HPC-2 assay failed to reveal interference by other major coagulation proteins.
We used gel-permeation studies to examine the binding of protein C to other plasma proteins. Although our gel-permeation chromatography system would not resolve free protein C from a complex with a small-Mr binding protein, it certainly would resolve free protein C from a high-Mr complex. Our studies demonstrated that protein C eluted from the column at the appropriate volume, and showed no significant binding to another plasma protein.

The correlation of immunological assays to functional assays is always difficult. In a recent comparative study of immunological and functional protein C assays, Mannucci et al. (31) demonstrated that the assays yielded different results for uncarboxylated forms of protein C in patients receiving warfarin therapy. Four out of the five functional assays they reviewed gave results significantly lower than the results of the immunoassays. This discrepancy was thought to reflect the poor adsorption of uncarboxylated protein C from plasma to the aluminum hydroxide and the barium salts used in the initial steps of these functional assays (31). In our studies, we attempted to correlate results from an assay modified from the amidolytic functional assay by Sala et al. (27) with those of our monoclonal RIA. The correlation value of 0.88 was in good agreement with that obtained by Sala et al. (r = 0.86) in their initial studies of healthy individuals and hospitalized patients. This r value is also comparable with those obtained by Mannucci et al., who compared results from the Sala assay with those from a Laurell rocket method (r = 0.91) and an enzyme immunoassay method (r = 0.87) for protein C (31).

With regard to reproducibility, Miletech and Broze (32), in a study of protein C antigen values in a large normal population, showed the importance of assay CVs for distinguishing low "normal" from high heterozygous protein C deficiency, on the basis of concentrations in plasma. They concluded that the interassay CV must be very tight for screening and identifying patients with congenital deficiencies. As was shown in Table 2, normal concentrations of protein C antigen fall in a wide range. Given that concentrations <65% of the pool value are usually considered abnormal (18), an individual potentially could be considered "normal" on the basis of data provided by one assay, yet "deficient" by another assay. As documented in Table 2, besides our assay, only two other immunoassays routinely achieve CVs of about 5%: those of Miletech and Broze (32) and Suzuki et al. (18). We are currently in the process of automating our assay, and anticipate even lower CV values in the near future.

In conclusion, the monoclonal competitive solid-phase RIA described here is sensitive, accurate, and highly reproducible for the measurement of protein C in plasma. Long-term stability is ensured through the use of a purified protein C standard and a monoclonal antibody.

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References


