D-Dimer is the term for any of a variety of split products that are derived from fibrin fibrils by the action of the fibrinolytic enzyme plasmin. They occur in the circulation in a wide range of molecular weights and carry various numbers of cross-linked D-domains, called D-Dimers (1). Although no commonly accepted standard is available, D-Dimers can be measured by immunoassays that use specific antibodies against the cross-linked D-domain. The concentration of D-Dimers in the blood plasma is correlated to the activity state of the coagulation and fibrinolytic system (2).

D-Dimer testing has been shown to be of value for diagnosis of clinically suspected thromboembolism (2–5). In a recent meta-analysis (6) including data from 2000 patients with suspected deep venous thrombosis and/or pulmonary embolism, increased D-Dimers as measured by ELISA were found to provide a diagnostic sensitivity of 97% for both diseases. However, the practical diagnostic value of D-Dimer testing by ELISA is limited by the long turnaround time and the special equipment required for the assay. Qualitative or semiquantitative latex agglutination slide-assays are suited for bedside testing, but are not recommended because of lower diagnostic sensitivity (6). Recently, a latex-based reagent for D-Dimer testing on clinical chemistry automated analyzers was developed. The diagnostic sensitivity of this assay for detection of deep venous thrombosis matched ELISA methods (7).

The recommended specimen for D-Dimer testing is citrate plasma, which is, however, not appropriate for many chemical analyses. Therefore, the advantage of D-Dimer testing on automated analyzers is hampered by the need for two different specimens when other analyses are requested.

To reduce costs, time, and the need for blood, we investigated whether D-Dimer testing is possible from heparinized plasma, which is suitable for routine chemical analyses.

One hundred pairs of blood samples anticoagulated with either citrate or heparin were used for the study. Samples were submitted from the emergency ambulance to the emergency laboratory for routine chemistry and coagulation examinations. For citrated plasma, 4.5 mL of blood was collected into Vacutainer Tubes containing 0.5 mL of 38 g/L sodium citrate (Becton Dickinson, cat. no. 606608); for heparinized plasma, we used evacuated tubes with lithium heparinate (Becton Dickinson, cat. no. 368484). Platelet-poor plasma was prepared by centrifugation at 2500 g for 10 min. After performance of the requested routine examinations, the remaining citrate and heparin plasma was stored in aliquots at −70 °C and tested for D-Dimer within 4 weeks.

D-Dimer was measured by ELISA (D-Dimer Gold®, Agen Biomedical) according to the manufacturer’s instructions, using a microplate reader and washer (MR 7000, Dynatech Laboratories). Each microtiter plate was calibrated, and all samples were tested in duplicate. For harmonization to other D-Dimer tests, all results were multiplied by the recommended conversion factor of 2.79 (8).

In addition, D-Dimer was measured on an automated chemistry analyzer (Hitachi 717) with the microlatex Tinaquant™ D-Dimer test kit (Boehringer Mannheim) according to the manufacturer’s instructions. The assay calibration was maintained for 2 weeks, and single determinations were made on all samples. Samples with >3 mg D-Dimer/L (3 μg/mL) were automatically reanalyzed with reduced sample volume (3 μL instead of 7 μL). The concentration of D-Dimer in the six calibrators was given as fibrin-equivalents, which are about twice the D-Dimer concentrations. We calculated an average conversion factor of 0.4 from the slopes obtained by method comparisons made with pool material and 100 patient samples.

Precision of the assays was evaluated with three pools of citrated plasma obtained from patients (n = 10) with low, medium, and high D-Dimer content, respectively (Table 1). A poor precision was observed for the ELISA method compared with Tinaquant.

Linear regression analysis by the method of Passing and Bablok (9) was used for comparison of D-Dimer values obtained by harmonized ELISA and the converted Tinaquant test, respectively, from citrate plasma samples of 100 patients (Fig. 1A). The Tinaquant results were slightly higher than the ELISA results (mean, 9.57 mg/L vs 8.92 mg/L); the slope of regression was 1.05 (confidence interval, 0.954–1.147); and the intercept was 0.07 (0.046–0.104). For the whole range of results (0–8 mg/L), the correlation coefficient was 0.917; the correlation coefficient was 0.826 in the low range (0–1 mg/L).

The comparison of D-Dimer results from the corresponding citrated and heparinized plasma samples measured with the Tinaquant is shown in Fig. 1B. The correlation coefficient was 0.998 for the whole range and 0.949 for the low range. Because addition of citrate solution to the blood diluted the plasma, the D-Dimer concentrations in citrated plasma were lower than in heparinized plasma (found median difference was 16%, which corresponded to an estimated plasma fraction of 58%, or a 42% hematocrit). The mean difference between the two types

<table>
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<tr>
<th>Table 1. Precision of the D-Dimer tests.</th>
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<td>Mean harmonized concentration, mg/L, n = 21</td>
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<tr>
<td>Low pool</td>
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<tr>
<td>0.24</td>
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<tr>
<td>(1.5%)</td>
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<tr>
<td>Medium pool</td>
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<tr>
<td>(2.4%)</td>
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<tr>
<td>High pool</td>
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<td>(1.4%)</td>
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The concentration of specimens was 0.15 ± 0.3 mg/L for the whole range and 0.03 ± 0.1 mg/L for the low range (<1 mg/L).

We conclude that citrated and heparinized plasma are equivalent specimens for D-Dimer testing with Tinaquant. For harmonization of D-Dimer concentrations between citrate and heparin plasma, a conversion factor of 0.84 for heparin plasma is recommended. The use of heparinized plasma allows measurements of chemical constituents as well as D-Dimer from blood in a single collection tube.

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References


Gel Diffusion Procedure for the Detection of Cryoglobulins in Serum, Toshio Okazaki,1,2* Tatsuo Nagai,2 and Takashi Kanno3 (1 Department of Biochemistry, Mitsubishi Kagaku Bio-Clinical Laboratories, Inc., Shimura 3-30-1, Itabashi-ku 174, Japan; 2 Department of Forensic Science, School of Allied Health Science, Kitasato University, 1-15-1 Kitasato, Sagamihara City 228, Japan; 3 Department of Laboratory Medicine, School of Medicine, Hamamatsu University, Handa-cho 3600, Hamamatsu 431–31, Japan; *author for correspondence: fax 81-03-5994-2925)

Cryoglobulin is a thermoprotein precipitated at low temperatures in serum (1). It consists mainly of immunoglobulins, which form various complexes. They are classified into three types (I, II, and III) according to their composition (2). In recent years, there have been reports on the relationship between type II cryoglobulinemia and hepatitis C (2–4), which is being widely studied. The common method of detection of cryoglobulins is as follows. After blood is collected at 37 °C and serum separated, 5 mL of the serum is allowed to stand in a cryocrit tube at 4 °C for 2–7 days, and formation of the precipitate is confirmed visually. When a