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Evaluation of the effect of storage temperature on D-dimer stability, using two different techniques

Abstract

Our study designed to assess the stability of D-dimer levels and storage conditions, using two different techniques. This is the first study to investigate the short-term storage stability of D-dimers in clinical citrated plasma samples containing a range of D-dimer concentrations. This study was performed with 60 samples that were collected randomly patients with suspected thromboembolism. Plasma samples were incubated for eight different time intervals (0 (i.e., fresh samples), 4, 24, 48, 72, 120, 168, and 240h) and at three different temperatures (25+2°C,4+2°C, and -20+2°C) to evaluate the effect of storage conditions on D-dimer stability. Plasma D-dimer concentrations were determined using two different techniques, a fluorescence-based sandwich immunodetection assay (i-CHROMA, Boditech Med Inc., Korea) and an enzyme-linked immunosorbent assay (ELISA) (VIDAS, bioMerieux, France). In accordance with our study, we suggest D-dimer analysis be performed on plasma samples stored for <48h at room temperature. Our findings indicate that i-CHROME and VIDAS D-dimer measurement methods have remarkably high sensitivity and are safe first-line tests that can be utilized to rule out pulmonary emboli in outcome studies multiple freeze-thaw.

Keywords: D-dimer, frozen plasma, fresh plasma, stability, room temperature

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Introduction

The D-dimer assay evaluates thrombin and plasmin activity and is specific for fibrin derivatives. In this assay, the presence of cross-linked D-dimer domains is a diagnostic marker of fibrin clot lysis. Furthermore, it is a highly sensitive marker for the formation of thrombin and indicates that factor XIII is activated by reactive fibrinolysis. Since fibrinogen derivatives do not contain a cross-linked D-dimer domain, they are not recognized by the D-dimer assay, even when present at high concentrations.[1-4] D-dimer assay protocols vary among laboratories, with some being superior to others. The method utilized depends not only on the equipment available, but also on a number of other factors including study design and analysis (i.e., chosen cut-off for D-dimer concentrations).[5-7]

The introduction of bedside analysis of D-dimer concentrations has facilitated rapid and reliable D-dimer tests convenient for clinical use, in which short sample turnaround time is essential. In routine clinical evaluations, D-dimer assays are performed using fresh plasma. However, storage is required in certain cases including reproducibility, reassessment, research, quality assurance purposes, and criminal cases. Although uncommon, testing is delayed in some cases. In all of these cases, proper storage is necessary. D-dimer stability in plasma in vitro is widely assumed but has not yet been documented by systematic studies evaluating samples covering a range of D-dimers. The Clinical and Laboratory Standards Institute (formerly the NCCLS) guidelines state that plasma samples may be stored for up to 2 weeks at -20°C and up to 6 months at -70°C.[8] Our study designed to assess the stability of D-dimer levels and storage conditions with two different techniques. To our knowledge, this is the first study to investigate the short-term storage stability of D-dimers in clinical citrated plasma samples containing a range of D-dimer concentrations.

Materials and methods

Plasma samples

This study was performed by the Department of Clinical Microbiology, Training and Research Hospital, in Sakarya, Turkey from August to November 2014. Samples were collected randomly from 60 patients (31 females and 29 males, age range: 42-58 years) with suspected thromboembolism. Initially, samples were obtained by standard phlebotomy techniques, placed into 5ml tubes containing 3.2% sodium citrate (Vacutainer, Becton Dickinson®, Franklin Lakers, NJ, USA), and then centrifuged (2000g) for 5min to yield plasma. Eligible samples were defined as those that contained ≥1.5ml plasma volume. The plasma samples were collected for routine laboratory tests for evaluation of related measurement(s), while the remainder was removed for the D-dimer tests.

Plasma sample conditions

Prior to the evaluation of D-dimer stability, all plasma samples were aliquoted into eight 200µL plastic sterile storage tubes. Plasma samples were incubated for eight different time intervals (0 (i.e., fresh samples), 4, 24, 48, 72, 120, 168, and 240h) and at three different temperatures (25±2°C, 4±2°C, and -20±2°C) to evaluate the effect of storage conditions on D-dimer stability. As the baseline measurement, fresh plasma samples were tested immediately (0h).

Study design

Plasma D-dimer concentrations were determined using two different techniques, a fluorescence-based sandwich immunodetection assay (i-CHROMA, Boditech Med Inc., Korea) and an enzyme-linked immunosorbent assay (ELISA) (VIDAS, bioMerieux, France). All assays were evaluated according to the manufactures' protocols. D-dimer assays were performed in a blinded fashion, without prior knowledge of the clinical details. For each day of analysis, two control concentrations were evaluated (i.e., that indicating normal levels and that indicating abnormal levels). Controls fell within the respective manufacturer's specified limits. D-dimer assays were performed by one experienced laboratory technician to avoid mistakes made due to a lack of experience. In this study, patients with cancer, cardiovascular disorders, hematological abnormalities, acute infections, granulomatous chronic disease, metabolic diseases, and a history of recent surgery were excluded.

Statistical analysis

Data analyses were performed using SPSS v. 21 (SPSS Inc., Chicago, IL, USA). Results are presented as means. The normality of distribution was tested using the Kolmogorov-Smirnov test. Relationships between both techniques and storage conditions were evaluated using chi-square, Mann-Whitney U, and Fisher's exact tests. P values ≤0.05 were considered statistically significant. This work was performed as part of a laboratory-based study, with no direct patient interactions.

Results

In total, 60 plasma samples were utilized. The results indicated a range of D-dimer concentrations, from normal to abnormal. D-dimer concentrations measured by i-CHROMA ranged from 96 to 6,073ng/L, with a mean of 746.12±788.44ng/L, and those measured by VIDAS ranged from 102 to 6,203ng/L, with a mean of 793.32±813.18 ng/L.

D-dimer levels in the plasma samples did not differ after 4 or 24 h compared with 0 h when stored at room temperature. However, longer storage times (48 and 72 h) at room temperature resulted in reduced D-dimer levels compared with 0 h (19.3% and 35.9%, respectively). Although D-dimer levels were not statistically significant after 48 or 72 h compared with 0 h (P=0.252 and P=0.08, respectively), there was a statistically significant difference observed at 120 h when stored at room temperature (P=0.012). For the other temperatures evaluated, we did not observe any significant differences between the 0 and 240 h time points. For both i-CHROMA and VIDAS, there were no differences regarding normal versus abnormal concentrations. However, we did observe a reduction in D-dimer stability using i-CHROMA (8%) or VIDAS (18%) when plasma was stored at room temperature for 48 h.

D-dimer levels in plasma samples stored at 4°C did not differ between 0 and 240h. There was a strong correlation between fresh samples and samples stored at 4°C for all time points evaluated (r>0.990-1.000; P<0.001). Similarly, D-dimer levels in plasma samples stored at -20°C.

Discussion

D-dimers are formed from the breakdown of intravascular fibrin and serve as a marker of fibrinolytic activity. The measurement of D-dimer levels is an important step in the diagnosis of clinically suspected venous thromboembolism not differ between 0 and 240h. There was a strong correlation between fresh samples and samples stored at -20°C for all time points evaluated (r>0.993-1.000; P<0.001). Increased storage time at 4°C and -20°C did not decrease D-dimer concentrations. The two methods utilized to measure D-dimer levels, i-CHROMA and VIDAS, did not reveal any significant differences in D-dimer concentrations between the 0 and 240h time points when stored at either 4°C or -20°C.

In addition to accurate execution of the method used to measure D-dimer levels, certain aspects of the technique (i.e., study design and analysis) play a role in the reliability of the results. A practical laboratory evaluation was performed to assess the suitability of different methods. The following aspects were considered: materials, quantity of materials, methods, analytical range, pre-analytical handling of the samples, estimated pre-analytical time, and duration of the analysis. Additionally, D-dimer testing is affected by numerous pre-analytical variables including time from collection to testing and storage temperature. Also, since D-dimer analysis is not a common test among all laboratories, the optimization of storage and transfer conditions is necessary to minimize pre-analytical errors.

In various studies, plasma samples have been stored at different temperatures. A study by Böhm-Weigert et al. reported that a reduction in D-dimer concentrations after frozen storage was not time-dependent. Repeated freezing did not significantly alter D-dimer values (mean change ≤5%). These data demonstrate that D-dimer levels in plasma are stable prior to freezing under the following conditions: ≤4h at room temperature or ≤24h at 2-8°C. Samples are also stable when stored for ≤3 years at -60°C.[9] Limited data exist regarding the impact of freezing on D-dimer stability. We sought to evaluate the effect of storage at different temperatures on the stability of D-dimer concentrations in plasma samples using two different techniques.

Initial studies showed that the i-CHROMA and VIDAS methods yield comparable results. Using both methods, we observed similar results at room temperature for the first 24h of storage. However, a decrease in D-dimer concentrations was observed after storage for 72h at room temperature. A statistically significant decrease in D-dimer concentrations was observed after 120h storage at room temperature. These results demonstrate that D-dimer concentrations in plasma are stable for ≤24h at room temperature. However, storage for >24h age at room temperature results in a time-dependent decrease in D-dimer levels have remarkably high sensitivity and are safe first-line tests that can be utilized to rule out pulmonary emboli in outcome studies.

I-CHROME, which is a point of care test, is suggested for samples stored under appropriate conditions. This and fuAccording to D-dimer stability comparisons at 4°C and -20°C using both I-CHROMA and VIDAS, the measurements were highly correlated (> 98%) between the two systems. These findings suggest that both D-dimer tests would be effective in diagnosing an individual suspected to have venous thromboembolism. Although we detected a slight reduction in D-dimer concentrations when samples were stored at 4°C and -20°C, this marginal change was not time-dependent.

A few limitations in our study merit consideration. First, we used a small sample size, and the patients' clinical information was not available. Therefore, whether the patients had deep vein thrombosis or pulmonary embolism was unknown. Second, only the small number of plasma samples that produced consistent results in the initial measurements were evaluated for stability at both 4°C and -20°C. Despite these limitations, our findings support the conclusion that D-dimer concentrations are stable when stored at 4°C and -20°C. Furthermore, we suggest the use of stored samples when performing new diagnostic tests to measure D-dimer levels. Additionally, our findings indicate that plasma samples may be stored for ≤240 h at -20 C, ≤168 h at 4°C, or ≤24 h at room temperature.

This clear demonstration of D-dimer stability in plasma during storage at -20°C should facilitate the storage of plasma samples used for laboratory research. This study has several strengths. First, the studies described in this work provide a comprehensive view of the stability of D-dimer concentrations in plasma stored over a wide range of temperatures (room temperature, 4°C, and -20°C). Second, to our knowledge, few studies have focused on the stability of D-dimer concentrations stored at room temperature over various time intervals. In accordance with our study, we suggest D-dimer analysis be performed on plasma samples stored for <48h at room temperature. Our findings indicate that i-CHROME and VIDAS D-dimer measurement methodture studies that compare different methods and storage conditions and test a number of variables will be necessary for the standardization of D-dimer tests.

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None.

Conflicts of interest

Authors declare there are no conflicts of interest.

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