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# Thromboelastographic Tracings in Retired Racing Greyhounds and in Non-Greyhound Dogs

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**Background:** Bleeding disorders in patients with normal coagulation test results are frequently reported in Greyhounds. The purpose of this study was to compare Greyhounds to non-Greyhounds by thromboelastography (TEG).

**Hypothesis:** TEG parameters in Greyhounds are different from those in non-Greyhounds.

**Animals:** Forty-three healthy dogs (28 Greyhounds and 15 non-Greyhounds) based on the results of physical examination, CBC, activated partial thromboplastin time, prothrombin time, fibrinogen, and platelet count.

**Materials and Methods:** Recalcified citrated native TEGs were performed in both groups; data were compared using Student's, Mann-Whitney, and Pearson's statistical tests.

**Results:** In Greyhounds, mean ± SD were as follows: R-time 4.3 ± 1.7 minutes, K-time 3.8 ± 1.4 minutes, angle (α) 50.0 ± 8.0°, maximum amplitude (MA) 47.6 ± 5.6 mm, clot strength (G) 4,647 ± 1,097 dyn/cm$^2$, and percent lysis at 60 minutes (LY60) 2.8 ± 5.0%. In the non-Greyhounds they were R-time 3.7 ± 1.6 minutes, K-time 2.5 ± 0.9 minutes, angle 59.8 ± 7.0°, MA 53.1 ± 5.6 mm, G 5,811 ± 1,256 dyn/cm$^2$, and LY60 3.1 ± 2.5%. All parameters were significantly different between the groups, except for R-time and LY60.

**Conclusion:** In Greyhounds, clotting kinetics are slower and clot strength are weaker than in non-Greyhounds, supporting the increased tendency to bleed observed after minor trauma or surgical procedures in the breed. The findings may also be attributed to blood viscosity or to the concentration of citrate in the sample (ie, Greyhounds have higher hematocrit and less plasma per unit volume).

**Key words:** Bleeding; Clot kinetics; Fibrinolysis; Hemostasis.

Greyhounds have unique physiological characteristics such as high PCV, hemoglobin concentration, and whole blood viscosity in addition to low white blood cell count, neutrophil count, platelet count, and serum protein and globulin concentrations.[1–3] Greyhounds also have high glomerular filtration rate, functional murmurs of aortic stenosis, higher blood pressure, higher serum creatinine concentrations, and lower serum $T_4$ and $fT_4$ concentrations than other breeds of dog.[4–8]

An issue of concern in the breed is the "Greyhound bleeder." This term typically has been used for those dogs that bleed, either spontaneously or after minor surgical trauma or procedures. We recently demonstrated that 26% of retired Greyhound racers bleed 24–48 hours after routine spay or neuter procedures; some bleed profusely and may require fresh frozen plasma or red blood cell transfusions as a life-saving measure, despite having normal one-stage prothrombin time (OSPT), activated partial thromboplastin time (APTT), and platelet counts.[a,b,9] Interestingly, in that study, bleeding was not associated with thrombocytopenia, platelet dysfunction, clotting factor deficiencies, or von Willebrand disease or syndrome.[a] Therefore, clot kinetics or strength, hemorrheologic issues, enhanced fibrinolysis, and vasculopathy are likely candidates for this hemostatic dysfunction.

The thrombelastograph[c] (TEG) is a whole blood coagulation analyzer that evaluates cell/protein interaction. It allows for a global analysis of the hemostatic system, including primary and secondary hemostasis, and the fibrinolytic system.[10] The TEG is a novel device in veterinary medicine used to evaluate patients with coagulopathies.[11–13] However, it is commonly used in human medicine for monitoring anticoagulant therapy in cardiac patients and liver transplant patients, and as an indicator for transfusion.[14–16]

Evaluation of the TEG tracing and data analysis allow one to isolate the different components of the hemostatic system. The TEG parameters used in this study are described as follows: The R-time is the time from addition of the agonist ($CaCl_2$) to the citrated whole blood in the cup until clot formation reaches a detectable level (2 mm trace amplitude), and represents the enzymatic portion of coagulation. The K-time is the time from detection of the endpoint R-time until the clot reaches a determined firmness (20 mm trace amplitude) and is a measure of the speed to reach a certain level of clot strength, representing the clot kinetics. The angle is related to the fibrinogen concentration and the rapidity of fibrin formation and cross linking, also related to the kinetics of clot formation. The MA is the maximum amplitude or ultimate strength of the fibrin clot and represents primarily the contribution of platelet aggregation to clot formation. G provides a measure of clot strength viscoelastographically in dyn/cm$^2$ and is calculated from the MA. Finally, LY60 represents the percent or proportion of clot lysis (ie, clot retraction and fibrinolysis) or decrease in amplitude from the MA at 60 minutes, under the area of the tracing.[17]

The purpose of this study was to evaluate whole blood hemostasis in healthy Greyhounds by means of TEG and

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to compare the results with those of healthy non-Greyhound dogs.

## Material and Methods

The 43 (28 Greyhounds and 15 non-Greyhounds) healthy dogs with no previous history of bleeding disorders used in the study were either enrolled in The Ohio State University Animal Blood Bank (OSUABB), were part of a 3rd year veterinary students' spay and neuter clinic, or were enrolled in the study after signed owner consent. The OSUABB and the Operative Practice Laboratory have current animal use protocols on file; the use of client-owned dogs was approved by the Veterinary Teaching Hospital Board, and the owners provided signed consent. Of the 28 healthy Greyhounds, there were 11 intact females and 7 intact males, and 2 spayed females and 8 castrated males. The mean age was 5.6 years (range 3–9 years).

Fifteen healthy non-Greyhound dogs were also evaluated; there were 6 mixed-breed dogs, 3 Labrador Retrievers, and 1 dog of each of the following breeds: Beagle, Dalmatian, German Shorthair Pointer, Presa Canario, Rotweiller, and Coonhound. Two were intact females and 1 an intact male; 4 were spayed females and 8 castrated males. The mean age was 4.5 years (range 2–14 years). All dogs were considered healthy based on absence of clinical signs of illness, results of physical examination, CBC, hemostasis panel (APTT, OPTT, fibrinogen), and platelet count. In addition, all the dogs enrolled in the OSUABB program had serum biochemistry profile results within the normal reference range.

Blood samples were collected by jugular venipuncture with a 21G needle and a 6-mL sterile syringe, then placed into two 2.7-mL Vacutainer[d] tubes containing 3.2% buffered sodium citrate to achieve a final proportion of anticoagulant to blood of 1 : 9, and mixed gently. They were stored for 30–45 minutes at room temperature in a tube rack. Citrated tubes were centrifuged (1,380 × *g* for 10 minutes) within 45 minutes of sampling to obtain plasma for hemostasis assays (OSPT, APTT, fibrinogen concentration) in an ACL-200.[e] Pooled plasma samples from 3 non-Greyhound healthy dogs were used as control group, and commercially available reagents[f] were used as previously described.[18] CBCs were performed with 0.8 mL of citrated blood in a LaserCyte,[g] as previously reported.[19]

### TEG Analysis

A single TEG test was performed per patient because we have only 1 instrument and could not run all samples in duplicate. Initially, 20 μL of $CaCl_2$ was placed in the prewarmed cup of the TEG-5000 then, 340 μL of citrated blood was added for a total volume of 360 μL. Tracings were obtained after 120–180 minutes of running time at 37 °C.[c]

### Statistical Analysis

All data were evaluated for normality by the Kolmogorov-Smirnov test. Statistical analysis was performed using an unpaired Student's test, and a Mann-Whitney *U* test was used to compare the distribution of the LY60 parameter between the study groups (data were not normally distributed). Correlation (Pearson) between HCT, and APTT, R, K, angle, MA, G, and LY60 also was evaluated. Statistical significance was set at $P < .05$ and the Prism statistical software package[h] was used to analyze the data.

## Results

The results of TEG obtained in the healthy Greyhounds and non-Greyhounds are provided in Table 1. In the healthy Greyhounds, the mean K-time was 3.8 ± 1.4 minutes; the angle was 50.0 ± 8.0°; the MA was 47.6 ± 5.6 mm; and the G was 4,647 ± 1,097 dyn/cm$^2$ (Table 1). The mean K-time in the non-Greyhounds was 2.5 ± 0.9 minutes; the angle was 59.8 ± 7.0°; the MA was 53.1 ± 5.6 mm; and the G was 5,811 ± 1,256 dyn/cm$^2$. Significant differences were observed between the groups for the following: K-time ($P = .0020$) was significantly longer (Fig 1A), and the angle ($P = .0003$) (Fig 1B), MA ($P = .0034$) (Fig 1C), and G ($P = .0030$) (Fig 1D) were significantly lower in the Greyhounds. The R-time (Fig 1E) and the LY60 (Fig 1F) were not significantly different between groups. A representative TEG tracing in a Greyhound and in a non-Greyhound dog is presented in Figure 2.

When comparing the results of routine hemostasis testing, there were no significant differences between groups for the OSPT ($P = .1061$) and fibrinogen concentration ($P = .4641$); however, the APTT and HCT (Table 1) were significantly higher ($P = .0020$ and $P = .0005$) (Figs 3A and B) and the platelet count was significantly lower in the Greyhounds ($P = .01$) (Fig 3C). There was

**Table 1.** TEG mean parameters and APTT, PT, fibrinogen, hematocrit, and platelet count mean values with respective control-plasma-test values and *P*-value of the study groups.

| Results | | Non-Greyhounds Mean ± SD (range) n = 15 | Greyhounds Mean ± SD (range) n = 28 | *P*-Value |
|---|---|---|---|---|
| R-time (minutes) | | 3.7 ± 1.6 (1.7–8.2) | 4.3 ± 1.7 (1.8–8.8) | .2471 |
| *K-time (minutes) | | 2.5 ± 0.9 (1.3–4.5) | 3.8 ± 1.4 (1.8–7.7) | .0020 |
| *Angle (degrees) | | 59.8 ± 7.0 (46.3–71.4) | 50.0 ± 8.0 (34.3–64.9) | .0003 |
| *MA (mm) | | 53.1 ± 5.6 (43.5–61.0) | 47.6 ± 5.6 (38.0–60.9) | .0034 |
| *G (dyn/cm$^2$) | | 5811 ± 1256 (3843–7810) | 4647 ± 1097 (3058–7772) | .0030 |
| LY60 (%) | | 3.1 ± 2.5 (0.0–8.6) | 2.8 ± 5.0 (0.0–19.2) | .0808 |
| *PLT (10$^9$U/L) | | 257 ± 88 (142–421) | 200 ± 49 (105–314) | .0100 |
| *HCT (%) | | 41.81 ± 4.3 (35.0–48.4) | 47.61 ± 5.1 (38.0–59.0) | .0005 |
| Test | Control | | | |
| PT (seconds) | 7.0 ± 0.5 | 6.9 ± 0.5 (6.1–8) | 6.7 ± 0.8 (6.1–7.9) | .1061 |
| *APTT (seconds) | 10.4 ± 0.8 | 12.17 ± 0.9 (9.9–14) | 13.3 ± 1.15 (11.1–15.7) | .0020 |
| Fibrinogen (mg/dL) | 99.3 ± 35.4 | 153.8 ± 51.3 (102–278) | 173.7 ± 97 (73–365) | .4641 |

The asterisks denote statistically significant differences between the groups.

TEG, thromboelastography; APTT, activated partial thromboplastin time; PT, prothrombin time; MA, maximum amplitude; LY60, percent lysis at 60 minutes; PLT, platelet count; HCT, hematocrit; n, number of dogs.

no correlation between HCT and APTT, R, G, or LY60 but there was a weak correlation between HCT and K ($r = 0.577$), HCT and angle ($r = 0.420$), and HCT and MA ($r = -0.386$).

## Discussion

We recently documented hemostatic complications in Greyhounds that underwent minor (eg, spaying and neutering) and major surgical (eg, amputation) procedures, consisting of excessive bleeding and bruising at the surgical site and in dependent areas (eg, axillary and inguinal regions).[a,b] Routine tests of hemostasis (APTT, OSPT, platelet count) and platelet function studies did not identify the cause of bleeding and bruising in those Greyhounds.[a,b] However, we speculated that individual cellular (eg, platelet count and function) and humoral (eg, OSPT, APTT, fibrinogen concentration) components used to routinely evaluate hemostasis may not be a reliable way to assess global hemostasis unless marked abnormalities are present.

Evaluation of hemostasis by TEG identified striking differences between Greyhounds and non-Greyhounds, although some of the results overlapped (Fig 1 and Table 1). The long K-time in Greyhounds suggests lower enzymatic function and slower clot kinetics. The decreased angle, lower MA, and lower G typically are associated with thrombocytopenia or decreased interaction between fibrin and platelet assembly, and reduced clot strength (ie, weaker clot). The diminished enzymatic clot function and kinetics observed potentially could explain the bleeding episodes in Greyhounds.

Greyhounds have several physiological peculiarities, including high blood pressure, large left ventricle, high aortic velocity, and high cardiac output, when compared with non-Greyhound dogs.[20–22] In this study, we demonstrated that Greyhounds have weaker clots than do non-Greyhounds. It is possible that the higher blood pressure

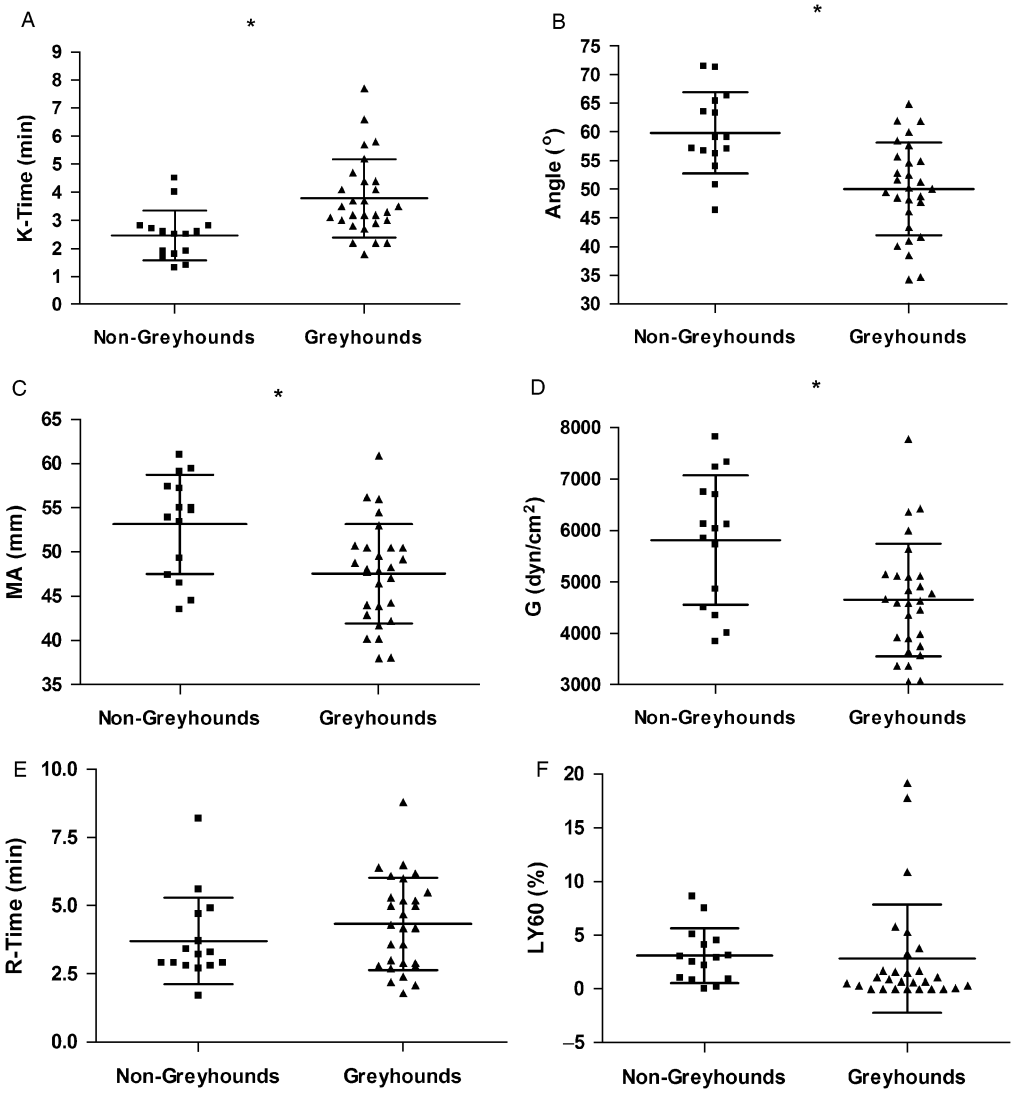


**Fig 1.** Vertical scatter plots showing the difference between the means for the K-time (A), angle (B), MA (C), G-value (D), R-time (E), and LY60 (F). Horizontal bars indicate the mean ± SD for each group, and the asterisk marks plots with significant differences between the groups.

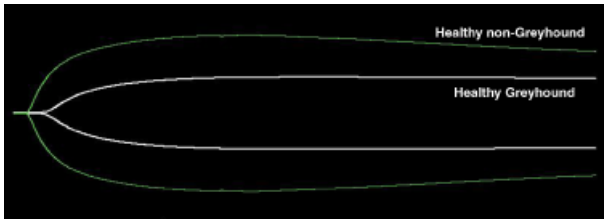


**Fig 2.** Representative superposed TEG tracings of a healthy non-Greyhound (outside tracing) and a healthy Greyhound (inside tracing).

or shear overrides the effectiveness of a weak clot and results in postoperative bleeding. Alternatively, the high whole blood viscosity in Greyhounds could artifactually cause a decrease in the MA and G because of restriction of pin movement in the instrument. In addition, because Greyhounds have less plasma per unit volume (ie, they have high HCT) and we did not adjust the concentration of sodium citrate, the lower MA and G may be the result of excessive anticoagulant, as suggested in some of the human studies.[i] However, according to the NCCLS Guidelines for Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays,[23] anticoagulant concentration should be adjusted in patients with HCT > 55%. Only 2 Greyhounds in this study had HCT > 55% (55.7 and 59%, respectively).[23]

In a recent study on transgenic polycythemic mice that carry the human erythropoietin cDNA driven by platelet-derived growth factor β promoter, the authors demonstrated that polycythemic mice have weaker clot strength (lower MA and G) and delayed kinetics of clot formation (ie, lower angle) when using TEG.[24] Moreover, the authors demonstrated that the changes in TEG tracings between mice with normal HCT and those with erythrocytosis were not caused by blood viscosity factors or dilutional effects from anticoagulant, but rather represented a true phenomenon. Polycythemic mice also had lower fibrinogen concentration and prolonged OSPT and APTT than did normal mice. The authors postulated that the hypocoagulability seen in polycythemic mice constituted a compensatory mechanism to prevent the thromboembolic events commonly seen in humans with erythrocytosis.[24] Moreover, similar to the situation in the Greyhounds, when the platelet count was adjusted for HCT, polycythemic mice were found to have normal corrected platelet counts.[24] Therefore, it is unlikely that the mild thrombocytopenia seen in the Greyhounds will explain the decreased angle and lower MA and G that we documented in this group.

Although statistically significant differences in APTT and platelet counts were observed between the Greyhounds and non-Greyhounds, those differences appear to be clinically irrelevant and are unlikely to have had an effect on the TEG tracing. For example, the mean difference for the APTT between groups was approximately 1 second, and all results were within the reference ranges. Altered APTT and PT results are considered clinically relevant only if they exceed by 25% those observed in the control samples. In this study, most control samples for

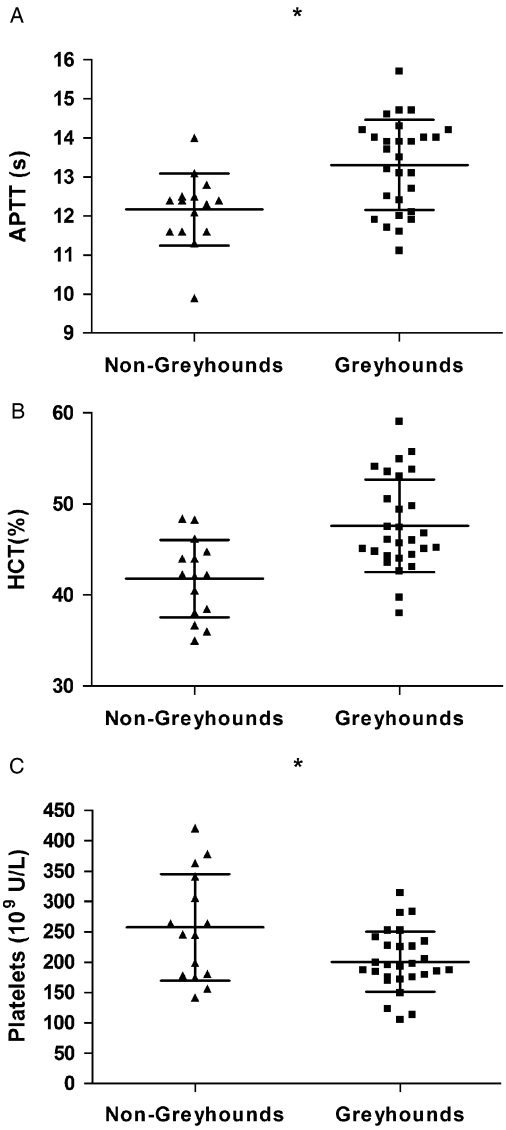


**Fig 3.** Vertical scatter plot showing the difference between the means for the activated partial thromboplastin time (APTT) (A), hematocrit (B), and platelet count (C). Horizontal bars indicate the mean ± SD for each group and the asterisk marks plots with significant differences between the groups.

APTT were in the 11–12 second range; clinically relevant prolongation of the APTT should have resulted in values of 14–16 seconds.[25] We propose that the differences in TEG parameters between Greyhounds and non-Greyhounds are clinically relevant, but we need to conduct additional studies in Greyhound bleeders to confirm this hypothesis.

The decreased clot strength and viscoelasticity and the slower clot kinetics in Greyhounds could be an adaptation to compensate for the cardiovascular and hemodynamic features of this breed. Approximately 15% of the blood volume is distributed to the rear limbs in resting Greyhounds, in comparison with 11% in non-Greyhounds.[26] Moreover, during exercise, Greyhounds undergo splenic contraction and a marked increase in HCT, thus further increasing blood viscosity.[27] Although

Greyhounds have lower peripheral resistance than mixed-breed dogs, a tendency to hypocoagulability may provide a mechanism for a steady blood flow through the muscles of the rear legs during exercise.[26] A basic tenet of hemostasis is that circulating blood does not clot; sludging of the blood in the muscles of the rear limbs could result in thrombosis if Greyhounds were not hypocoagulable. Finally, human patients with polycythemia or erythrocytosis are at high risk for thromboembolic events. As discussed, Greyhounds have erythrocytosis, and this phenomenon may also apply to the breed.[28]

Obtaining TEG tracings in normal Greyhounds and non-Greyhounds before and after routine surgical procedures may shed light on the pathogenesis of bleeding in the breed. The TEG may also allow us to characterize the potential role of enhanced fibrinolysis in Greyhounds that bleed postoperatively.[29] Additional studies using the TEG and clotting activators (eg, tissue factor, kaolin) to localize alterations of the coagulation cascade as well as an in-depth evaluation of the effects of viscosity on the hemostasis protein-cell interaction in whole blood may allow characterization of abnormalities in clot formation observed in Greyhounds.

## Footnotes

[a] Lara-García A, Couto CG, Iazbik MC, Brooks MB. Hemostatic assessment of postoperative bleeding in retired racing Greyhounds. J Vet Intern Med 2007; 21: 574–576 (abstract)

[b] Marin L, Couto CG, Iazbik MC et al. Hemostatic complications after limb amputation in retired racing Greyhounds. J Vet Intern Med 2007; 21:573 (abstract)

[c] Thrombelastograph, TEG Haemoscope, Niles, IL

[d] 2.7 mL, 3.2% buffered sodium citrate (0.3 mL; 0.109 M) Vacutainer, BD, Franklin Lakes, NJ

[e] ACL-200 Automated Coagulation Laboratory. Instrumentation Laboratory, Lexington, MA

[f] IL Test APTT-C Activated Partial Thromboplastin Time and IL Test PT-Fibrinogen, Instrumentation Laboratory

[g] LaserCyte, IDEXX Laboratories, Westbrook, ME

[h] Prism version 4.0, GraphPad Software Inc, San Diego, CA

[i] Tuman K, Naylor B, Spiess B, McCarthy R and Ivankovich A. Effects of hematocrit on thromboelastography and sonoclot analysis. Anaesthesiology 1989:71 (Suppl) (abstract A414)

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