

# Parameters of Thromboelastography in Healthy Newborns

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## Abstract

*Thromboelastography (TEG) aids in monitoring a patient's global hemostatic system by measuring the rate of clot formation, clot strength, and stability. The usefulness of TEG in pediatric settings, especially with neonates, is limited owing to a lack of neonatal reference values. In this study, neonatal TEG reference intervals were developed and results correlated with other coagulation test parameters. Samples were from women who delivered a neonate after at least 34 weeks of gestation in normal pregnancies. From the recovered placenta, cord blood from the umbilical vein or artery was collected within 30 minutes after delivery and tested. Neonatal TEG reaction time (time clot formation begins), clot firmness (shear elastic modulus strength), and platelet function analysis closure times were significantly lower than those in adult ranges ( $P < .001$ ). When compared with the values for children, TEG reaction time, angle, coagulation index, clot firmness value, and clot kinetics (time from clot formation to time amplitude reaches 20 mm) were significantly different ( $P < .001$ ) among neonates. TEG can be used to interpret the data for newborns by using reference values obtained in the present study.*

All patients with a bleeding or thrombotic history need to undergo a thorough coagulation evaluation to determine the cause. For bleeding, first-line coagulation tests involve prothrombin time (PT), activated partial thromboplastin time (PTT), and fibrinogen level. A history of thrombosis may prompt a request for a thrombophilia panel. Based on the results, more comprehensive tests may follow. Then, transfusion medicine physicians and clinicians would have to gather all available data and may need to order other tests as well. There is a need for assays that provide a quick understanding of a patient's hemostatic system to help guide clinicians.

The thromboelastography (TEG) assay provides a graphic and numeric representation of a patient's primary and secondary hemostatic system and fibrinolysis. TEG results usually correlate with the results of other coagulation parameters such as PT, PTT, fibrinogen level, and platelet count, but sometimes results do not correlate, probably owing to the difference in the specimen type, ie, whole blood for TEG and plasma for PT, PTT, and fibrinogen level. TEG measures the kinetic changes, the rate of clot formation, clot strength, and clot stability that occur in a cup containing whole blood, plasma, or platelet-rich plasma. The cup rotates 4°45' in a cycle lasting 10 seconds. The motion of the cup is monitored by a pin suspended by a torsion wire. Once bonding between platelet and fibrin is formed, the pin will start to move in conjunction with movement of the cup until both cup and pin move at the same rate. An electrical transducer converts the movement made by the pin to an electrical signal that is recorded by the TEG software. The strength of the clot is directly related to the motion of the cup and pin. As the clot weakens over time, the TEG also measures the rate of clot retraction or lysis as the pin motion decreases.

TEG has been shown to be useful in monitoring hemostasis during liver transplantation and cardiac surgery and in reducing unnecessary blood component use.<sup>1,2</sup> In the past 5 years, applications of TEG in adult settings are well described, especially owing to ongoing clinical research.<sup>3</sup> However, the usefulness of TEG in pediatric populations, especially neonates, is limited, mainly owing to the lack of reference ranges and standardization. Neonates up to 1 month of age are known to have a hemostatic system that is significantly different from that of infants 1 to 12 months of age and adults. During the first 3 to 6 months of life, most of the coagulation parameters change to levels that may match the adult hemostatic profile. TEG may provide additional valuable information to assess the coagulation profile of newborns.

The goal of this study was to develop neonatal TEG reference intervals and to correlate TEG results with those of other coagulation test parameters such as PT, PTT, fibrinogen level, platelet function analysis (PFA) by using the PFA-100 (Dade Behring, Miami, FL), and the CBC count and activated clotting time (ACT).

## Materials and Methods

### Samples

Samples were from 59 women who delivered a neonate after at least 34 weeks of gestation in a normal pregnancy. From the recovered placenta, cord blood from the umbilical vein or artery was collected. Collection of cord blood samples was performed from January 2006 to November 2006. The Baylor College of Medicine (Houston, TX) Institutional Review Board approved this study.

For the study, 59 cord blood samples were collected, 15 after vaginal deliveries and 44 after cesarean sections. The mean gestation was 38.6 weeks. Among the neonates, there were 25 boys and 34 girls. To verify that the blood collected

was from the neonate, hemoglobin F was measured by hemoglobin fractionation using high-performance liquid chromatography (HPLC). Children control values were obtained from data collected in a previous institutional study involving a population of 46% African Americans. Adult values were obtained from data collected for the institution's reference interval.

### Blood Sampling and Processing

After delivery of the placenta, it was immediately transferred to a separate room where specimens were to be collected. By using gauze, traces of maternal blood and amniotic fluid were wiped off the surface of the umbilical cord. A 21-gauge needle was inserted into the umbilical vein or artery, and 10 mL of cord blood was collected within 30 minutes after delivery. Specimens for ACT were collected into Hemochron tubes (International Technidyne, Edison, NJ); specimens for TEG, PT, PTT, fibrinogen, and PFA were collected into tubes with 3.2% sodium citrate (0.129 mol/L) as the anticoagulant (9:1 vol/vol ratio); and specimens for CBC counts and hemoglobin fractionation were collected into EDTA-anticoagulated tubes.

The ACT was performed immediately after specimen collection by using Hemochron Junior (International Technidyne). Specimens were hand delivered to the coagulation laboratory within 30 minutes for immediate testing or processing for specimen storage. Specimens were stored at room temperature before testing or processing for storage.

### TEG Assay Procedure

The TEG assay was performed by using a Thromboelastograph Hemostasis analyzer (Haemoscope, Skokie, IL). Citrated whole blood was tested 30 minutes after collection to allow specimen equilibration at room temperature. Then, 20  $\mu$ L of calcium chloride (0.2 mol/L) was added to a standard specimen cup. After the sample was adequately mixed, 340  $\mu$ L of citrated whole blood was added to the cup, and the assay was run for at least 60 minutes until completion of the measurement of clot lysis at 30 minutes. TEG was performed within 3 hours of specimen collection. As part of routine coagulation and hematology testing, specimens were carefully checked for fibrin clots and other contaminated materials. If fibrin clots or contaminated materials were found in the specimen, it was discarded.

### TEG Profile Measured Parameters

The hemostatic profile displayed by TEG can be interpreted by using several measured parameters. This study focused on 5 TEG parameters: clot reaction time (R), clot kinetics (K, time from clot formation to time amplitude reaches 20 mm), angle ( $\alpha$ , size of the angle is measured from a tangent line drawn to the curve of the TEG tracing starting from the point

**Table 1**  
Demographics for 59 Neonates\*

Variable	Result
Mean (range) gestation (wk)	38.6 (36.2-41.0)
Sex	
M	25 (42)
F	34 (58)
Mode of delivery	
Vaginal	15 (25)
Cesarean	44 (75)
Ethnicity <sup>†</sup>	
African American	9 (16)
Asian	6 (10)
White	37 (64)
Hispanic	6 (10)

\* Data are given as number (percentage) unless otherwise indicated.

<sup>†</sup> Data were available for 58 neonates.

of clot reaction time), maximum amplitude (MA, amplitude measured at the widest point of TEG tracing), lysis at 30 minutes, clot firmness (G, shear elastic modulus strength), and the coagulation index (CI, derived from other TEG parameters that describe a patient's global coagulable state [ $CI = -0.2454R + 0.0184K + 0.1655MA - 0.0241\alpha - 5.0220$ ]) **Figure 1**.

### PFA-100 Procedure

The PFA-100 is an instrument and test cartridge system that mimics in vitro primary hemostasis following vessel injury in which the process of platelet adhesion and aggregation occurs. Anticoagulated whole blood is aspirated from the sample reservoir through the capillary and the aperture with a membrane coated with collagen (Col)/epinephrine or Col/adenosine diphosphate, which exposes platelets to high-shear flow conditions.<sup>4,5</sup> The instrument measures the closure time of the aperture by platelet thrombus that gradually causes the flow of blood to cease.

### Other Coagulation Tests

The remaining coagulation specimens were processed to achieve platelet-free plasma (PFP). The following steps were used to process PFP: capped specimen tubes were centrifuged for 30 minutes at 1,500g, plasma was transferred to plastic tubes, and the platelet count was verified as 5,000/ $\mu$ L. PT, PTT, and fibrinogen assays were performed immediately on a STA-R analyzer (Diagnostica Stago, Parsippany, NJ) using reagents from Diagnostica Stago. The remaining PFP was divided into aliquots to be frozen at  $-70^{\circ}\text{C}$ .

### Other Laboratory Tests

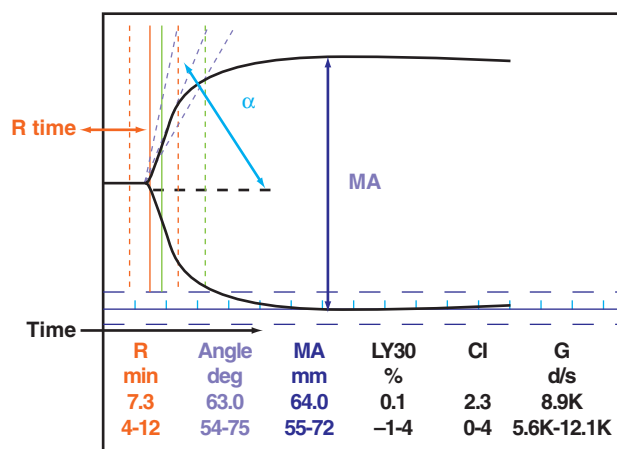
The CBC count was measured by using the Sysmex XE2100 (Sysmex, Mundelein, IL). To make sure that the specimen was from the neonate, hemoglobin fractionation was performed by HPLC. Hemoglobin profiles to determine hemoglobin F concentrations were obtained by using Primus HPLC (Primus, Kansas City, MO). ACT was measured using Hemochron Junior. All testing was performed according to guidelines defined by the Texas Children's Hospital Pathology Department (Houston).

### Statistical Analysis

Values reported are mean and 1 SD. Data were analyzed by using the 2-sample *t* test. *P* values of less than .05 indicate statistically significant differences, and *P* values of less than < .001 are considered highly significant.

## Results

The hemoglobin F in the specimens was 82% (range, 70%-94%), indicating that the specimens were from neonates and were not maternal specimens. This range was similar to



**Figure 1** Profile parameters for thromboelastography. The study was done without kaolin. CI, coagulation index; d/s, dynes per second; G, clot firmness (shear elastic modulus strength); K, clot kinetics (time from clot formation to time amplitude reaches 20 mm); LY30, lysis at 30 min; MA, maximum amplitude; R time, reaction time.

the published range of hemoglobin F in neonates of 60% to 90%.<sup>6</sup> The TEG parameters for healthy neonates and control groups are detailed in **Table 2**. The range for reaction time was significantly shorter for neonates than that for children and adults ( $P < .001$ ). The angle and coagulation index for neonates were significantly higher than for children ( $P < .001$ ). The G values for neonates were higher than those for children ( $P < .001$ ) and lower than those for adults ( $P < .001$ ). The maximum amplitude for neonates was slightly higher than for children ( $P < .05$ ) (Table 2).

Comparison of neonatal coagulation and hematologic parameters with adult values is shown in **Table 3**. The PT and PTT were significantly longer than for adults ( $P < .001$ ). The mean closure times in PFA were significantly shorter than the adult range ( $P < .001$ ). Shorter closure times are seen in both cartridge types, Col/epinephrine and Col/adenosine diphosphate (Table 3). Although the published data for non-TEG tests were not obtained by using the same reagents and analyzers as in our laboratory, they were inserted in Table 3.

## Discussion

Despite the fact that the neonatal PT and PTT are longer and the fibrinogen level is lower than in adults, the reaction time of the TEG is shorter. This significant difference may be explained by the unique and delicate balance in the neonatal hemostatic system. The dynamic equilibrium that exists is due in part to lower neonatal levels of

**Table 2**  
**Comparison of Thromboelastography Parameters in Studied Groups\***

	Newborns, 0 d (n = 59) <sup>†</sup>	Children, 6.5 ± 4.8 y (n = 44)	Adults, 39.1 ± 10.4 y (n = 21)
Reaction time (min)	5.3 ± 1.3	8.7 ± 2.6 <sup>‡</sup>	8.0 ± 2.0 <sup>‡</sup>
Angle (°)	67.2 ± 4.5	61.6 ± 7.1 <sup>‡</sup>	64.7 ± 5.3
Maximum amplitude (mm)	61.8 ± 4.6	59.6 ± 4.2 <sup>§</sup>	63.5 ± 4.2
Lysis at 30 min (%)	0.7 ± 0.7	0.2 ± 1.3	1.3 ± 2.0
Cl	2.3 ± 0.8	1.3 ± 0.9 <sup>‡</sup>	2.0 ± 0.9
G (K dynes/s)	8.4 ± 1.6	7.4 ± 1.3 <sup>‡</sup>	8.9 ± 1.6 <sup>‡</sup>
K (min)	1.6 ± 0.4	2.1 ± 0.7 <sup>‡</sup>	1.8 ± 0.4

Cl, coagulation index; G, clot firmness (shear elastic modulus strength); K, clot kinetics (time from clot formation to time amplitude reaches 20 mm).

\* Values are given as mean ± SD. The highest and lowest data points were excluded. The laboratory at Texas Children's Hospital, Houston, established the ranges for children and adults.

<sup>†</sup> Newborns were delivered at 38.6 ± 1.2 weeks of gestation.

<sup>‡</sup> P < .001 compared with values for newborns.

<sup>§</sup> P < .05 compared with values for newborns.

**Table 3**  
**Comparison of Other Neonatal Hemostatic Parameters With Parameters in Adults\***

	Newborns, Day 0	Adults
PT (s)	14.9 ± 1.0	13.1 ± 0.7 <sup>†</sup>
PTT (s)	41.2 ± 6.3	29.0 ± 2.4 <sup>†</sup>
Fibrinogen (g/L)	2.09 ± 0.37	3.30 ± 0.53
PFA Col/Epi (s)	87.7 ± 16.6	133.4 ± 24.7 <sup>†</sup>
PFA Col/ADP (s)	57.9 ± 10.0	97.4 ± 25.9 <sup>†</sup>

ADP, adenosine diphosphate; Col, collagen; Epi, epinephrine; PFA, platelet function analysis; PT, prothrombin time; PTT, partial thromboplastin time.

\* Values are given as mean ± SD. The highest and lowest data points were excluded.

Values for fibrinogen are given in Système International values; to convert to conventional values (mg/dL), divide by 0.01. The laboratory at Children's Hospital of Texas, Houston, established the ranges for adults.

<sup>†</sup> P < .001 compared with values for newborns.

plasma coagulation inhibitors such as antithrombin, protein C, and protein S, whereas some procoagulant factor levels, such as factor VIII, von Willebrand factor (vWF), and factor XIII levels, are comparable to the levels in adults.<sup>7,8</sup> The significant prolongation of PT and PTT compared with adults is due to the lower procoagulant factors at birth, such as factors II, VII, IX, X, and XI.<sup>9</sup> Also, the contributions of RBCs, WBCs, and, especially, platelets in clot formation are not observed in coagulation tests involving plasma only. Elevated plasma levels of vWF and enhanced vWF multimeric properties contribute to the procoagulant activity of platelets.<sup>7</sup> This elevation of vWF and elevated hematocrit levels may also explain the shorter PFA closure times in neonates than in adults. Although the usefulness of TEG for neonates and children is an open field that needs to be evaluated further, TEG can be used to interpret the data for neonates by using the reference values obtained in this study.

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*Haemoscope, Skokie, IL, loaned the Thromboelastography Hemostasis Analyzer for this study.*

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