

# Reference Values for Kaolin-Activated Thromboelastography in Healthy Children

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**BACKGROUND:** The hemostatic system of children changes with age and differs significantly from the hemostatic system of adults. Age-specific reference values are therefore required for most hemostatic variables. Thromboelastography (TEG®) is a point-of-care coagulation test that may provide superior evaluation and management of coagulopathies after cardiac surgery, when large-dose unfractionated heparin is administered for cardiopulmonary bypass. In this study, we established reference values for kaolin-activated TEG in healthy children, to facilitate accurate interpretation of pediatric TEG results.

**METHODS:** Kaolin-activated TEG was performed on 100 healthy children undergoing elective day surgery and 25 healthy adult volunteers. The following TEG variables were recorded: reaction time, coagulation time,  $\alpha$  angle, maximum amplitude, percentage lysis 30 min after maximum amplitude was reached, and the coagulation index. Differences between age-groups were evaluated using analysis of variance.

**RESULTS:** Age-specific reference values for kaolin-activated TEG in healthy children between 1 mo and 16 yr of age are presented. No significant differences between children and adults were observed.

**CONCLUSIONS:** TEG results, from a particular clinical setting, must be compared to age-specific, as well as analyzer- and activator-specific, reference values to allow for correct interpretation of the results. Reference values provided here will be of use in acute clinical situations where a practical monitor of hemostasis is required.

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The concept of developmental hemostasis underpins many facets of current research in the field of pediatric hematology. Founded on a series of papers by Andrew et al. (1–4), developmental hemostasis establishes significant differences between pediatric and adult hemostatic systems, and describes how age-related changes occur as the hemostatic system matures. The effects of these differences must be considered when diagnosing and treating hemostatic disorders in children (5). However, further studies are required to clarify the extent of these effects in order for clinicians to optimize the care of children with such conditions.

Management of the pediatric population is further complicated by the unreliability of hemostatic tests currently used to monitor standard anticoagulation therapy. Recently published data suggests that there are varying degrees of correlation between clinical tests and specific laboratory assays of unfractionated

heparin activity (6). Considering that suboptimal anticoagulation is associated with increased morbidity and mortality from thromboembolic and hemorrhagic sequelae, there remains a pressing need for more accurate, practical means of monitoring the unfractionated heparin effect.

Thromboelastography (TEG®) is a point-of-care, whole blood coagulation test that provides a global assessment of hemostasis from clot initiation and development, to fibrinolysis. Used predominantly in the past for research purposes, pediatric use of TEG has been described in the contexts of cardiac surgery (7), extra-corporeal life support (8), neurosurgery (9), and orthotopic liver transplantation (10).

Only two studies have defined reference values for TEG in children (11,12). The first, by Miller et al., performed TEG on native blood samples from surgical patients younger than 2 yr old, whereas the second, by Pivalizza et al. (12), evaluated celite-activated TEG in patients younger than 9 yr old. Age-specific reference values have not been determined for kaolin-activated TEG. Values from native and celite-activated TEGs, however, are significantly different from those acquired when kaolin is used as an activator (13). Therefore, kaolin-specific reference values are essential for meaningful interpretation of TEG variables in children.

In light of these differences, and to better reflect the demographics of the patient population in a tertiary

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pediatric hospital, our study was designed to determine reference values for kaolin-activated TEG in children from 1 mo to 16 yr of age.

## METHODS

### Subjects

After approval from the Royal Children's Hospital, Melbourne, Ethics in Human Research Committee (EHRC reference number: 20031E) and obtaining written informed parental consent, blood samples were collected by venous cannulation from 100 healthy children about to undergo minor, elective day surgery. These patients did not have a personal or family history of thromboembolic events and/or bleeding disorders, and were not receiving any anticoagulant therapy at the time of sample collection. The age-groups used for this study (<1 yr, 1–5 yr, 6–10 yr, 11–16 yr) are in agreement with previous studies outlining age-specific hemostatic reference ranges (4,14). Blood was also collected from healthy adult volunteers for the purposes of comparison between pediatric and adult values.

### Thromboelastography

TEGs were performed using the Thrombelastograph® Analyzer 5000 (Hemoscope Corp., IL) immediately after sample collection, using disposable standard cuvettes and pins from Medtel (for Hemoscope Corp., IL). One milliliter of collected blood was placed into a 1% kaolin vial (Medtel; for Hemoscope Corp.), which was then inverted five times to ensure appropriate activation of the sample. After activation, 360  $\mu$ L of blood was pipetted into the cuvette and the test was conducted at 37°C. The tracing was automatically stopped 60 min after attainment of maximum amplitude (MA). Test results were generated by the TEG computer software (Version 4) and recorded for later analysis.

Five TEG variables were taken as a representation of hemostasis: *R* (reaction time), *K* (coagulation time),  $\alpha$  angle, MA, and LY30 (percentage lysis 30 min post-MA). *R* is the time from initiation to initial fibrin formation, arbitrarily defined as the trace amplitude of 2 mm. *K* measures the time taken for the amplitude to increase from 2 mm to 20 mm, whereas  $\alpha$  is the angle formed between the midline and the tangent to the main body of the trace. MA is the amplitude at the widest point of the trace, and LY30 is calculated as the percentage reduction in amplitude 30 min after MA is reached. The rate and dynamics of coagulation are assessed with *R*, *K*, and  $\alpha$  angle, with overall clot strength and fibrinolysis reflected by MA and LY30 respectively.

The *R*, *K*,  $\alpha$  angle, and MA variables can also be incorporated into a coagulation index (CI) as defined by the equation:  $CI = -0.6516R - 0.3772K + 0.1224MA + 0.0759\alpha - 7.7922$  (TEG 5000 User's Manual; Hemoscope Corp., IL). The CI functions as an

overall assessment of coagulation, with values less than  $-3.0$  said to signify a hypocoagulable sample and values over  $+3.0$  said to signify a hypercoagulable sample (TEG 5000 User's Manual; Hemoscope Corp.).

### Statistical Analysis

The data were analyzed using the statistical software package STATA, Release 9.0 (Stata Corp., College Station, TX). Differences among age-groups were evaluated using one-way analysis of variance, with subsequent 2-sided, unpaired Student's *t*-tests performed if significance was found. Significance was defined as a *P* value  $<0.05$ .

## RESULTS

Blood collection was unsuccessful in four children and technical difficulties were encountered with the TEGs of 16 other children, necessitating their exclusion from the study. These problems included preclotting of the blood sample (four samples), baseline/disposable errors (two occurrences) as determined by the TEG computer software, and the inadvertent stopping of two TEGs before all of the variables could be measured. Specific causes could not be attributed to the grossly abnormal appearance of the remaining eight excluded traces, leaving 100 successful pediatric TEGs. These 100 patients underwent a range of elective, non-cardiac surgical procedures, with the most common being ophthalmologic (esotropia repair, examination under anesthesia—25%), urological (circumcision, hypospadias repair, cystoscopy—19%), plastic surgery (17%), and orthopedic operations (removal of frames, fracture repair—14%).

Adult controls were derived from a population of 25 volunteers, who had a median age of 31.24 yr and an interquartile range of 14.17 yr. The overall gender distribution of participants was approximately equal, despite imbalances within certain age-groups.

Reference values for measured TEG variables and the CI are shown in Tables 1 and 2 respectively, and are expressed as the mean and boundary encompassing 95% (between the 2.5th and 97.5th centiles) of the population. Significant differences between age-groups were not identified in the TEG variables or the CI.

## DISCUSSION

Clinical use of TEG has increased in recent years, due to the need for rapid analysis of hemostasis in many medical and surgical situations. Current practice allows for considerable variations of methodology, particularly in the activators used for testing. Applications of celite-, kaolin-, and tissue factor-activated TEGs to different clinical settings have been described in the literature (7,15). Each activator is found to exert differing effects on given TEG variables (13). Accurate interpretation of results therefore relies on comparisons with reference values specific for the

**Table 1.** Kaolin-Activated TEG® Reference Values

	<1 yr <i>n</i> = 24, 13M/11F	1–5 yr <i>n</i> = 24, 12M/12F	6–10 yr <i>n</i> = 26, 12M/14F	11–16 yr <i>n</i> = 26, 13M/13F	Adults <i>n</i> = 25, 12M/13F
<i>R</i> (min)	7.7 (4.5–11.6)	8.3 (5.7–10.9)	7.8 (5.3–11.0)	6.9 (3.8–11.1)	7.5 (5.3–9.3)
<i>K</i> (min)	1.8 (1.2–2.3)	2.0 (1.4–3.3)	2.0 (1.4–2.8)	1.9 (1.2–2.9)	2.0 (1.4–3.5)
$\alpha$ (°)	66.5 (58.8–73.4)	63.6 (53.8–70.3)	63.9 (54.3–70.7)	65.1 (54.9–73.2)	64.3 (48.8–72.2)
MA (mm)	67.2 (60.7–73.2)	65.2 (57.6–71.3)	65.0 (57.3–72.8)	66.5 (56.8–74.4)	63.0 (55.3–69.3)
LY30 (%)	3.8 (0.3–8.4)	3.0 (0.2–7.8)	3.3 (0.2–6.2)	3.7 (0.5–8.0)	4.3 (0.8–8.6)

Results are expressed as the mean and boundary encompassing 95% of the population.

M = males; F = females; *R* = reaction time; *K* = coagulation time;  $\alpha$  = measure of the rate of clot formation; MA = maximum amplitude; LY30 = percentage lysis 30 min post-MA.

**Table 2.** Coagulation Index

	<1 yr	1–5 yr	6–10 yr	11–16 yr	Adults
CI	–0.2 (–2.9 to 3.4)	–1.2 (–4.1 to 2.0)	–0.8 (–4.0 to 1.6)	0.1 (–4.2 to 3.5)	–0.9 (–4.3 to 1.3)

Results are expressed as the mean and boundary encompassing 95% of the population.

CI = coagulation index.

activator used. Our study is the first to provide such values for kaolin-activated TEG in healthy children.

Kaolin-activated TEG predicts excessive coagulopathic bleeding after cardiac surgery and may reflect the efficacy of corrective, prohemostatic interventions (15). However, Avidan et al. (13) observed that celite- and kaolin-activated TEGs are significantly affected by aprotinin, resulting in prolongation of the *R* time, a decrease in  $\alpha$  angle, and reduced MA. Tissue factor-activated TEG is not affected by aprotinin and thus may be more reliable for identifying underlying coagulopathies during and after cardiac surgery, where aprotinin is routinely administered (13).

Although the study by Avidan et al. established that aprotinin significantly affects the *R*,  $\alpha$  angle, and MA values of celite- and kaolin-activated TEGs, no mention was made of the influence, if any, on variables of fibrinolysis that are measured by the TEG. Considering the insensitivity to aprotinin by the tissue factor-activated TEG, this test may not provide a true representation of fibrinolysis, on which aprotinin itself has a powerful inhibitory effect (16). Use of celite or kaolin as an activator should therefore be considered when evaluating the physiological fibrinolytic state of a patient, as part of overall postoperative management of bleeding.

Age-related differences in kaolin-activated TEG variables were not identified in our data. One possible explanation for this is that TEG, being a global assessment of hemostasis, is inherently insensitive to developmental changes in the hemostatic system. These changes are minor compared to the severe coagulopathies observed in the settings of cardiac surgery and orthotopic liver transplantation, where hemostatic monitoring with TEG has mainly been validated (7,10). Alternatively, maturational increases in certain coagulation factor levels may offset decreases in other coagulation factors or increases in coagulation inhibitors, resulting in negligible overall impact on hemostasis and TEG results.

Additionally, our study involved TEG conducted on kaolin-activated whole blood samples, whereas the hemostatic tests performed for past developmental hemostasis studies (1–4,14) used citrated platelet-poor plasma. Processes such as citration, centrifugation, and activation of blood samples are all major variations in test methodology that could affect the degree to which age-related hemostatic differences are reflected in the actual results. Further investigations are required to investigate these hypotheses in the absence of any supportive or contradictory evidence in the literature. Such studies should also examine the hemostatic system of children younger than 1 year of age in greater detail, as it is during this period that most developmental changes occur (1).

In other comparisons, kaolin-activated TEG reference values for children younger than 1 year of age were significantly different from corresponding celite-activated TEG reference values, as reported by Pivalizza et al. (12). Specifically, *R* and *K* times were shorter with kaolin compared to celite as an activator ( $P = 0.0052$  and  $P = 0.0004$ , respectively), whereas  $\alpha$  was smaller for kaolin-activated TEG ( $P < 0.0001$ ). The CI for celite-activated TEG was higher compared to kaolin ( $P < 0.0001$ ). These results reinforce the importance of consulting activator-specific TEG reference values when interpreting results, even when using the same type of TEG analyzer.

Detailed comparisons of kaolin-activated TEG data to previously published reference values for native and celite-activated TEG in healthy children (11,12) and tissue factor-activated TEG in pediatric cardiac surgery patients (17) were not possible because of differences in the age-groups of participants. We divided our patients into the age-groupings reported in all previous major papers reporting changes in developmental hemostasis, and for which we already have normative data for our sample population. Had there been significant differences in TEG results, this would enable correlation with more specific aspects of the

coagulation system (1,2,4,14). Furthermore, ours is the only study to assess TEG variables over the age range of 1 month to 16 years, throughout which significant developmental changes in the hemostatic system still occur. Standardization of age-grouping in future studies is recommended to facilitate comparisons of activator-specific TEG data as well as the age-related trends observed for other hemostatic tests.

In summary, we present reference values for kaolin-activated TEG in healthy children between 1 month and 16 years of age. Although significant age-related differences in kaolin-activated TEG variables do not appear to be present, pediatric TEG results must still be compared to age-, analyzer- and activator-specific reference values to ensure accurate interpretation and clinical management of hemostasis. Further investigations are required to validate the current and future use of kaolin-activated TEG in specific medical and surgical settings.

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