TEG® 6s coagulation testing with a novel heparin neutralization cartridge

Technical validation and determination of normal reference ranges

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ABSTRACT

Objectives: We sought to establish normal reference ranges (NRRs) for a novel TEG 6s cartridge (TEG 6s Citrated: K, KH, RTH, FFH [Global Hemostasis]) (Haemonetics Corporation, Boston, MA, US).

Methods: Healthy volunteers (≥18 years of age) included in this single-arm study provided single samples of whole blood. Primary end points included TEG parameters in the citrated kaolin (CK), CK with heparinase (CKH), RapidTEG with heparinase (CRTH), and functional fibrinogen with heparinase (CFFH) assays.

Results: Evaluable data were contributed by 164 volunteers (48.8% female; 62% White/Caucasian). The following NRRs were established: CK maximum amplitude (MA), 51.0 to 67.6 mm; CKH-MA, 51.8 to 67.9 mm; CRTH-MA, 53.0 to 68.9 mm; CFFH-MA, 15.3 to 34.4 mm; CK reaction time, 5.0 to 9.1 minutes; CKH reaction time, 4.9 to 9.4 minutes; CKH lysis 30 minutes after MA, 0% to 3.2%. Duplicate measurements demonstrated high reproducibility. CFFH-MA correlated with Clauss fibrinogen concentration (Pearson correlation coefficient, 0.74). Laboratory-based studies demonstrated maintenance of the relationship between CFFH-MA and fibrinogen up to 1344 mg/dL (hyperfibrinogenemic samples) and acceptability of heparin neutralization up to concentrations of low molecular weight and unfractionated heparin of 1.3 IU/mL and 5 IU/mL, respectively.

Conclusions: This study established NRRs for the Global Hemostasis cartridge and serves as a proof of concept for the validity of results obtained using this cartridge.

KEY POINTS

- A novel heparin neutralization TEG 6s cartridge has been developed.
- Normal reference ranges were established, and reproducibility of assays was demonstrated.
- Functional fibrinogen with heparinase maximum amplitude and Clauss fibrinogen results were shown to be positively correlated.

KEY WORDS

hematology; coagulation; quality

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INTRODUCTION

Heparin is the most widely used anticoagulant.[1-3] Its primary mechanism of action is the binding of antithrombin and subsequent blockade of thrombin and factor Xa.[2-4] Available in unfractionated and low-molecular-weight (LMW) forms, heparin is used in the prevention and treatment of thrombotic events such as deep vein thrombosis and pulmonary embolism in a range of clinical settings that include surgical procedures.[1-4] In addition to therapeutically administered heparin, endogenous heparin and heparin-like entities (heparinoids) have the potential to affect coagulation status. Decreased clearance of endogenous heparinoids can lead to increased levels of endogenous heparinoids in circulation, increasing the likelihood of excessive bleeding.[5] These circumstances are frequently encountered in liver disease, meaning that endogenous heparinoids may complicate coagulation management in patients undergoing liver transplantation.[5-7]

To guide and optimize coagulation management in patients who have received heparin or have high levels of endogenous heparinoids, hemostatic testing may be required. Conventional tests include activated partial thromboplastin time, prothrombin time/international normalized ratio, platelet function testing, dimerized plasmin fragment D levels, and anti–factor Xa assays. Fibrinogen levels can also be assessed using the Clauss fibrinogen assay. Although several of these tests are affected by the presence of heparin, with anti–factor Xa assays most sensitive, none provide a complete picture of the effect of heparin/heparinoids due to their nonspecific mechanisms of action.[3,8,9] Furthermore, prolonged turnaround times can limit their use in guiding treatment in acute and surgical settings.[10]

Viscoelastic hemostatic assays (VHAs) provide information about clot strength and the kinetics of clot formation and breakdown, enabling comprehensive assessment of coagulation in whole blood.[10-13] These assays can be performed at the point of care, with shorter turnaround times than conventional tests, and have been shown to improve coagulation management and patient outcomes in clinical practice.[14,15] The applicability of VHAs to clinical settings, including cardiovascular surgery and liver transplantation surgery, has been well demonstrated.[15-17] Devices used to perform VHAs have evolved over time, and the current TEG 6s system (Haemonetics Corporation, Boston, MA, US) facilitates rapid and accurate testing in close proximity to the patient.[12,17] Microfluidic cartridges enable TEG 6s coagulation testing in a range of clinical settings. The TEG Citrated: K, KH, RT, FF Multi-channel Cartridge (hereafter referred to as the *CM cartridge*) includes citrated kaolin (CK), where kaolin is used to activate the intrinsic coagulation pathway; the citrated RapidTEG (CRT) assay, where tissue factor is added to kaolin and the extrinsic pathways are activated to accelerate clotting; and the citrated functional fibrinogen (CFF) assay, in which a glycoprotein IIb/IIIa inhibitor enables measurement of the fibrin component of clot strength (a surrogate for the fibrinogen concentration). The CK with heparinase (CKH) assay is the fourth of the CM cartridge. The principle of using heparinase—an enzyme that rapidly and specifically neutralizes the effects of heparin and heparinoids to negate their impact on hemostatic testing—has long been recognised.[6,18-20] Combined consideration of results from the CK and CKH assays enables the presence of heparin to be identified and provides an overview of the underlying hemostatic state in the absence of heparin.

A novel cartridge has been developed to expand the heparin neutralization (HN) capability of the cartridge-based TEG 6s system. The cartridge (TEG 6s Citrated: K, KH, RTH, FFH [Global Hemostasis—HN] Cartridge, hereafter referred to as the *HN cartridge*) contains reagent concentrations that are consistent with that of the CM cartridge but with the addition of heparinase to the CRT and CFF assays. By including heparinase in the CRT and CFF assays (CRTH and CFFH, respectively) alongside the CK and CKH assays, the cartridge may enhance the information available to support clinical decision-making in patients with known or suspected heparin in circulation.

Introduction of the HN cartridge to clinical practice requires the establishment of normal reference ranges (NRRs) and validation vs existing laboratory parameters. A study was therefore undertaken in healthy volunteers to fulfil these requirements.

METHODS

This trial (ClinicalTrials.gov identifier NCT06261580) was conducted in cooperation with Machaon Diagnostics (Oakland, CA), and healthy volunteers were enrolled at 3 centers in the United States (Oakland; Chicago, IL; and New Orleans, LA). The study was conducted with adherence to the ethical principles of the International Council for Harmonisation Good Clinical Practice guidelines and the applicable national and local laws and regulatory requirements. The study protocol was approved by the institutional review boards of each participating site, and written informed consent was obtained from all the volunteers.

The primary objective was to establish NRRs for TEG 6s parameters with the HN cartridge in healthy donors. Secondary objectives were (1) to establish the relationship of Clauss fibrinogen with the maximum amplitude (MA) parameter (a measure of clot strength) from the CFFH test; (2) to evaluate the agreement between MA on the CRTH and CKH tests (CRTH-MA and CKH-MA, respectively); and (3) to evaluate the reproducibility and replicability of all the TEG 6s HN cartridge parameters. The secondary objectives also related to establishment of these relationships in healthy donors.

The main inclusion criteria for the study were age 18 years and older and general good health (self-identified). Individuals with acute illness or chronic disease (including coagulation or bleeding disorders) and individuals taking medications with known anticoagulant effects were excluded (see Supplementary Methods, Supplementary Table 1 for details [all supplementary material is available at *American Journal of Clinical Pathology* online]). The number of volunteers enrolled was based on the sample size required to produce representative NRRs. The sample size of 180 was determined to ensure that there was a minimum of 120 samples, even after potential data loss, in line with standard practice on establishing reference ranges.[21] Participants were selected to yield

a sample that approximately reflected US general population demographics, with equal numbers of male and female individuals and representation of 3 age ranges: 18 to 35 years, 36 to 50 years, and older than 50 years (≥20 participants per age group). Demographic information, including the medications and dietary supplements that the donors were taking, was recorded.

Each study participant provided a single sample of whole blood (no more than 15 mL) for TEG and Clauss fibrinogen assessments, which was collected in a Beckton Dickinson blue-top tube containing 3.2% sodium citrate, with one 3-6–mL discard tube or equivalent taken first. After blood collection, the sample tubes were gently inverted 5 times; aliquots from these blood samples were used separately for TEG and Clauss fibrinogen assessment. TEG samples were processed no sooner than after a 10-minute incubation time and no later than 2 hours of the collect time to maintain in-use stability. TEG duplicates were run as closely together in time as possible, and no more than 10 minutes apart. The study sponsor provided each center with 2 TEG 6s devices and HN cartridges for completing the study. TEG 6s devices were calibrated as specified in the user manual (see Supplement for details). The blood samples for Clauss fibrinogen assessment were spun down to produce plasma samples, with analysis conducted at a central Machaon Diagnostics laboratory using the STAGO Clauss fibrinogen assay (STA-Fibrinogen kit and analyzer). Because Clauss fibrinogen was a secondary analysis, there was no protocol requirement to freeze the plasma samples and test them within a certain time frame, but all frozen plasma samples were shipped, handled, and analyzed according to Machaon Diagnostics standard operating procedures.

The primary study end points were parameters measured on the TEG 6s device with the HN cartridge: CK-MA, reaction time in the CK assay (time taken for an amplitude of 2 mm to be achieved, or CK-R), CKH-MA, CKH-R, lysis 30 minutes after MA in the CKH assay, CRTH-MA, and CFFH-MA. Clauss fibrinogen measurement was undertaken as an exploratory end point. Brief descriptions of these parameters and their clinical value can be found in Supplementary Table 2. Participant safety was assessed by recording any adverse events related to the blood draw procedure.

The NRRs for the primary end points were estimated from the Final Analysis Data Set (FADS). The FADS contained only the first replicate of the 2 measurements. If either of the 2 replicates was missing or data quality requirements (eg, difference between the 2 measurements divided by the mean between −0.4 and 0.4) were not met, the sample was excluded from the FADS. Provided that the data were normally distributed, the NRR was estimated as mean ± 1.96 * SD. In the case of non-normal distribution, the NRR was estimated using nonparametric quantile estimation (2.5th and 97.5th percentiles). In both cases, the intention was for the NRR to represent 95% of the normal population. Secondary analyses included assessment of the relationship between Clauss fibrinogen and CFFH-MA using a linear or nonlinear mixed-effect regression framework. Bland-Altman analysis was used to examine the level of agreement between duplicate measurements of the same parameter.

A post hoc analysis examined the agreement between NH cartridge parameters (CKH-MA vs CRTH-MA and CK-MA vs CKH-MA) using the Bland-Altman method. In this post hoc analysis, the average of replicates was used instead of the FADS. The rationale for this change was that the average of the 2 replicates represents the best assessment of each parameter in the comparison.

Supporting Laboratory-Based Studies

Additional studies assessing the performance of the HN cartridge in contrived samples with different concentrations of fibrinogen and heparin were conducted to further characterize cartridge performance.

The bench-top study 'Assessment of Correlation Between CFFH-MA and Fibrinogen Within the Upper Level of the Analytical Measuring Range (AMR) on HN Cartridge' assessed the relationship between CFFH-MA values and fibrinogen concentration on the HN cartridge from the normal reference range to the upper end of the AMR (approximately 25-60 mm). The study involved spiking 11 samples of citrated whole blood from a single donor with escalating volumes of cryoprecipitate. After CFFH-MA was measured on these samples in duplicate, the samples were spun down to produce plasma for Clauss fibrinogen assessment. An ordinary least square regression analysis was performed to construct the best-fit model to define the correlation between CFFH-MA values against the fibrinogen concentration (mg/dL). The Pearson correlation coefficient was calculated to describe the strength and direction of the relationship between CFFH-MA and fibrinogen concentration. See Supplementary Material for further method details.

The 'TEG 6s Heparin Neutralization Requirement Verification' study assessed the consistency of HN cartridge results in samples from 4 healthy donors spiked with different concentrations of LMW heparin (LMWH [enoxaparin, Lovenox]; 0.008 mg/mL and 0.013 mg/mL—equivalent to 0.8 IU/mL and 1.3 IU/mL, respectively[22]) and unfractionated heparin (1 IU/mL and 5 IU/mL). A minimum of 200 HN cartridges were tested on samples from 4 donors in replicate (10 per above experimental groups plus an "unspiked" control group). Values for each HN parameter from the 10 replicates were average and compared with the acceptance criteria, which were based on the reference ranges established in the NRR study. See Supplementary Material for further method details.

RESULTS

Population

Out of 181 healthy volunteers enrolled in the study, 164 contributed evaluable data. Reasons for exclusion included protocol deviations and difficult venipuncture or incomplete blood draw. Demographic characteristics of the population with evaluable data are shown in TABLE 1.

Primary End Point

The NRRs for the primary study end points are presented in FIGURES 1, 2, and 3. The results were calculated using a parametric methodology for CK-MA, CK-R, and CKH-MA, meaning that these parameters were distributed normally. The remaining

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TABLE 1 Demographic Characteristics of Participants Providing Evaluable Data (n = 164)

Category	Evaluable population (n = 164)
Age group, No. (%)	
18-35 y	75 (45.7)
36-50 y	41 (25.0)
>50 y	48 (29.3)
Sex, No. (%)	
Female	80 (48.8)
Male	84 (51.2)
Race, No. (%)[a]	
American Indian or Alaska Native	5 (3.1)
Asian	31 (19.0)
Black or African American	19 (11.7)
White or Caucasian	101 (62.0)
Not reported or unknown	7 (4.3)
Ethnicity, No. (%)	
Hispanic or Latino	18 (11.0)
Not Hispanic or Latino	145 (88.4)
Not reported or unknown	1 (0.6)
Weight range, lb, No. (%)	
100-150	50 (30.5)
151-200	85 (51.8)
201-250	25 (15.2)
>250	4 (2.4)

[a]n = 163; multiple races reported for 1 participant, so that participant was removed from the total number in the table.

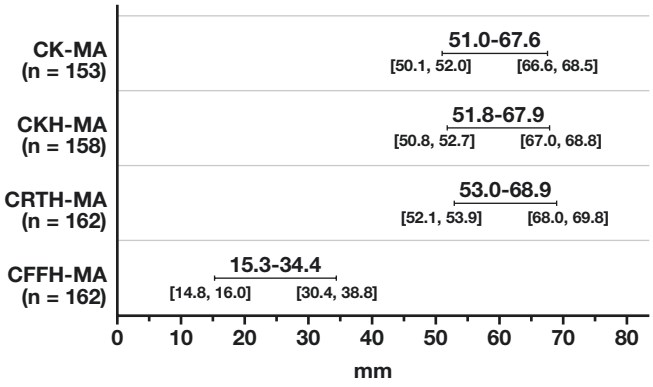

FIGURE 1 Normal reference ranges for TEG 6s HN cartridge MA parameters.Numerical values defining each range are shown above the line, and 95% CIs for the upper and lower limits are shown below the line. Sample numbers for each parameter differ due to data quality requirements—see "Methods" for details. CFFH, citrated functional fibrinogen with heparinase; CK, citrated kaolin; CKH, citrated kaolin with heparinase; CRTH, citrated RapidTEG with heparinase; HN, heparin neutralization; MA, maximum amplitude.

data were calculated using nonparametric methods. The normal ranges for CK-MA, CKH-MA, and CRTH-MA were numerically similar FIGURE 1, with similarity also observed between CK-R and CKH-R FIGURE 2.

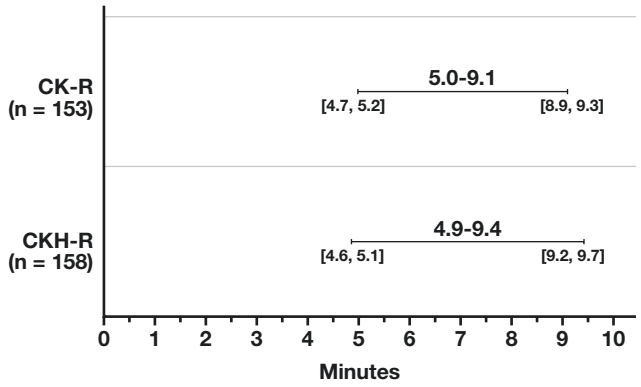

FIGURE 2 Normal reference ranges for TEG 6s HN cartridge reaction time parameters.Numerical values defining each range are shown above the line, and 95% CIs for the upper and lower limits are shown below the line. Sample numbers for each parameter differ due to data quality requirements—see "Methods" for details. CK, citrated kaolin; CKH, citrated kaolin with heparinase; R, reaction time.

Secondary/Exploratory End Points

Correlation was observed between CFFH-MA and Clauss fibrinogen concentration. Results for the first of the 2 TEG 6s measurements are shown in FIGURE 4, where the Pearson correlation coefficient was 0.74. Corresponding results from analysis using averages of the 2 duplicate CFFH-MA measurements were similar (correlation coefficient = 0.74) (Supplementary Figure 1).

Agreement was demonstrated between CRTH-MA and CKH-MA (see the Bland-Altman agreement plot in FIGURE 5); the 95% CI for the CKH-MA − CRTH-MA Δ (−4.58 to 0.6) suggests acceptable agreement in the framework of bias acceptance criteria. The acceptable bias for the NRR of CKH-MA (±4.3) is close to the CI limits, with only 4 to 5 points (of 156) observed to lie outside the acceptable bounds. In line with this finding, CRTH-MA and CKH-MA were shown to correlate, with a Deming regression analysis slope for the first measurements of 0.99 (95% CI, 0.94-1.05) (Supplementary Figure 2). The mean difference between the 2 parameters was 1.99 mm (95% CI, 1.68-2.12). Analysis of the averages of the duplicate measurements yielded similar results (Deming slope = 1.01 [95% CI, 0.95-1.06]; mean difference = 1.99 mm [95% CI, 1.78-2.2]) (Supplementary Figure 3). Agreement was also demonstrated between CK-MA and CKH-MA (Supplementary Figure 4). The bias acceptance criteria for the NRR of CK-MA (±4.3) are larger than the CI limits on the Bland-Altman plot, suggesting strong agreement.

Comparisons of the first and second duplicate measurements indicated that the TEG 6s HN results were highly reproducible, with Deming regression analysis slopes close to 1 (range, 0.97-1.04) TABLE 2. Mean differences between the 2 measurements were close to zero (range, −0.08 to 0.21).

Safety

Two adverse events related to the blood draw procedure were reported during the study, both comprising dizziness of mild severity. No serious adverse events or deaths occurred.

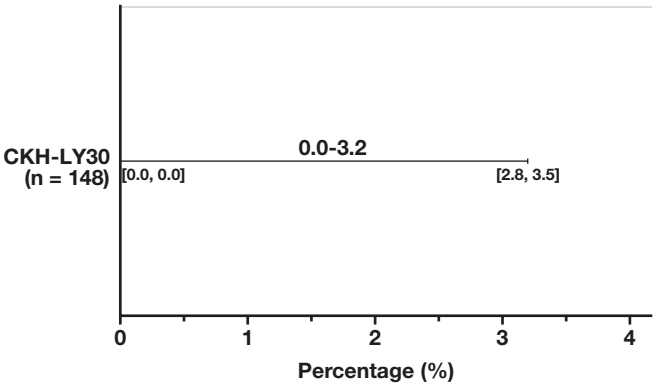

FIGURE 3 Normal reference range for the TEG 6s HN cartridge CKH-LY30 parameter.Numerical values defining each range are shown above the line, and 95% CIs for the upper and lower limits are shown below the line. CKH, citrated kaolin with heparinase; HN, heparin neutralization; LY30, lysis 30 minutes after maximum amplitude.

Supporting Data From Laboratory-Based Studies

HN Cartridge Performance in Hyperfibrinogenemic Samples

The CFFH-MA results showed a positive linear Pearson correlation, with fibrinogen concentration up to 1344 mg/dL in the blood sample FIGURE 6 (Supplementary Table 3), with a regression analysis for CFFH-MA vs fibrinogen showing a statistically significant ($P < .001$) linear positive correlation ($r = 0.99$) for CFFH-MA values within the normal reference range and up to the upper AMR value.

HN Requirement Verification

In the presence of concentrations of LMWH up to 0.013 mg/mL or 1.3 IU/mL and unfractionated heparin up to 5 IU/mL, mean HN cartridge parameters were all within the normal reference range, with the exception of CK-R, which is expected because heparinase is absent from the channel TABLE 3. All parameters met acceptance criteria (mean values within 50% of the NRR range) in the 4 donors (Supplementary Tables 4-8 and Supplementary Figures 5-9). The

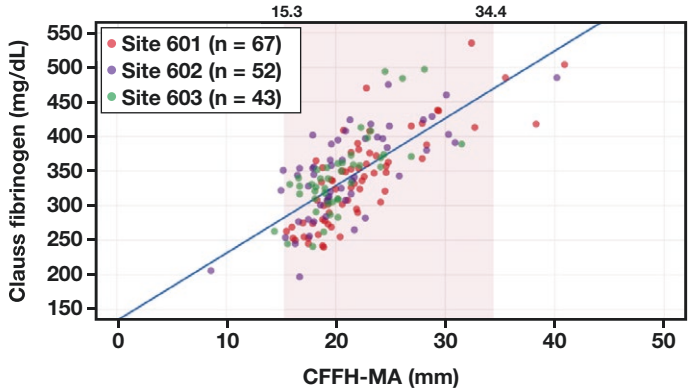

FIGURE 4 Relationship between Clauss fibrinogen and the TEG 6s HN parameter CFFH-MA (first replicate measurement) (162 data points). The shaded red area represents the 95% normal reference range for CFFH-MA. Pearson correlation = 0.74. CFFH, citrated functional fibrinogen with heparinase; HN, heparin neutralization; MA, maximum amplitude.

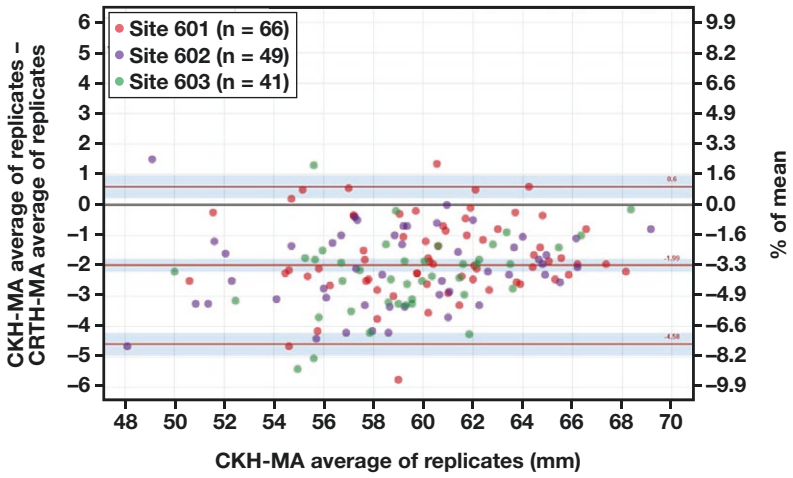

FIGURE 5 Bland-Altman agreement plot of CKH-MA vs CRTH-MA (n = 156).The red horizontal lines represent the 95% CI for the Δ (y-axis), while the shaded blue area is the 95% error CIs for the Δ limits. CKH, citrated kaolin with heparinase; CRTH, citrated RapidTEG with heparinase; HN, heparin neutralization; MA, maximum amplitude.

TABLE 2 Reproducibility of TEG 6s Heparin Neutralization Measurements

Parameter	No.	Regression analysis slope (95% CI)	Bland-Altman analysis, mean Δ (95% CI)
CK-R	153	0.98 (0.76-1.20)	0.21 (0.06-0.36)
CK-MA	153	1.01 (0.96-1.06)	−0.08 (-0.28 to 0.11)
CKH-R	157	1.01 (0.77-1.25)	−0.01 (−0.18 to 0.15)
CKH-MA	158	1.03 (0.97-1.09)	0.19 (−0.04 to 0.43)
CKH-LY30	148	1.04 (0.96-1.11)	−0.1 (−0.18 to −0.03)
CRTH-MA	162	1 (0.96-1.03)	0.04 (−0.11 to 0.19)
CFFH-MA	162	0.97 (0.94-0.99)	0.09 (−0.03 to 0.22)

CFFH, citrated functional fibrinogen with heparinase; CK, citrated kaolin; CKH, citrated kaolin with heparinase; CRTH, citrated RapidTEG with heparinase; LY30, lysis 30 minutes after maximum amplitude; MA, maximum amplitude; NRR, normal reference range; R, reaction time.

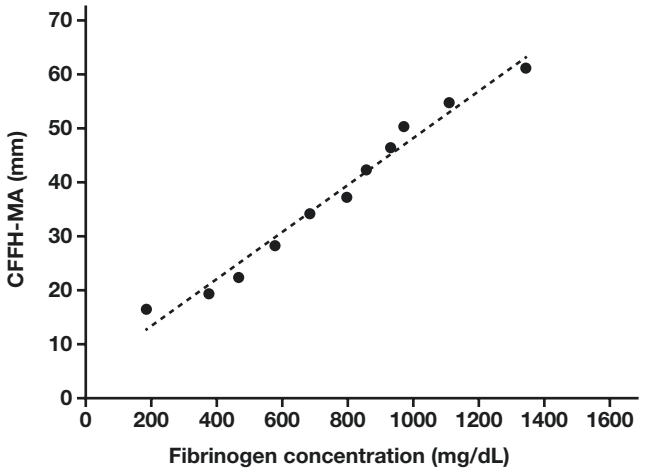

FIGURE 6 CFFH-MA vs fibrinogen concentration (average of duplicates) from HN cartridge performance in hyperfibrinogenemic samples study. Y = 0.0434X + 4.8644; R^2 = 0.9769; r = 0.988.CFFH, citrated functional fibrinogen with heparinase; HN, heparin neutralization; MA, maximum amplitude.

impact of heparin is apparent from the CK-R/CKH-R parameters, with the average of the 4 means for CK-R and CKH-R at 18.9 and 7.8 minutes, respectively, for LMWH 0.008 mg/mL or 0.8 IU/mL, with CK-R values not reportable (beyond the analytical range—that is, the device run time with the HN cartridge) for the other LMWH/unfractionated heparin concentrations.

DISCUSSION

This study describes the first results from whole-blood samples with the novel TEG 6s HN cartridge. Based on data from healthy volunteers, we have determined normal ranges for parameters from all 4 assays using this cartridge. As expected, similar ranges were observed with the CK, CKH, and CRTH assays. Differences between duplicate TEG 6s measurements were small, while measurements of clot strength in the CFFH assay showed a positive correlation with the conventional laboratory parameter, Clauss fibrinogen.

The NRRs presented in this study are consistent with the reference ranges for the CM cartridge[20]; this finding was expected because establishment of NRRs is conducted in healthy (ie, nonheparinized) donors and the design of the cartridge is substantially equivalent for the corresponding assays. In line with this, agreement was observed between results from the CK and CKH assays in the present study. Previous data have shown correlations between the CK and CRT assays.[23] The present study adds to this knowledge, with agreement observed between clot strength (MA) in the CRTH and the CKH assays with minimal bias, confirming the feasibility of accelerated assessment of clot strength with the RapidTEG assay with neutralization of heparin effect. Because the RapidTEG results are available much faster than the CK results, it is likely of more utility than CK in the dynamic environment of the surgical setting. There may be a perception among some physicians, however, that because the results are available faster, they are less reliable. In FIGURE 4, we show close agreement between the heparinized CRTH and CKH assays, thereby supporting the implementation of CRTH in a clinical environment where there is likely to be substantial quantities of heparin in the patient's blood.

Positive correlation between CFFH-MA and Clauss fibrinogen was also as expected. Correlation between CFF-MA and Clauss fibrinogen has been demonstrated previously in cardiovascular procedures, obstetric hemorrhage, and trauma patients,[17,24-27] and results in the present study confirmed maintenance of this relationship in healthy donors when heparinase is added to the assay. Supporting data from contrived hyperfibrinogenemic samples provide evidence of the performance of the CFFH assay in concentrations of fibrinogen more than twice that of the upper end of the normal expected range. Nevertheless, this relationship will be further evaluated in a clinical study of the HN cartridge in cardiac surgery/liver transplant patients.

The results of this study show minimal differences between duplicate TEG 6s measurements. This demonstration of reproducibility highlights the high precision of results obtained with the HN cartridge. This finding is in line with a previous validation study conducted using samples from intensive care unit patients, which showed TEG 6s CM cartridge results to be consistent across different devices.[12]

Although the primary function of the present study was to provide a validation of the TEG 6s HN cartridge, it also provides a basis for comparison with future studies that will determine

TABLE 3 TEG 6s Heparin Neutralization Results From Contrived Blood Samples Containing Different Concentrations of Low-Molecular-Weight Heparin and Unfractionated Heparin, Range of Means for Results From the 4 Donors Who Provided Samples[a]

CK-R, min (NRR: 5.0-9.1)	CKH-R, min (NRR: 4.9-9.4)	CKH-LY30, % (NRR: 0.0-3.2)	CRTH-MA, mm (NRR: 53.0-68.9)	CFFH-MA, mm (NRR: 15.3-34.4)
0.008 mg/mL or 0.8 IU/mL LMWH				
11.9-26.9	7.2-8.7	0.2-2.8	59.5-64.0	19.4-22.6
0.013 mg/mL or 1.3 IU/mL LMWH				
Not reportable[b]	6.9-8.7	0.1-2.8	61.4-63.7	19.8-23.0
1.0 IU/mL unfractionated heparin				
Not reportable[b]	7.1-7.8	0.1-2.8	61.6-64.0	19.9-22.6
5.0 IU/mL unfractionated heparin				
Not reportable[b]	8.0-9.3	0.2-2.9	61.6-64.3	19.8-22.6

CFFH, citrated functional fibrinogen with heparinase; CK, citrated kaolin; CKH, citrated kaolin with heparinase; CRTH, citrated RapidTEG with heparinase; LY30, lysis 30 minutes after maximum amplitude; LMWH, low-molecular-weight heparin; MA, maximum amplitude; NRR, normal reference range; R, reaction time.
[a]Presented is a summary of the data in Supplementary Tables 5-8; for each parameter, the lowest and highest mean values (average of 10 replicates) from the group of 4 donors who provided samples are reported.
[b]Beyond duration of device run time with the HN cartridge (47 min).

HN parameters in surgical patients as well as the potential of the HN cartridge to guide clinical management in clinical settings where heparin use or a high level of endogenous heparinoids is common, such as cardiovascular surgery or liver transplantation. Supporting the applicability of the HN cartridge for this role are the results from the heparin spiking study, which confirmed consistency of results from the cartridge at heparin concentrations in excess of what would be expected in the blood of patients who have received therapeutic or prophylactic doses. The difference between TEG readings with and without heparinase is notable from the CK-R/CKH-R parameters, with readings approximately 2.5 times higher without heparinase in the presence of enoxaparin 0.008 mg/mL or 0.8 IU/mL. Although the function of the study was solely to verify the HN capacity of the cartridge, the ability to monitor and quantify heparin activity using CK/CKH has been previously shown using the CM cartridge in healthy donors.[20] It should be noted, however, that this is not an approved use or claim of the TEG 6s cartridges.

Strengths of this study include the large number of participants, inclusion of volunteers with a wide and representative range of ages and races, and the rigorous exclusion criteria employed to establish the NRRs with minimal influence of confounding factors. Performance of the study within the United States only was a limitation because we cannot be certain of applicability to other countries.

In conclusion, this study has established NRRs for the novel TEG 6s HN cartridge, and the validity of results obtained using this cartridge was demonstrated by high reproducibility, positive correlation between Clauss fibrinogen and CFFH-MA, and consistency of results between different HN cartridge channels. The establishment of NRRs without the influence of any anticoagulants or applicable disease or clinical states provides a basis for further clinical studies.

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