

# Can We Rely on the Activated Clotting Time to Measure Heparin Anticoagulation? A Clinical Evaluation of Two ACT Monitors

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**Abstract:** The sensitivity to heparin during cardiopulmonary bypass (CPB) is determined by patient-specific characteristics and is assessed by the whole blood activated clotting time (ACT). We aimed to examine reliability measures between two different ACT monitors using Bland–Altman analysis: bias should not exceed  $50 \pm 50$  seconds for measurements performed during CPB or  $10 \pm 10$  seconds before and after CPB. The ACT response should be linear in relation to the concentration of heparin in plasma. Twenty patients ( $n = 20$ ) aged 20–80 years and admitted for coronary artery bypass surgery were enrolled to this clinical observational study. ACT values and antifactor Xa were sampled: 1) before induction of anesthesia, 2) after heparin bolus, 3) during CPB at the start of rewarming, 4) at weaning from CPB, and 5) after heparin reversal. The evaluation comprised the Hemostasis Management System Plus™ (HMS, Medtronic Inc., Minneapolis, MN) and i-STAT™ (Abbott, Point of Care Inc., Princeton, NJ).

Bias for the HMS Plus™ vs. i-STAT™ was  $+105 \pm 119$  seconds for measurements during CPB and  $+2.8 \pm 11.7$  seconds before and after CPB. Associated limits of agreement for the observed bias were  $\pm 235$  and  $\pm 23$  seconds, respectively. Inter-device correlation of ACT values was .46 ( $p < .001$ ) during CPB; otherwise .48 ( $p = .02$ ). Both devices produced ACT values unrelated ( $<10\%$ ) to the measured heparin concentration. The use of multivariable regression analysis demonstrated an independent association between the ACT measurement and hematocrit, however, not with the plasma concentration of heparin. ACT monitors demonstrate unacceptable bias differences, combined with wide limits of agreement. The ACT response correlated with hematocrit, but not with the actual heparin concentration. **Keywords:** anticoagulation, activated clotting time, heparin and heparin concentration, cardiopulmonary bypass, point of care. *J Extra Corpor Technol. 2020;52:212–7*

Blood in contact with the artificial surfaces of the extracorporeal circuit during cardiopulmonary bypass (CPB) triggers the internal coagulation pathway (1). Administering unfractionated heparin is the standard therapy to inhibit this reaction and to prevent thrombus formation (2). Measurement of the activated clotting time (ACT) was first described by Hattersley (3) and later implemented clinically by Bull et al. (4). The new method relocated coagulation assessments to the operating room and made dosing of heparin more predictable mainly because of the quick response times. It soon became the

gold standard for anticoagulation management during CPB.

Today, a range of different point-of-care devices for bedside applications is available. However, reliability and precision measures deviate significantly (5–9). The reported shortcomings may seem remarkable, especially because dosing of anti- and procoagulants, including decisions on transfusions, partly relies on the results produced from these analyses. This is in contrast with instruments used by the hospital's chemistry laboratory for coagulation assessments, which must pass a formal test procedure before being used (10).

The present observational clinical study aimed to compare mechanical (11,12) with electro-chemical (11,12) ACT assessment methods based on the two following hypotheses: 1) bias based on Bland–Altman analysis should not exceed  $50 \pm 50$  seconds for measurements performed during CPB and  $10 \pm 10$  seconds before and after CPB, and 2) the ACT response should be linear in relation to the concentration of heparin in plasma.

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## METHOD

### Study Design

Twenty patients ( $n = 20$ ) aged 20–80 years admitted for coronary artery bypass surgery were enrolled to this clinical observational study after the patient's informed consent. The exclusion criteria comprised emergencies; verified coagulation disorder; medication with warfarin, direct thrombin inhibitors, or dual antiplatelet therapy within 5 days prior surgery; diabetes mellitus; kidney dysfunction (estimated glomerular filtration rate  $< 50$  mL/min); and allergy to fish, seafood, and protamine. The Regional Ethical Review Board at the University of Umeå, Sweden, approved the study protocol (DNr 2016/449-31). The investigation conforms to the principles outlined in the Declaration of Helsinki.

### Conduct of CPB

An intravenous bolus dose of heparin (350 IU/kg) was administered to prolong the ACT above 480 seconds. Iterations were made if indicated as verified by repetitive ACT analyses. CPB was performed using an S5 roller pump (LivaNova, Munich, Germany), interlinked with a Quadrox-i Softline-coated oxygenator and an open venous cardiomy reservoir (Getinge AB, Gothenburg, Sweden). Hypothermia at 34°C was aimed for. Patients were rewarmed to 37°C before weaning from CPB. The circuit was primed with 1,000 mL Ringer acetate® (Fresenius-kabi, Uppsala, Sweden), 60 g Mannitol® (Fresenius-kabi, Uppsala, Sweden), 10,000 IU heparin, and 160 mmol sodium chloride. Pericardial blood was returned to the cardiomy reservoir using cardiomy suckers. The remaining blood volume in the CPB circuit after completion was transferred to a transfusion bag and thereafter re-transfused.

Arterial access was achieved by placing a cannula in the ascending aorta and a dual stage cannula in the right atrium for venous return. The non-pulsatile blood flow was adjusted to maintain the venous oxygen saturation more than 75%. The surgical procedure followed generally accepted principles for coronary artery bypass grafting.

### Blood Sampling and ACT Analysis

Blood samples for ACT analysis were collected from the indwelling radial artery catheter before and after CPB, otherwise from the sampling port of the CPB circuit on the following occasions: 1) baseline at induction of anesthesia, 2) heparin bolus administered, 3) rewarming of the patient during CPB, 4) weaning from CPB, 5) after reversal of the heparin effect by protamine (13). ACT measurements were completed in the operating room directly ( $< 60$  seconds) after specimen collection and in accordance with the manufacturers' instructions. This study examined the HMS

Plus™ (Medtronic Inc.) and i-STAT™ (Abbott, Point of Care Inc.). The HMS Plus™ served as reference device for heparin dosing. Both devices use kaolin as the coagulation activating agent. The HMS Plus™ uses an automatic process, where blood withdrawn in a 3-mL Monoject® syringe (Covidien, Dublin, Ireland) is dispensed into a two-channel cartridge (high range activated clotting time). Movements of the plunger descended in the specimen register changes in viscosity. On clot formation, the plunger movement halts, which defines the measured ACT value (14). The i-STAT™ device uses an electro-chemical process, which by amperometry detects fibrin strands in the formed thrombin substrate. After addition of whole blood (40  $\mu$ L) to the measurement cartridge, the process is fully automated. At the end of measurement, the actual clotting time is calculated (15). Duplicate samples from two i-STAT™ devices were used to equate with the two-channel system of the HMS Plus™. In both cases, the mean ACT value was used for statistics.

### Plasma Antifactor Xa

The laboratory method of quantifying heparin in plasma is a one-step chromogenic method based on a synthetic chromogenic substrate and anti-Xa inactivation (ACL TOP 700, LAS Instrumentation Laboratory, Bedford, MA). When the heparin–antithrombin complex is formed, two competing reactions occur: anti-Xa is inactivated by the heparin–antithrombin complex and anti-Xa that is not inactivated is quantitated with a synthetic chromogenic substrate that leads to the release of paranitroaniline. The released quantity is kinetic at a wavelength of 405 nm and is inversely proportional to the heparin concentration in the sample.

Anti-Xa samples above the measuring range ( $> 2.5$  IU/L) were manually diluted with saline and addition of  $< .1\%$  sodium azide. Samples for analysis of anti-Xa and ACT were collected simultaneously. Analysis of anti-Xa was performed postoperatively.

### Statistics

Plots and the Shapiro–Wilk test were used to examine and verify distribution patterns for data on the ratio scale. Missing values were replaced using the median of nearby points. Intergroup differences were analyzed using Student's *t*-test and degree of agreement with linear regression analysis and Pearson's correlation coefficient. Residual and multicollinearity analyses were performed to fulfill the requirements associated with using linear regression analysis. In addition, Bland–Altman analysis was used to further explore the levels of agreement (LOA) and bias between the two instruments examined (16). Results are presented as means  $\pm$  SD, if not otherwise stated. A *p*-value of  $< .05$  was considered statistically

significant. The statistical analysis was performed using IBM SPSS statistics for Mac version 25 (IBM Corp., Armonk, NY).

## RESULTS

Data were analyzed using a complete dataset containing one replaced missing value. Patient characteristics are presented in Table 1.

Results of the ACT values and anti-Xa measured before, during, and after CPB are presented in Table 2. Anti-Xa increased from .04 to 4.6 IU/mL after heparin bolus and attained baseline levels after heparin effect reversal with protamine as verified by the concomitant ACT response.

The ACT values were not statistically different between the HMS Plus™ and i-STAT™, except for the two measurements made at rewarming and weaning from CPB. The mean differences were  $150 \pm 90$  seconds ( $p < .001$ ) and  $163 \pm 82$  seconds ( $p < .001$ ), respectively. Of note is the large spread of the ACT values observed after heparin bolus. The interquartile range reached 138 seconds (Figure 1).

Pearson's correlation coefficient was .46 ( $p < .001$ ) between the HMS Plus™ and i-STAT™ for high-range ACTs during systemic heparinization and .48 ( $p = .02$ ) for the low-range ACT assessments before and after CPB. Bias for the HMS Plus™ vs. i-STAT™ was  $+105 \pm 119$  seconds for measurements performed during CPB. LOA attained  $\pm 235$  seconds based on a 95% confidence interval. For non-heparinized blood, bias was  $+2.8 \pm 11.7$  seconds and LOA  $\pm 23$  seconds (Figure 2).

Scatter plots showing the association between the heparin concentration and ACT measurements using the HMS Plus™ and i-STAT™ instruments are presented in Figure 3. The unadjusted degree of explanation ( $R^2$ ) was .04 ( $p = .114$ ) for the HMS Plus™ and .09 ( $p = .021$ ) for the i-STAT™ device. When introducing both the heparin plasma concentration and on-bypass measured hematocrit

**Table 1.** Patient and perioperative characteristics.

Characteristic	n = 20
Age (years)	67 ± 10
Gender (female, %)	10
Body weight (kg)	85 ± 11.5
Body height (cm)	175 ± 8
Body surface area (m <sup>2</sup> )	2.02 ± .16
Hemoglobin (g/L)	140 ± 12
Platelets (10 <sup>9</sup> /L)	266 ± 69
Fibrinogen (g/L)	3.8 ± 1.0
Antithrombin (KIU/L)	1.08 ± .18
Total heparin dose (IU)	44,375 ± 5,553
Total protamine dose (mg)	220 ± 35
CPB time (minute)	85 ± 25
Cross-clamp time (minute)	51 ± 21

KIU, kilo-international units; IU, international units.

**Table 2.** Measurement of ACT and antifactor Xa.

	HMS Plus™ (s)	i-STAT™ (s)	Anti-Xa (IU/mL)
Baseline	136 ± 11	132 ± 12	.04 ± .05
Heparin bolus	612 ± 133	609 ± 127	4.6 ± .95
CPB rewarming	670 ± 110	520 ± 90	3.2 ± .94
CPB weaning	608 ± 84	444 ± 68	3.0 ± .90
Heparin reversal	125 ± 11	124 ± 9	.5 ± .09

ACT values are presented in second(s).

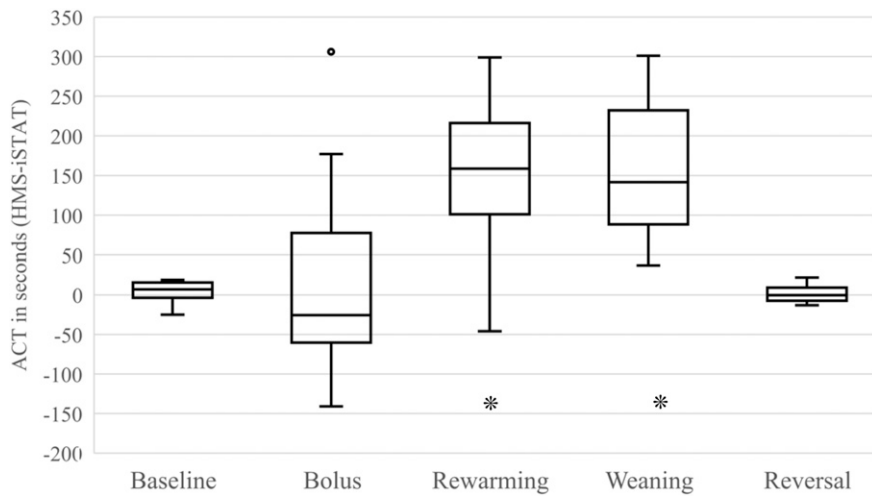
in a multivariable linear regression model, only hematocrit was independently associated with the measured ACT level. These statistically significant associations were apparent for both the HMS Plus™ and i-STAT™. No statistically significant associations were detected between the plasma heparin concentration and the ACT level for neither of the tested ACT devices (Table 3).

## DISCUSSION

The results from this clinical observational study demonstrated significant differences in performance between the two commercially available ACT monitors. Neither bias nor LOA attained the targets defined by our hypothesis. The HMS Plus™ overestimated the results received from the i-STAT™ device by  $+105 \pm 119$  seconds, with an associated LOA of  $\pm 235$  seconds. In addition, both used monitors showed ACT values unrelated to the concentration of heparin in plasma, where the degree of agreement was less than 10%. These findings point at two serious limitations regarding the reliability of ACT values and translating the information as an effect of heparin. Acknowledged drawbacks may have consequences for anticoagulation strategies and patient safety during CPB.

The poor outcome from our evaluation may seem remarkable, especially because similar limitations related to the ACT method are known from previous publications (8,9,17,18). It ultimately challenges current quality assessment methods used to test ACT devices or even the ACT method per se. Enforcement from governing authorities of a certification process of ACT monitors would seem warranted to overcome the present mismatch dilemma (10).

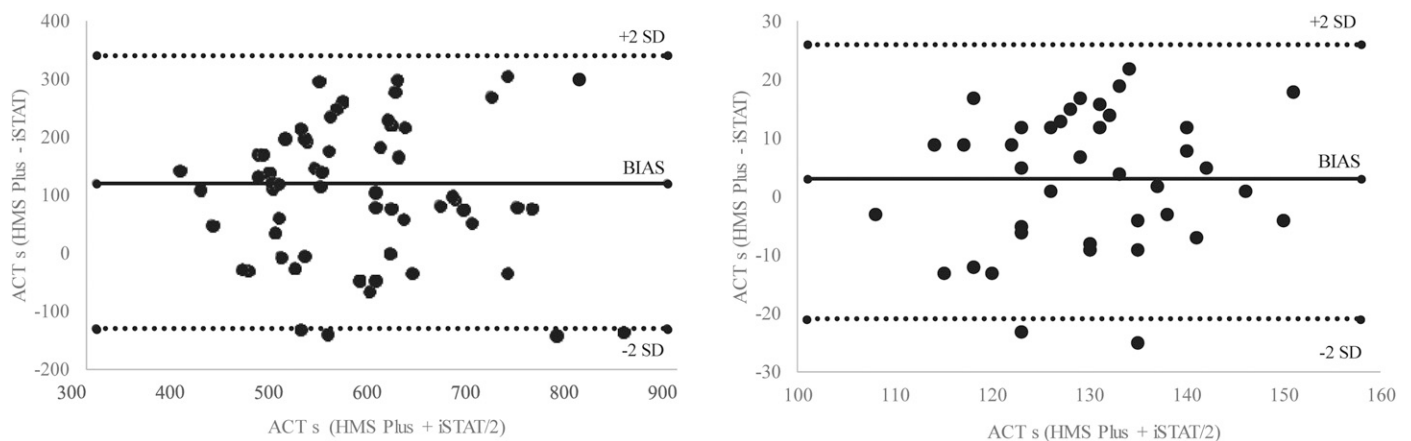
Heparin is typically administered in a sufficiently large dose to prolong the ACT above 480 seconds (2). It is generally recognized as an effect entirely depending on heparin. But, this is apparently not the case because the heparin concentration appears unrelated to the ACT value (8). One key factor that may have a significant effect on the ACT is the degree of hemodilution (19). The ACT values measured after bolus injection of heparin differed insignificantly between the HMS Plus™ and i-STAT™ but deviated profoundly after commencement of CPB. Hemodilution of heparinized blood associated with CPB



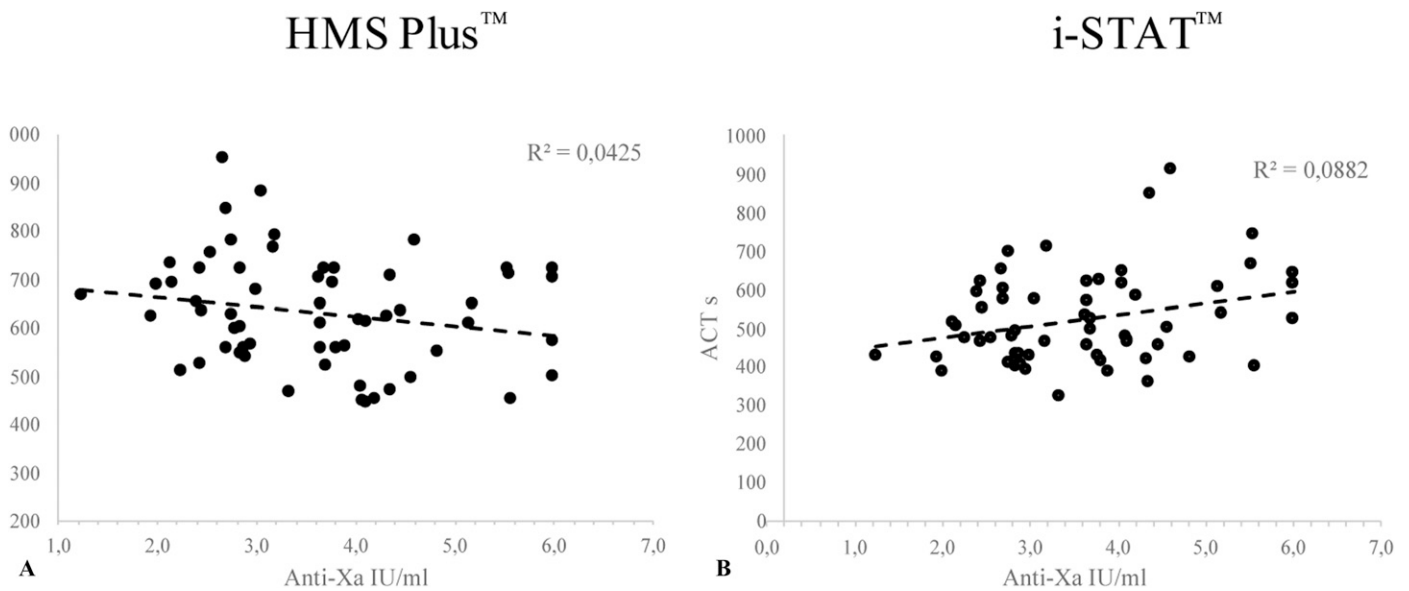
**Figure 1.** Observed differences of the activated clotting time between the HMS Plus™ and i-STAT™ at specific time points. Asterisks (\*) indicate a statistical significant difference ( $p < .001$ ).

decreases the concentration of essential coagulation factors, most notably antithrombin and fibrinogen (19). Activation and aggregation of platelets will further activate thrombin and interact with antithrombin (20). The consumption of antithrombin in addition to the lower concentrations of other vital coagulation factors will slow down the coagulation activity (20). However, the exact coagulation mechanisms involved following hemodilution remain unclear. The heparin effect is dependent on the cofactor antithrombin. When the concentration of antithrombin decreases, antithrombin-independent effects via cofactor II should be considered. This is predominately seen as a direct inhibition of factor Xa (21). It should be emphasized that the impeding effects of hemodilution requires the presence of heparin (19). The disproportional response between the HMS Plus™ and i-STAT™ after initiating CPB could be an effect of hemodilution, especially because both devices showed a significant correlation to the degree of hemodilution. This is in contrast to the

finding of Lewandrowski et al., where the electro-chemical technique applied by the i-STAT™ monitor was less influenced by hemodilution, temperature, and fibrinogen levels (12,22). The good agreement measures for the i-STAT™ reported by Lewandrowski et al. (12) were later confirmed by Maslow et al. (23), this time in a comparison with the Hemochron™ ACT device. Both these studies based their findings on variants of linear regression analyses. Of note is that inclusion of both low to very high ACT values in the same analysis will produce a seemingly high degree of association. However, the spread differs significantly between low- and high-range ACTs, which is why the lower spread for low readings will improve the correlation significantly. In our case, Pearson's correlation coefficient was less than .5, when low- and high-range ACTs were analyzed separately, but increased to .92 (not reported), when analyzed together. It is tempting to believe that the overall high degree of association is applicable for all test situations. This is apparently not the case, which is important



**Figure 2.** Bland–Altman analysis showing limits of agreement (dotted lines) within a 95% confidence interval and bias (filled line) for the HMS Plus™ and i-STAT™ ACT devices. Panel (A) Sample points heparin bolus, rewarming, and weaning. Panel (B) Sample points baseline and heparin reversal.



**Figure 3.** Association between plasma heparin concentration (anti-Xa) and the ACT for two different ACT devices. (A) HMS Plus™  $R^2 = .04$  ( $p = .114$ ). (B) i-STAT™  $R^2 = .09$  ( $p = .021$ ).

to consider. The frequently cited Lancet publication by Bland and Altman (16) addressed the limitations associated with correlation analyses and how results can give false impressions and therefore jeopardize correct conclusion making. Both Lewandrowski et al. (12) and Maslow et al. (23) applied the method of Bland and Altman for assessment of reproducibility between ACT machines. Lewandrowski did neither report bias nor the 95% confidence limits of agreement, but the graph would indicate a significant negative bias accompanied with a spread of  $\pm 100$  seconds over the measuring range. The 95% confidence limits of agreement fluctuated over 300 seconds in Maslow's report, with a clear negative bias and a spread increasing with the heparin concentration (23). Because blood specimens were collected before initiating CPB, influences of hemodilution could not be determined.

There is no doubt that ACT devices are sensitive to heparin but react in an unspecific manner to the measured concentration (23) under the conditions of CPB and hemodilution. Anticoagulation guidance by ACT should therefore be seen more as a global assessment, where the

effect of heparin represents only one of a series of possible components engaged in controlling the ongoing coagulation activity. Combining the ACT with heparin concentration analyses is nevertheless indicated because it will validate the effect of heparin. The heparin concentration is also used as an alternative reference instead of the ACT to manage anticoagulation during CPB (17,24).

The marked bias and LOA encountered in this study preclude interchangeability between the two devices evaluated. It would influence decisions regarding heparin iteration and dosing of protamine, with consequences for the postoperative phase regarding blood transfusions, pharmacological intervention, and redo surgery.

Of note, bias for the low-range ACT assessments fulfilled the stipulated  $\pm 10$ -second target. However, because the LOA was  $\pm 23$  seconds, device agreements may seem unacceptable.

Why characteristics between different ACT devices are inconsistent remains an open question. There is no obvious answer but most likely multifactorial. Apart from influences by hemodilution, one very reasonable explanation

**Table 3.** Association between heparin concentration and hematocrit vs. ACT during CPB.

	HMS Plus™	<i>p</i> -value	i-STAT™	<i>p</i> -value
Model <i>R</i> -value	.384	.052	.406	.035
Heparin				
Standardized beta coefficient	-.122	.433	.070	.649
Hematocrit				
Standardized beta coefficient	-.345	.031	-.412	.010

Heparin concentration refers to the antifactor Xa. The multivariable regression models used the ACT from the i-STAT™ and HMS Plus™ as dependent variables and hematocrit and the plasma heparin concentration as predictors. The overall model *R*-value describes the correlation coefficient between observed and predicted values of the dependent variable. The standardized beta refers to the regression coefficients, when all variables are expressed in standardized *z*-scores.

would be differences in the analyzing methodology. The i-STAT™ device uses an electro-chemical technique sensitive to thrombin cleavage (11,12), whereas the HMS Plus™ awaits complete clot formation sensed by movements of plungers (11,12). The use of different agents to activate the coagulation represents still another plausible reason. However, both HMS Plus™ and i-STAT™ use kaolin. Reasons for this unpredictability between ACT devices warrant further investigation. Instruments capable of compensating for changes in hematocrit represent one alternative how precision and bias can be improved (25).

### Limitations

Because no “gold standard” for the ACT methodology exists, it was not possible to compare tests of individual monitor characteristics to such a reference. Limits of agreement stated in our hypothesis were chosen arbitrarily because we lack official recommendations. Because the sample size was relatively small, confirmation of results may seem warranted.

### Conclusion

A clinical evaluation of the HMS Plus™ and i-STAT™ showed significant inter-device differences defined by measures of bias and LOA for the ACT. The ACT was unrelated to the systemic concentration of heparin during CPB but correlated with the measured hematocrit in the systemic circulation.

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