CELITE ACTIVATED CLOTTING TIME/ (CELITEACT)

The i-STAT® Celite® Activated Clotting Time test, CeliteACT, is a measure of the time required for complete activation of the coagulation cascade.[1]

In traditional ACT tests, coagulation is initiated by mixing a whole blood sample with a particulate activator, and complete activation is indicated when extensive or localized clots form as activated thrombin converts fibrinogen to fibrin. These clots are mechanically detected.

The i-STAT CeliteACT test is similar to traditional ACT tests except that the endpoint is indicated by the conversion of a thrombin substrate other than fibrinogen and an electrochemical sensor is used to indicate the event of this conversion. The substrate used in the electrogenic assay has an amide linkage that mimics the thrombin-cleaved amide linkage in fibrinogen.

The substrate is H-D-phenylalanyl-pipecolyl-arginine-*p*-amino-*p*-methoxydiphenylamine which has the structure:

Phenylalanine - Pipecolic acid - Arginine -- NH - C_6H_4 - NH - C_6H_4 - OCH_3

Thrombin cleaves the amide bond at the carboxy- terminus of the arginine residue (denoted by the two dashes) because the bond structurally resembles the thrombin-cleaved amide linkage in fibrinogen. The product of the thrombin-substrate reaction is the electrochemically inert tripeptide Phenylalanyl - Pipecolyl - Arginine and the electroactive compound NH_3^+ - C_6H_4 - NH - C_6H_4 - OCH_3 . The formation of the electroactive compound is detected amperometrically, and the time of detection is measured in seconds. The test reports the Activated Clotting Time (ACT) as a whole blood time (WBT) in seconds.

The i-STAT CeliteACT test is calibrated to match the Hemochron Celite FTCA510 using prewarmed tubes. However, users of the i-STAT®1 analyzer may choose to customize their individual i-STAT locations to report ACT results as calibrated against the Hemochron Celite ACT using non-prewarmed (ambient) temperature tubes. This customization affects the Patient path only, and will not be applied to the Control or the Proficiency Testing pathway.

The customization in effect (prewarm or non-prewarm calibration mode) is identified on the analyzer screen as PREWRM or NONWRM, respectively. Please note that different locations within a given hospital may utilize different customization profiles. Prior to patient sample testing, ensure the appropriate calibration mode is employed. For a comprehensive discussion of this customization feature, please see the Technical Bulletin entitled “ACT Test Result Calibration Options: PREWARMED vs. NON-PREWARMED Result Calibration Modes for the i-STAT®1 Analyzer”.

If results appear inconsistent with the clinical assessment, the patient sample should be re-tested using another cartridge.

Intended Use

The i-STAT Celite Activated Clotting Time (CeliteACT) test is an *in vitro* diagnostic test that uses fresh, whole blood, and is useful for monitoring patients receiving heparin for treatment of pulmonary embolism or venous thrombosis, and for monitoring anticoagulation therapy in patients undergoing medical procedures such as catheterization, cardiac surgery, surgery, organ transplant, and dialysis.

Contents

Each i-STAT [Celite]ACT cartridge provides a sample collection chamber, sensors to detect the coagulation endpoint, and dry reagents necessary to initiate and allow coagulation. Stabilizers and reagents are coated on a section of the sensor channel and include the following reactive ingredients:

Reactive Ingredient	Minimum Quantity
Diatomaceous Earth	14.4 µg
Thrombin Substrate	0.36 µg

Metrological Traceability

The i-STAT System test for Celite Activated Clotting Time measures the time interval required for complete activation, by Celite®, of the coagulation cascade in arterial or venous whole blood (dimension seconds) for *in vitro* monitoring of moderate- and high-level heparin therapy. Presently, no international conventional reference measurement procedure or international conventional calibrator for [Celite]ACT is available. [Celite]ACT values assigned to i-STAT's controls are traceable to i-STAT's selected reference measurement procedure, which employs diatomaceous earth (Celite) activated glass reagent tubes, an automated timer and traditional viscometric clot detection and is run under specified temperature and sample conditions. i-STAT System controls are validated for use only with the i-STAT System and assigned values may not be commutable with other methods. Further information regarding metrological traceability is available from Abbott Point of Care Inc..

Expected Values

Test/Abbreviation	Units	Reportable Range	Reference Range (PREWRM)	Reference Range (NONWRM)
Activated Clotting Time/ACT	seconds	50 - 1000*	74 - 125	84 - 139

*The range from 80 - 1000 seconds has been verified through method comparison studies.

Clinical Significance

The ACT is primarily used to monitor a patient's state of anticoagulation due to heparin that is administered during a medical or surgical procedure. It is commonly employed in cardiac catheterization, Percutaneous Transluminal Coronary Angioplasty (PTCA), renal dialysis, hemodialysis, and extra-corporeal circulation during bypass.

Performance Characteristics

The typical performance data summarized below was collected in health care facilities by health care professionals trained in the use of the i-STAT System and comparative methods. All data uses the PREWRM calibration, unless otherwise noted.

Precision data were collected at Abbott Point of Care Inc. and during clinical trials following a protocol recommended by i-STAT and using plasma control material. Similar results can be expected in future performance studies provided the same experimental design and data analysis procedures are followed.

Plasma Control	n	Mean	SD	%CV
Level 1	329	221 seconds	18 seconds	8.1
Level 2	438	456 seconds	22 seconds	4.8

Method comparison data were collected using a modification of the CLSI guideline EP9-A[2]. Venous or arterial blood samples were collected in plastic syringes and analyzed in duplicate on the i-STAT System and in duplicate using the comparative methods. All samples were analyzed immediately upon collection. The patient populations in the studies were those in which ACT is routinely used. This includes baseline, heparin-treated, and heparin-reversed samples from from patients undergoing cardiac catheterization and cardiac bypass.

Deming regression analysis[3] was performed on the first replicate of each sample. In the method comparison table, *n* is the number of specimens in the data set, *Sxx* and *Syy* refer to estimates of the imprecision based on the duplicates of the comparative and i-STAT methods respectively, *Sy.x* is the standard error of the estimate, and *r* is the correlation coefficient.

Method comparisons will vary from site to site due to differences in the sample handling, reagent and instrument systems in use, and other site-specific variables.

Cath Lab	Medtronic HR-ACT	Hemochron CA510/FT CA510
n	270	418
Sxx	10.1	19.7
Syy	10.7	13.5
Slope	1.15	0.86
Int't	-30	-3
Sy.x	32.5	22.5
Xmin	73	63
Xmax	523	763
r	0.848	0.903

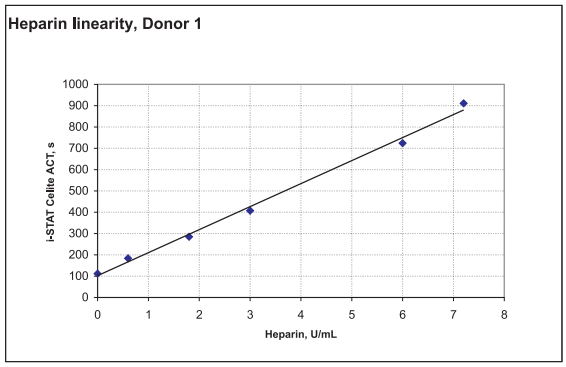
	Hemchron CA510/FT CA510		
CVOR	Site 1	Site 2	Site 3
n	35	30	24
Sxx	15.8	34.2	24.4
Syy	13.0	11.5	20.8
Slope	0.85	1.10	1.19
Int't	4	-52	-73
Sy.x	43.8	17.4	62.1
Xmin	118	94	125
Xmax	671	735	767
r	0.912	0.952	0.891

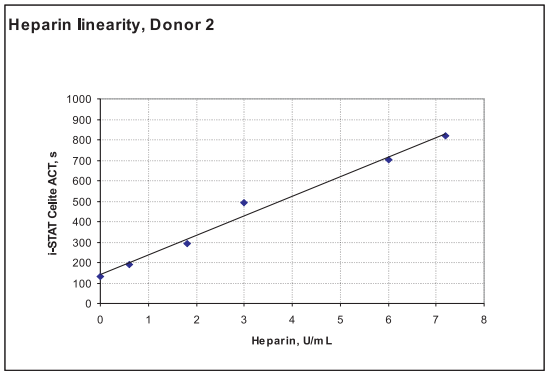
Factors Affecting Results*

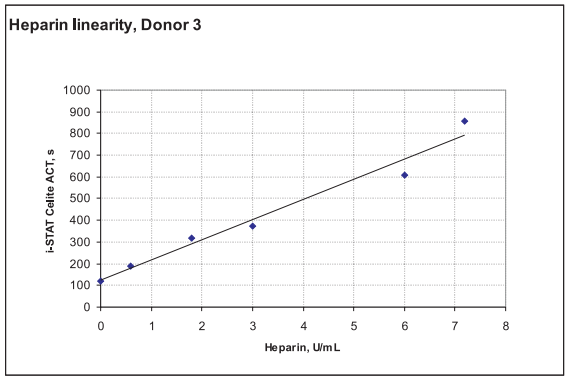
*It is possible that other interfering substances may be encountered. These results are representative and your results may differ somewhat due to test-to-test variation. The degree of interference at concentrations other than those listed might not be predictable.

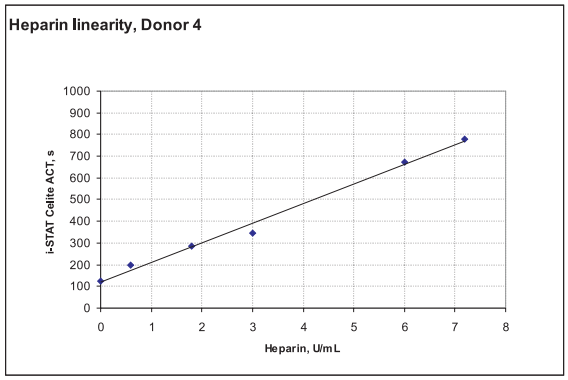
Heparin sensitivity was demonstrated using whole blood samples to which varying concentrations of heparin were added *in vitro*.

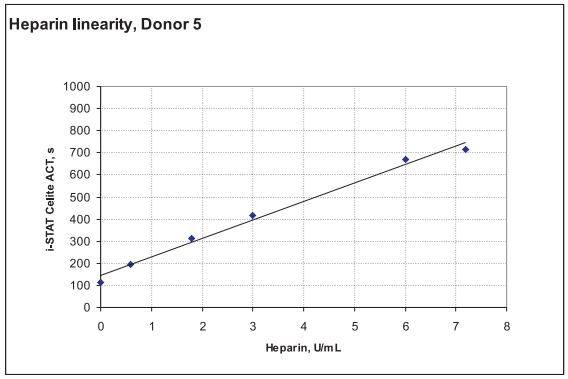
The five graphs below each indicate the response of a different donor with respect to heparin concentration:



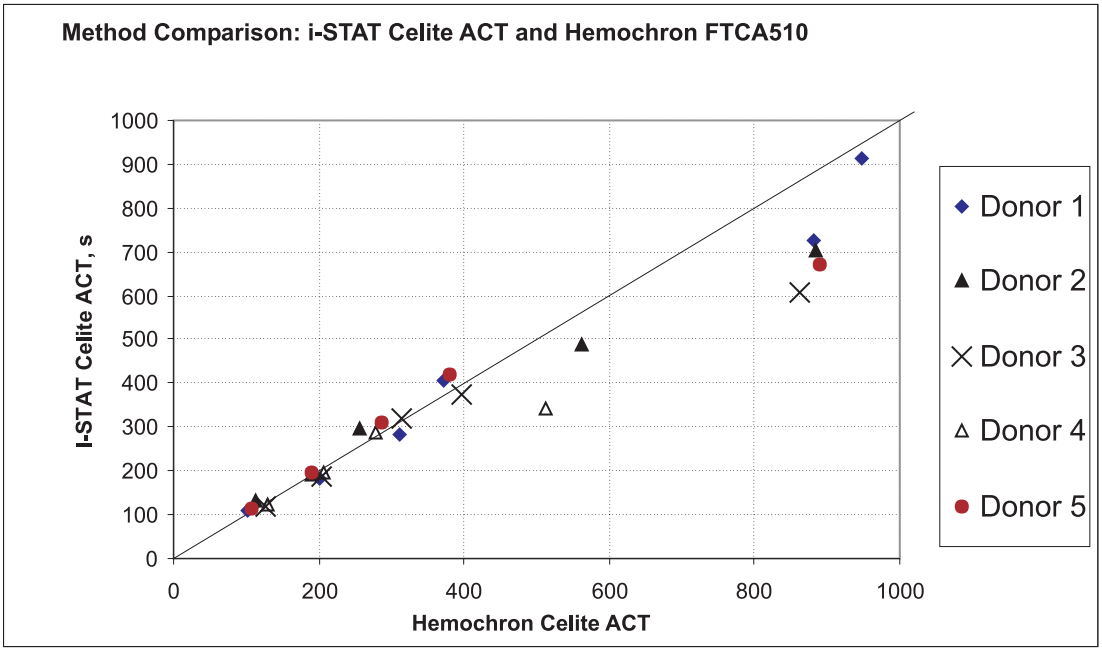


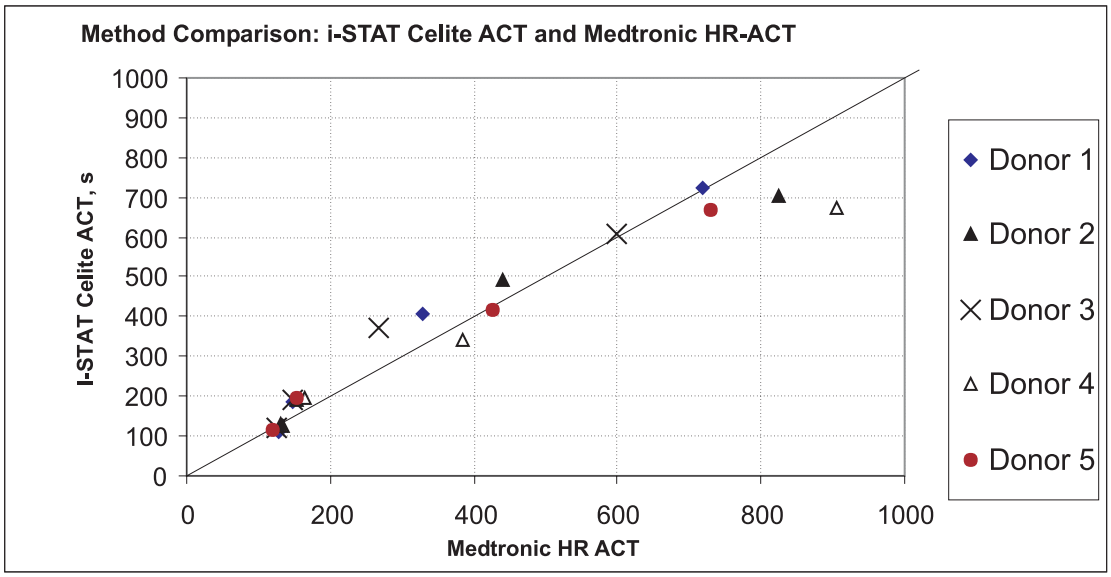




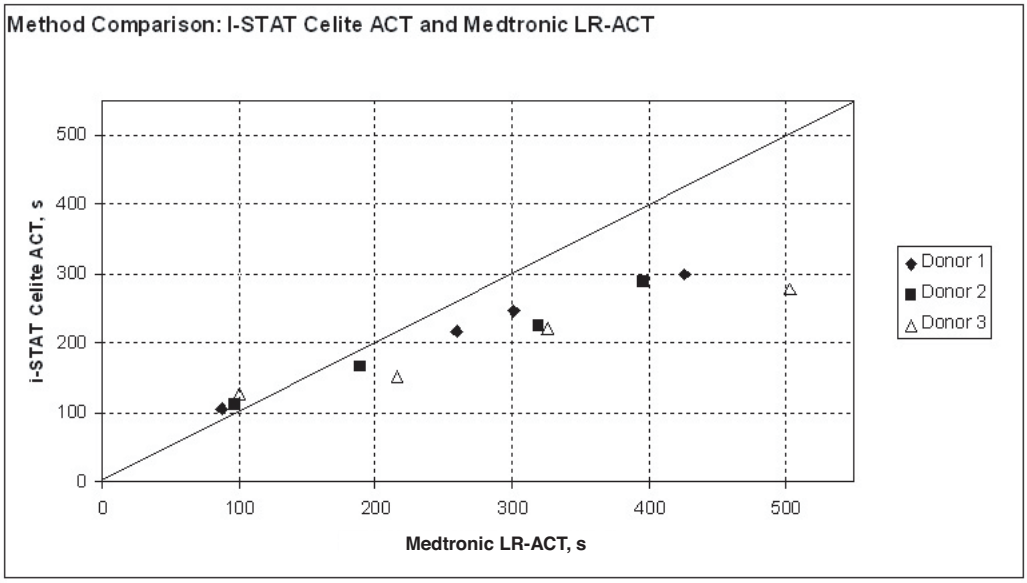


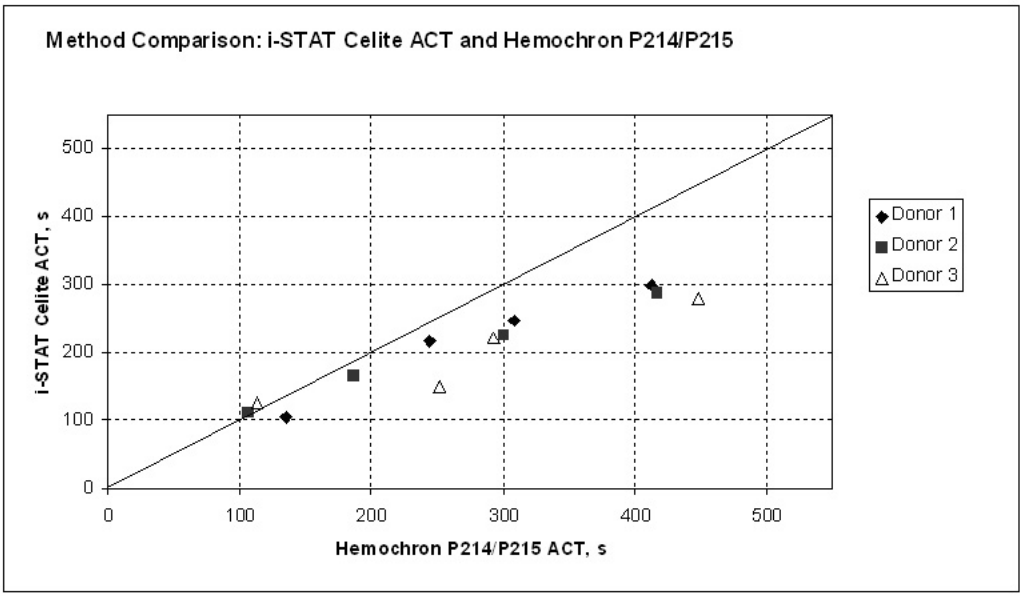
The graphs below indicate the response of the same five donors with respect to the ACT result on the Medtronic HR-ACT and the Hemochron Celite FTCA 510.





Performance of the i-STAT Celite ACT at lower levels of heparin is shown below with two “Low Range” ACT methods included for comparison:





Test Limitations

The i-STAT [Celite]ACT test is to be used with fresh venous or arterial whole blood samples. The presence of exogenously added heparin, citrate, oxalate, or EDTA will interfere with test results. Poor technique in sample collection may also compromise the results. Samples drawn from insufficiently flushed catheters or from traumatic venipunctures may be contaminated with interfering substances. Samples should be collected into plastic syringes or tubes. Collection into glass may prematurely activate coagulation resulting in accelerated clotting times.

The i-STAT ACT test uses Celite brand diatomaceous earth as the activator of the intrinsic pathway. The result may, therefore, be prolonged in the presence of aprotinin.[4] **The test is not recommended for use with patients receiving aprotinin.**

The analyzer should remain on a level surface with the display facing up during testing. If the analyzer is not level, the ACT result may be affected by more than 10%. A level surface includes running the handheld in the downloader/recharger.

Hemodilution may affect test results.

Platelet dysfunction, hereditary or acquired, may affect the results of this test. This includes the administration of pharmacological compounds known as platelet inhibitors which affect platelet function. Factor deficiencies, dysprothrombinemias, other coagulopathies, and other pharmacological compounds may also affect the results of this test.

The i-STAT ACT test is not affected by hematocrit in the range of 20 - 70%, fibrinogen concentration in the range from 100 - 500 mg/dL, or sample temperature from 15 - 37ºC.

Specimen Collection and Preparation

The i-STAT Celite ACT test can be performed using venous or arterial samples.

Venipunctures and Arterial Punctures

- Collection technique resulting in good blood flow must be used.
- The sample for testing should be drawn into a **plastic collection device** (either a plastic syringe or plastic evacuated tube).
- The collection device **cannot contain anticoagulants** such as heparin, EDTA, oxalate, or citrate.
- The collection device cannot contain clot activators or serum separators.
- The sample should be immediately dispensed into the sample well of a cartridge.
- If a second measurement is required, a fresh sample must be obtained.

Note: Some experts recommend drawing and discarding a sample of at least 1 mL prior to drawing sample for coagulation testing.[5]

Indwelling line

- Fluid drip through the line must be discontinued.
- If blood must be drawn from an indwelling line, possible heparin contamination and specimen dilution should be considered. The line should be flushed with 5 mL of saline and the first 5 mL of blood or six dead space volumes should be discarded.
- Withdraw the sample for testing into a fresh **plastic** syringe.
- The collection syringe **cannot contain anticoagulants** such as heparin, EDTA, oxalate, or citrate.
- The sample should be immediately dispensed into the sample well of a cartridge.
- If a second measurement is needed, draw a fresh sample.

Extracorporeal line

- Flush the extracorporeal blood access line by withdrawing 5 mL of blood into a syringe and discard the syringe.
- Withdraw the sample for testing into a fresh **plastic** syringe.
- The collection syringe **cannot contain anticoagulants** such as heparin, EDTA, oxalate, or citrate.
- The sample should be immediately dispensed into the sample well of a cartridge.
- If a second measurement is needed, draw a fresh sample.

References

1. Hattersly, P. Activated coagulation time of whole blood. Journal of the American Medical Association 136:436-440, 1966.

2. CLSI. *Method Comparison and Bias Estimation Using Patient Samples*; Approved Guideline. CLSI document EP9-A (ISBN 1-56238-283-7). CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087, 1995.

3. P.J. Cornbleet and N. Gochman, "Incorrect Least-Squares Regression Coefficients in Method Comparison Analysis," Clinical Chemistry 25:3, 432 (1979).

4. Wang, JS; Lin, CY; Hung, WT; Thisted, RA; Carp, RB. In vitro effects of aprotinin on activated clotting time measured with different activators. Journal of Thoracic Cardiovascular Surgery 104(4):1135-40, 1992.

5. Corriveau, Donna: Fritsma, George (ed.): Hemostasis and Thrombosis in the Clinical Laboratory. Ed, J.B. Lippinncott Company, Philadelphia, 1988, pp 70-71.

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