The i-STAT® Kaolin Activated Clotting Time test, KaolinACT, 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 KaolinACT 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:

\[
\text{Phenylalanine - Pipecolic acid - Arginine -- NH - C}_6\text{H}_4 - \text{NH - C}_6\text{H}_4 - \text{OCH}_3
\]

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 - Pipecoly - Arginine and the electroactive compound \(\text{NH}_3^+ - C_6\text{H}_4 - \text{NH - C}_6\text{H}_4 - \text{OCH}_3\). 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) in seconds.

The i-STAT KaolinACT test is calibrated to match the Hemochron Celite FTCA510 using prewarmed reagent 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 (prespark or non-prespark 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 Kaolin Activated Clotting Time (KaolinACT) test is an *in vitro* diagnostic test that uses fresh, whole blood, and is used to monitor high-dose heparin anticoagulation frequently associated with cardiovascular surgery.
Contents
Each i-STAT Kaolin 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:

<table>
<thead>
<tr>
<th>Reactive Ingredient</th>
<th>Minimum Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaolin</td>
<td>23.4 μg</td>
</tr>
<tr>
<td>Thrombin Substrate</td>
<td>0.09 μg</td>
</tr>
</tbody>
</table>

Metrological Traceability
The i-STAT System test for Kaolin Activated Clotting Time measures the time interval required for complete activation, by kaolin, of the coagulation cascade in arterial or venous whole blood (dimension seconds) for in vitro monitoring of high-level heparin therapy. Presently, no international conventional reference measurement procedure or international conventional calibrator for Kaolin ACT is available. Kaolin ACT values assigned to i-STAT’s controls are traceable to i-STAT’s selected reference measurement procedure, which employs 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

<table>
<thead>
<tr>
<th>Test/Abbreviation</th>
<th>Units</th>
<th>Reportable Range</th>
<th>Reference Range (PREWRM)</th>
<th>Reference Range (NONWRM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated Clotting Time/ACT</td>
<td>seconds</td>
<td>50 - 1000*</td>
<td>74 - 137</td>
<td>82 - 152</td>
</tr>
</tbody>
</table>

* The range from 77 - 1000 seconds (PREWRM mode) 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 i-STAT 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.

<table>
<thead>
<tr>
<th>Plasma Control</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>119</td>
<td>169 seconds</td>
<td>4 seconds</td>
<td>2.0</td>
</tr>
<tr>
<td>Level 2</td>
<td>113</td>
<td>409 seconds</td>
<td>21 seconds</td>
<td>5.2</td>
</tr>
</tbody>
</table>
Method comparison data were collected using a modification of the CLSI guideline EP9-A². 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 and included both aprotinin and non-aprotinin receiving patients. All were undergoing cardiac surgery. Sample types included baseline, heparin-treated, and heparin-reversed samples.

Deming regression analysis³ was performed on the first replicate of each sample. In the method comparison table, $n$ is the number of specimens in the data set, $S_{xx}$ and $S_{yy}$ refer to estimates of the imprecision based on the duplicates of the comparative and i-STAT methods respectively, $S_{yx}$ 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.

<table>
<thead>
<tr>
<th></th>
<th>CVOR</th>
<th>Site 1</th>
<th>Site 2</th>
<th>Site 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>104</td>
<td>118</td>
<td>106</td>
<td></td>
</tr>
<tr>
<td>$S_{xx}$</td>
<td>9.1%</td>
<td>6.8%</td>
<td>7.6%</td>
<td></td>
</tr>
<tr>
<td>$S_{yy}$</td>
<td>3.6%</td>
<td>4.0%</td>
<td>3.6%</td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>0.96</td>
<td>1.05</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>-12</td>
<td>-38</td>
<td>-39</td>
<td></td>
</tr>
<tr>
<td>$X_{min}$</td>
<td>68</td>
<td>111</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>$X_{max}$</td>
<td>1286</td>
<td>1310</td>
<td>1102</td>
<td></td>
</tr>
<tr>
<td>$r$</td>
<td>0.906</td>
<td>0.940</td>
<td>0.971</td>
<td></td>
</tr>
</tbody>
</table>

Factors Affecting Results*

The i-STAT $^{\text{Kaolin}}$ACT test is not significantly prolonged in the presence of aprotinin (Trasylol).

*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 following three graphs below each indicate the response of a different donor with respect to heparin concentration:
The following two graphs indicate the response of the same three donors with respect to the ACT result on the Medtronic HR-ACT and the Hemochron Kaolin FTK-ACT.
Test Limitations

The i-STAT Kaolin 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 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 fibrinogen concentration in the range from 100 - 500 mg/dL, or sample temperature from 15 - 37°C.

Specimen Collection and Preparation

The i-STAT Kaolin 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.

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


i-STAT is a registered trademark of the Abbott Group of Companies in various jurisdictions. Celite is a registered trademark of Celite Corporation, Santa Barbara, CA, for its diatomaceous earth products. Hemochron is a registered trademark of International Technidyne Corporation, Edison, NJ.