How Does the Age of a Blood Sample Affect Its Activated Clotting Time? Comparison of Eight Different Devices

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Abstract: Monitoring activated clotting time (ACT) during extracorporeal procedures is virtually universal. The ACT test is usually performed immediately following blood collection. However, certain situations may occur that delay rapid measurement. It is unknown how an aged blood sample affects the ACT measurement. It is hypothesized that the ACT will be affected as a blood sample ages. Multiple blood samples were taken from six patients undergoing cardiopulmonary bypass (CPB). Samples were divided into two groups, heparinized (H) and unheparinized (UH). ACT/HMT tests were performed with each sample on eight devices (Array Actalyke, Gem PCL, Hemochron Jr. Signature, Hemochron Response, Hemochron 801, Hemotec HMS, Rapidpoint Coag, and Sonoclot II) at three different sample ages [<60 s (fresh blood), 10 min, and 15 min after sample collection]. ACT/HMT results of aged samples (10 min and 15 min after sample collection) were compared to ACT/HMT results for fresh blood using a repeated measures analyses of variance (ANOVA) with Student’s–Newman-Keuls post hoc test. In the unheparinized group, no device produced an ACT significantly different from the fresh sample counterpart at the 10 min time point. At the 15 min time point, the Hemochron 801 produced a significantly lower average ACT when compared to the fresh sample. (120±25 vs. 135±15 s). In the heparinized group, the Actalyke device produced results with 10 and 15 min aged blood that were significantly longer than fresh blood sample results (ACT <60 s

The activated clotting time is a parameter that is universally monitored for assessment of anticoagulation during cardiopulmonary bypass (CPB)(1,2). Currently, more than 10 different point-of-care (POC) devices exist that can perform ACT tests. These devices provide quick, accurate results at the patient’s bedside when used correctly. To ensure accuracy, it is recommended that the ACT test be performed immediately following blood sample withdrawal (3–5). However, in the clinical setting, there are circumstances that may preclude immediate testing and lead to sample aging. Sample aging could occur because of device preparation, incorrect initiation of the test requiring the technician to restart the tests, and inability to run the test immediately following blood draw because of an overriding clinical priority.

The purpose of this investigation was to evaluate eight commonly available POC ACT devices and determine the effect of time delays on the ACT measurements. This investigation could aid the clinician to devise guidelines regarding the management of blood samples with respect to age of sample.

MATERIALS AND METHODS

Patients
Following approval from the Institutional Review Board for the Protection of Human Subjects, informed consent was obtained from six adult patients undergoing elective cardiopulmonary bypass (CPB) for CABG or valve repair/replacement. Patient inclusion criteria were as follows: age ≥ 18 years, body surface area ≥ 1.2 m², and pre-CPB hematocrit of ≥ 35%. Patient exclusion criteria were as follows: redo sternotomy, abnormal laboratory coagulation profile, and pre-operative intravenous heparin.

Blood Collection
Before anesthetic administration, a radial artery catheter was placed in the left arm of each patient for hemo-
dynamic monitoring and blood sampling purposes. Multiple blood samples were drawn into 30-mL plastic syringes at two time periods during the case (before heparinization and post heparinization). Blood samples were left undisturbed between tests but were gently agitated before initiating tests with aged blood. Before heparin administration, 2–4 blood samples (UH group) were drawn from each patient’s radial arterial line. Following full heparinization (300 U/kg), and initiation of CPB, 2–4 blood samples (H group) were taken from the manifold of the extracorporeal circuit (ECC).

**ACT Devices**

The following eight different ACT testing devices were utilized for blood analysis: Array Actalyke (Array Medical, Somerville, NJ), Gem PCL (Instrumentation Laboratories, Lexington, MA), Hemochron Jr. Signature, Hemochron Response, Hemochron 801 (International Technidyne Corporation, Edison, NJ), Hemotec HMS (Medtronic, Inc. Minneapolis, MN), Rapidpoint Coag (Bayer/Chiron, Medfield, MA), and Sonoclot II (Sienco, Inc. Wheat Ridge, CO). Before investigation on each testing day, quality control tests were performed on each machine in accordance with manufacturer’s recommendations. Table 1 provides specific information regarding the devices and tests. The same operator drew all samples.

**Protocol**

ACT tests were initiated at three specific times for each blood sample. The three times corresponded with three different sample ages—<60 s (fresh blood), 10 min, and 15 min. Between test times, samples were left undisturbed. Immediately before test initiation, the syringe was gently agitated to ensure that the sample was uniform throughout.

**Statistical Analysis**

All data are expressed as mean ± standard deviation of the mean (SD). Comparative data were analyzed using repeated measures analyses of variance (ANOVA) with Student’s–Newman-Keuls post hoc test. Statistical significance was accepted at a $p < .05$.

### RESULTS

**Patient Parameters**

The average pre-CPB hematocrit (HCT) was 37.5 ± 4.7% (range = 35–42.3%). During CPB, the average HCT was 27.3 ± 3.2% (range = 22.3–28.8%). In five of six patients, temperatures were allowed to drift to 34°C during CPB. A single patient had blood cooled to 20°C.

**UH Group**

Comparisons of unheparinized blood sample average ACT results at the three time point are shown in Table 2. For all devices, there were no statistically significant differences in average ACT measurements between fresh unheparinized blood samples when compared to samples that had aged 10 min. At the 15 min sample age, one device, the Hemochron 801, showed a significant difference in the ACT when compared to fresh blood (120 ± 25 s vs. 135 ± 15 s; $p < .0001$). The remaining seven machines produced no statistically significant difference at the 15 min time point.

**H-Group**

With heparinized blood samples, no significant differences were seen in ACT measurements of fresh blood, 10 min aged samples, and 15 min aged samples for seven of eight devices tested (Table 3). The Actalyke was the single device that did show statistically significant differences between fresh, 10 min aged, and 15 min aged samples (426 ± 66, 457 ± 82, 451 ± 68 s, respectively; $p = .0004$).

**DISCUSSION**

In this study, differences in ACT measurements between fresh blood sample and aged sample time points occurred in only three cases: 1) Hemochron 801, UH-group at 15 min time point; 2) Actalyte, H-group, 10 min time point; and 3) Actalyte, H-group, 15 min time point. Closer evaluation of these three instances shows that for the Hemochron 801 the difference seen at the 15 min time point in the UH-group was a significant reduction in the ACT (120 ± 41 vs. 135 ± 13 s) In contrast, for H-group

<table>
<thead>
<tr>
<th>Device</th>
<th>Test</th>
<th>Volume</th>
<th>Activator</th>
<th>Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Array Actalyke</td>
<td>MAX-ACT</td>
<td>0.5 mL</td>
<td>Celite, Kaolin, &amp; glass particle “cocktail”</td>
<td>Mechanical</td>
</tr>
<tr>
<td>Gem PCL</td>
<td>ACT</td>
<td>1 Drop</td>
<td>Silica &amp; kaolin</td>
<td>Photo-optical</td>
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<tr>
<td>Hemochron Jr. Signature</td>
<td>ACT+</td>
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<td>Silica &amp; kaolin</td>
<td>Photo-optical</td>
</tr>
<tr>
<td>Hemochron Response</td>
<td>FTCA 510</td>
<td>2 mL</td>
<td>Celite</td>
<td>Mechanical</td>
</tr>
<tr>
<td>Hemochron 801</td>
<td>FTCA 510</td>
<td>2 mL</td>
<td>Celite</td>
<td>Mechanical</td>
</tr>
<tr>
<td>Hemotec HMS</td>
<td>ACT</td>
<td>1 mL</td>
<td>Kaolin</td>
<td>Photomechanical</td>
</tr>
<tr>
<td>Rapidpoint Coag</td>
<td>HMT</td>
<td>1 Drop</td>
<td>Celite</td>
<td>Photo-optical</td>
</tr>
<tr>
<td>Sonoclot II</td>
<td>SonACT</td>
<td>360 µL</td>
<td>Celite</td>
<td>Electromechanical</td>
</tr>
</tbody>
</table>

POC = point-of-care devices, ACT = activated clotting time.
samples performed on the Actalyte device, the significant difference seen in the 10 and 15 min time points was from an increase in ACT from fresh sample time point values (ACT<60s/H11505 426 ± 66, 10 min/H11505 457 ± 82, 15 min/H11505 450 ±88s , p<.05). This observation has no clear interpretation. The Hemochron 801 showed no effect of sample age in the H-group and the Aktalyte device also showed no effect in the UH-group.

It has been suggested that such other factors as hemodilution and temperature can affect the accuracy of ACT measurements (6,7). In this study, no correlation or trends were identified when data were examined with respect to these variables.

Product literature suggests that it is good practice to initiate ACT measurements immediately after the blood sample is drawn. However, there are few, if any published studies that have examined the effects of time delays on the accuracy of the ACT. It was hypothesized that drawing a blood sample and allowing a delay of 10 to 15 min before measurement would significantly alter the ACT results when compared to the fresh sample measurement. This hypothesis was not supported and, overall, based on this small study, it seems that both heparinized and unheparinized blood samples remain stable inside of a syringe and accurate ACTs can be performed for time periods up to 15 min in most devices.

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**REFERENCES**