

The Influence of Sampling Technique on ACT Plus[®] Results

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Abstract: The manufacturer of the ACT Plus[®] Automated Coagulation Timer, Medtronic Inc., recommends that test cartridges be prewarmed and the activating reagent resuspended (tapped/mixed) before patient testing. In a busy clinical environment, these recommendations may be overlooked or disregarded. In this study, the impact of sampling technique on ACT Plus[®] test results was investigated. In Series 1, two test cartridges were split into four individual chambers. Two ACT Plus[®] machines were used, allowing for three separate comparisons to be made. The sample results from test Chambers 2 (cold/tapped), 3 (warmed/not tapped), and 4 (cold/not tapped) were compared individually against the result from test Chamber 1, the recommended tech-

nique (warm/tapped). In Series 2, the manufacturer's recommendations were tested using a single double cartridge (warm/tapped). Results were interpreted using the Bland-Altman method of analysis. The prewarming and tapping of cartridges before use independently influenced the agreement of results when compared with cartridges that were not prewarmed and tapped. Each factor (temperature and mixing) when excluded was found to affect the standard deviation and decrease the agreement of results. By following the manufacturer's recommendations to standardize the sampling technique, ACT Plus[®] test results are more accurate. **Keywords:** activated clotting time, activated coagulation time. *JECT. 2012;44:194–197*

The Activated Clotting Time (ACT) is a simple test to perform and provides a rapid result at the patient's bedside or within the operating room. It has been used for decades as a general reference for ACT monitoring to assess heparin therapy. Various devices, with different reagents and test tubes or cartridges, have been used over time to obtain ACT results. One such device is the Medtronic ACT Plus[®] Automated Coagulation Timer (Minneapolis, MN). In Australia alone, over 90 ACT Plus[®] analyzers may be found in various operating suites, catheter laboratories, and intensive care units (personal correspondence, Medtronic, Australia). The ACT Plus[®] (ACT+) has a testing well designed for a double-chamber device enabling two tests to be run simultaneously. The difference of the two tests must be <12% of the mean in

high-range heparinized patients using the proper technique (1). If two simultaneous results differ by more than 12%, the ACT+ analyzer will issue a warning. The ACT+ test cartridge (Figure 1) contains a liquid biological buffer, which suspends the kaolin activator. In addition, the test cartridge contains calcium chloride to ensure adequate calcium for coagulation to occur and sodium azide to prevent bacterial growth during cartridge storage.

The ACT+ operator's manual states that: 1) cartridges should be prewarmed to 37°C for 3–5 minutes in the instrument's heating block before collecting the test sample; and 2) the activating reagent should be resuspended, by tapping the cartridge, before adding the test sample (1).

In a busy clinical environment, these recommendations may be overlooked or disregarded. In this study, the impact of sampling technique on ACT Plus[®] test results was investigated.

MATERIALS AND METHODS

Each ACT+ analyzer used in this study was tested routinely using electronic and liquid controls on a monthly

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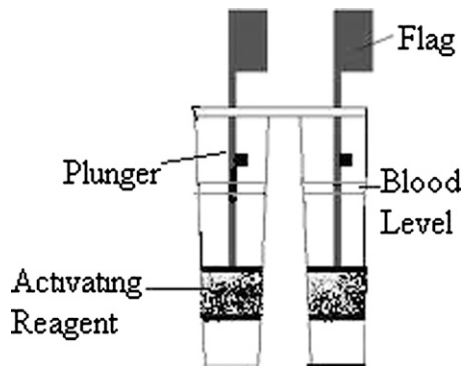


Figure 1. Medtronic ACT Plus® cartridge.

basis and with each change in cartridge lot number. Electronic controls verify quantitative and qualitative results by testing proper mechanical operation of the analyzer. Liquid (sheep's blood) controls verify instrument and cartridge performance as well as operator technique (1). In addition, a temperature verification cartridge was used to ensure the heat block temperature was maintained between 36.5 and 37.5°C. The ACT+ machines used were approximately two years old and had recently undergone a preventive maintenance service. The photo-optic sensors of each ACT+ machine were cleaned daily before patient samples were analyzed. Each set of cartridges was taken from identical lot numbers. Human Research Ethics Committee approval was granted for this project (July 18, 2011).

In Series 1, each double test cartridge was split into two single chambers allowing for four different techniques to be tested using only two ACT+ machines.

Each patient blood sample was divided among these four single test chambers as follows:

Test Chamber 1: cartridges were prewarmed and tapped (manufacturer's recommendation)

Test Chamber 2: cartridges were not prewarmed but were tapped (testing for the influence of prewarming alone)

Test Chamber 3: cartridges were prewarmed and not tapped (testing for the influence of mixing alone)

Test Chamber 4: cartridges were not prewarmed and not tapped (testing for the combined influence of prewarming and mixing.)

In Series 1, 138 patient samples provided 552 recorded results. Samples were taken consecutively in heparinized patients during cardiopulmonary bypass. Multiple samples were taken from several patients. Fifteen test cartridges (11%) failed to display a final result eliminating all four tests from the study. Test results of >900 seconds or

<300 seconds from any cartridge in the group excluded all four tests from the study. There were 22 cartridges (16%) that displayed a result in this range. A test result >900 seconds would not allow for large differences between samples because the ACT+ display is limited to three digits (highest number 999). A result <300 seconds was highly suspicious with testing repeated in these patients. In total, 101 samples had acceptable results in all four chambers for inclusion in the study. The prewarmed and tapped ACT result was used to determine patient heparin requirements.

The Bland-Altman method was used to analyze the agreement found among samples. The mean of two measurements was calculated and plotted against the difference scores from this mean (2). A logarithmic scale was chosen to reflect and illustrate the large range of data found. Back-transformation of data from the plotted logarithm to a percentage figure allowed for easier discussion of findings.

In Series 1, three separate plots provide a graphical illustration of the agreement found under the four different conditions. A mean was calculated combining the result of test Cartridges 2 (cold/tapped), 3 (warm/not tapped), and 4 (cold/not tapped) with the result from test Cartridge 1, the manufacturer's recommendations (warm/tapped).

In Series 2, a further 113 patient samples were tested. Only one ACT+ analyzer was required and cartridges were not separated. Each ACT+ cartridge in this second series was prewarmed and tapped and the mean of the two results recorded and plotted against their difference scores. Conducting this second series of testing provided a graphical illustration of the agreement found under control conditions whereby the manufacturer's recommendations were adhered to. In this control series, 12 samples were excluded with a result >900 seconds. There were no failed test results displayed; neither were there results <300 seconds.

RESULTS

The upper and lower limits of relative agreement for the warm/tap vs. warm/tap (Series 2) were found to be 13.4% (Figure 2). In terms of absolute values, 2 standard deviations (SDs) equate to a ± 73.1 -second difference with an average result of 606 seconds. The mean difference was -4.7 seconds.

The upper limit of relative agreement for the warm/tap vs. cold/not tapped was found to be 18.1% with a lower limit of 23.2% (Figure 3). In terms of absolute values, 2 SDs equate to ± 123.3 seconds with our average test result of 614 seconds. The mean difference was -27.6 seconds.

The upper limit of relative agreement for the warm/tap vs. warm/not tapped was found to be 21.6% with a lower

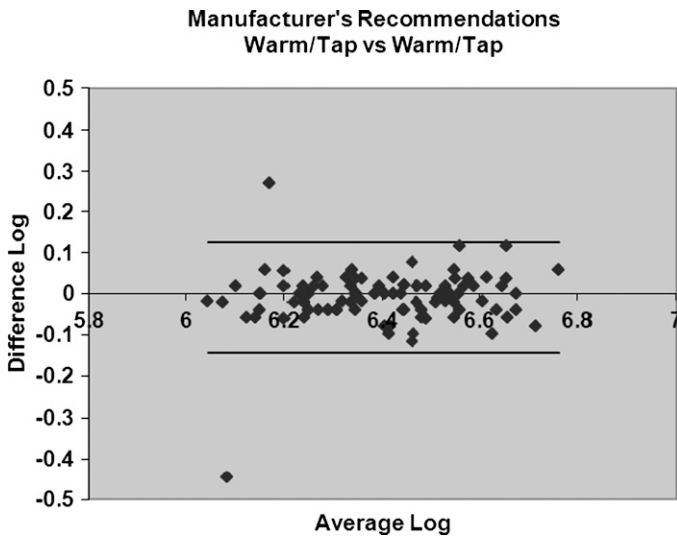


Figure 2. Series 2; one cartridge was used, both chambers were prewarmed and tapped.

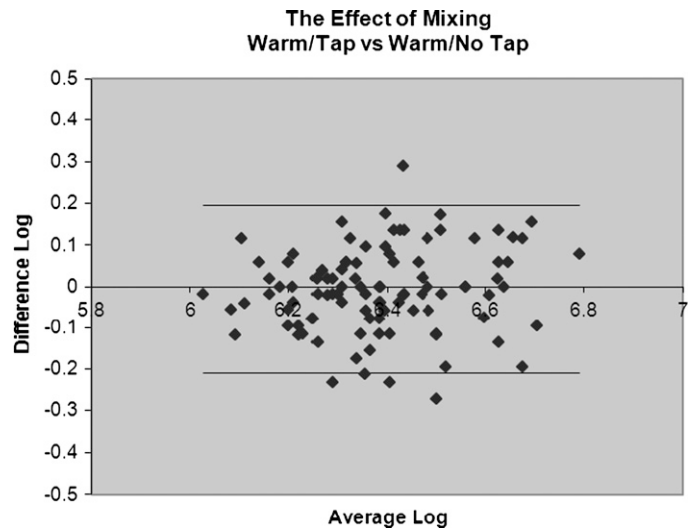


Figure 4. Series 1, one chamber prewarmed and not tapped analyzed with one chamber warmed and tapped.

limit of 18.9% (Figure 4). In terms of absolute values, 2 SDs equate to ± 127.8 seconds with an average test result of 601 seconds. The mean difference was -3.0 seconds.

The upper limit of relative agreement for the warm/tap vs. cold/tap was found to be 18.8% with a lower limit of 18.1% (Figure 5). In terms of absolute values, 2 SDs equate to ± 112 seconds with an average test result of 604 seconds. The mean difference was -8.7 seconds.

DISCUSSION

The limits of agreement (± 2 SDs) reflect 95% of the differences between the two methods used. The smaller the range between these two limits, the better the agreement.

When both cartridges are prewarmed and tapped before use (as per the manufacturer's recommendations), results show a 13.4% upper and lower limit of agreement. The manufacturer accepts a limit of 12% difference in duplicate samples. Our results were affected by two aberrant outliers. Warm/tap vs. warm/tap cartridge results were found to fall within the expected range 98% of the time with 2% of results having a $>12\%$ difference.

By ignoring the recommendation to tap/resuspend the reagent within the test cartridge, the level of agreement was adversely affected. Instead of a 13.4% upper and lower level of agreement, the upper level of agreement became 21.6% with a lower limit of 18.9%. There was less agreement and an increase in 2 SDs.

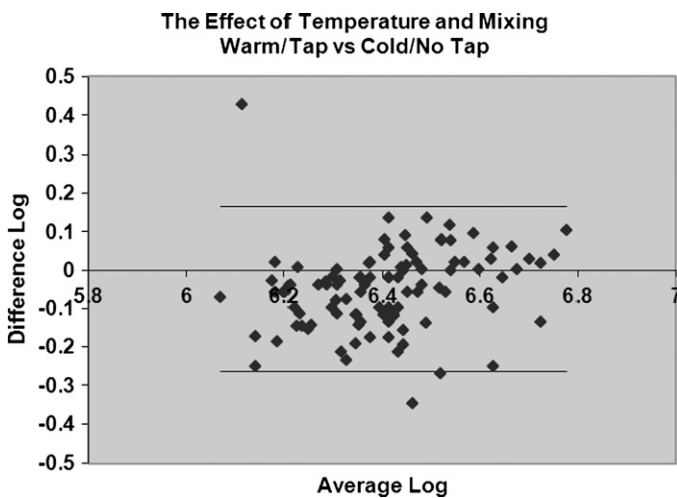


Figure 3. Series 1; one chamber not prewarmed and not tapped analyzed with one chamber warmed and tapped.

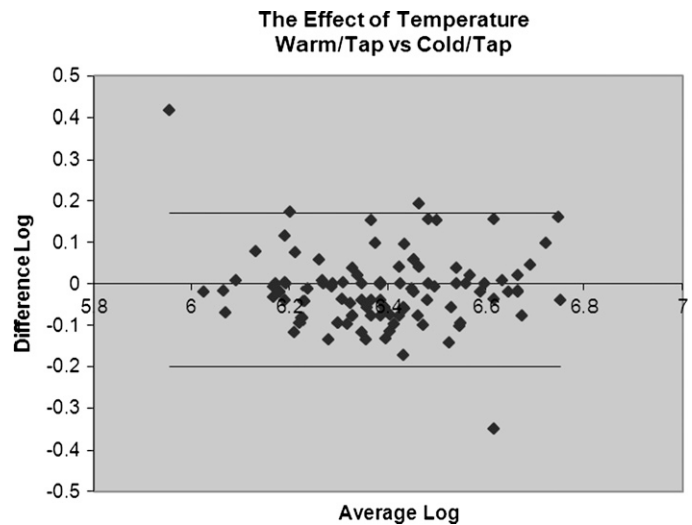


Figure 5. Series 1, one chamber not prewarmed and not tapped analyzed with one chamber warmed and tapped.

By ignoring the recommendation to prewarm the test cartridge, the level of agreement was again adversely affected. Instead of a 13.4% upper and lower level of agreement, the upper level of agreement became 18.8% with a lower level of agreement of 18.1%. The expected lengthening of ACT results was evident, but not as profound as one might expect, with a mean difference of -8.7 seconds. Hypothermia is a well-known factor lengthening ACT results (3,4). It has been suggested that the prewarming phase of microsample (<2 mL) coagulation devices may negate the hypothermic effects seen in macrosample (2 mL) devices (5). For this reason, it was expected that ACT results would be lengthened when cartridges were not prewarmed before use. One explanation for our mean difference not being more negative may relate to our surgeons' preferences to maintain patient temperatures $32-34^{\circ}\text{C}$ for routine cases. The possible effects of hypothermia are not as evident simply because patients are not, at our institution, routinely experiencing low temperatures.

Finally, by ignoring both recommendations to prewarm and tap cartridges before adding a blood sample, the level of agreement was adversely affected. Instead of the 13.4% levels of agreement seen in our control, the upper level of agreement became 18.1% with a lower level of 23.2%. An additive effect was not evident. Here it is of interest to note that the mean difference between warm/tapped and cold/not tapped was a negative value of -27.6 seconds, indicating that on average, the ACT results were longer when the cartridges were not both prewarmed and tapped.

If results are falsely high, heparin will be withheld by clinicians potentially leading to inadequate anticoagulation during cardiopulmonary bypass. Insufficient heparin may result in microscopic deposits of fibrin within the bypass circuit as well as thrombin-altered fibrinogen in the plasma. In addition, insufficient heparin anticoagulation causes the consumption of coagulation factors and may increase bleeding postoperatively (6). Alternatively, excessive heparin may contribute to postoperative bleeding if heparin is not adequately neutralized by protamine.

It should be noted that the manufacturer not only recommends the prewarming and tapping of cartridges, but as well the use of duplicate samples to improve the consistency of results. In an early article by Gravlee (7), a recommendation of taking the mean of duplicate samples was suggested based on the variability of individual ACT

results. In our series, comparing warm/tap vs. warm/tap, we have shown that 98% of the time, duplicate samples were $<12\%$ of each other. In effect what we were testing in our first series is the influence of changing the sampling technique of one test in a pairing. A more comprehensive assessment would have compared the mean of duplicate samples under each test condition. The cartridge is designed to run duplicate samples simultaneously with the analyzer displaying the mean result. If four ACT Plus® machines were available, duplicate samples may have provided a more accurate analysis. Our study design was limited by the availability of only two ACT Plus® machines.

In this study, the prewarming and tapping of cartridges before use independently influenced the agreement of results when compared with cartridges that were not prewarmed and tapped. Each factor (temperature and mixing) when excluded was found to affect the SD and decrease the agreement of results. It is evident that by following the manufacturer's recommendations to standardize sampling technique, ACT Plus® test results are more accurate.

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