Original Article

Activated Clotting Time (ACT) Testing: Analysis of Reproducibility

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ABSTRACT

Activated Clotting Time (ACT) has been the standard for monitoring heparin anticoagulation in cardiac surgery for three decades. Although a 10% coefficient of variation (CV) is the referenced standard for the test, no recent reports of precision are available. The precision of Hemochron FTCA510 (celite) and KACT (kaolin) ACT test tubes was evaluated using a retrospective analysis of results from both laboratory studies and routine clinical usage.

Laboratory studies of reproducibility included analysis of the CV from repetitive testing using multiple lots of ACTs. Substrates used included 40 consecutive lots of control plasma and freshly heparinized donor blood. Across the lots of control plasma, the celite ACT yielded an average CV of 5.4% for the normal control level and 4.0% in the abnormal control level (range 3.6–9.7% and 2.7–6.3%, respectively). The KACT showed similar performance for the normal (mean = 4.5%, range 2.2–7.8%) and abnormal (mean = 3.8%, range 2.0–10.0%). These values, significantly less than 10%, reflect the combined variability of both the ACT tests and the lyophilized, single use vial, control material. Fresh whole blood samples exhibited improved ACT precision when compared to this artificial substrate. CVs for the celite ACT ranged from 0.6–6.0% at one unit heparin/ml blood to 2.4–11.6% at 5 units/ml where clotting times exceed 650 sec. The KACT showed even lower CVs at all heparin levels, with values of 2.4– 7.0%.

Clinical evaluations included samples (N = 56) collected from cardiac surgery patients with celite ACT values ranging to 744 sec. Duplicate values differed by an average of 7.5 sec or 1.8%. There was only one clinically significant difference in paired values; a 376 sec paired with a 406 sec, 400 sec being the clinical target time. This retrospective data analysis demonstrates that Hemochron ACT variability is significantly less than 10%.

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INTRODUCTION

Since its introduction by Hattersley in 1966, the activated clotting time (ACT) has become the standard of care for monitoring heparin anticoagulation (1). The Hemochron®[a] ACT rapidly gained acceptance as the first standardized automated ACT system after its clinical equivalence to the manual method was demonstrated (2–4). Early evaluations of ACT variability have led to the widespread acceptance of a 10% coefficient of variation between ACT replicates. This value was developed from publications (5–7) that describe the difference between replicate ACTs performed both before and after heparin administration. ACT variability was defined by calculating the mean absolute value of the difference between duplicates. This value was then expressed as a percentage of the average of the duplicates (8).

Over the years, several other automated ACT systems have become available for clinical practice. These systems differ from the original in activator types, detection systems, and sample size. As each new system is introduced, the reproducibility of the assay is frequently compared to the 10% "standard" variability expected from an ACT. This study was designed to examine the validity of this 10% value using data that had been collected under standard laboratory and clinical practice conditions.

MATERIALS AND METHODS

To ensure a nonbiased assessment of variability, a retrospective analysis as performed on data previously collected for reasons unrelated to ACT variability, as described in each section below. None of these studies included a reproducibility endpoint at the time of data collection.

LABORATORY EVALUATIONS

All laboratory evaluations were performed as a part of routine quality control programs at the manufacturer's facility.

Control plasma (CPL[a]) reproducibility data were drawn from standard quality control records of 40 sequential lots of either normal or abnormal control material. The CPL product is a single use vial of lyophilized plasma used for routine clinical quality control of the Hemochron ACT. For each lot of CPL examined, 10 vials were tested in each tube type (FTCA510,[a] celite activated, or KACT,[a] kaolin activated) following the manufacturer's directions. These values are routinely collected to develop acceptance ranges for the quality control material when used in the clinical field. Samples were tested as described in the manufacturer's instructions for use.

For heparin dose response analyses, fresh whole blood samples were obtained from a single normal, healthy donor not taking any medications. The samples were spiked with increasing concentrations of beef lung heparin and eight celite and eight kaolin ACT tests were performed at heparin each level. A 2 ml sample was introduced into a room temperature ACT test tube and the test was initiated as described in the manufacturer's directions for use. Heparin dose response curves were generated.

CLINICAL EVALUATIONS

Data were examined from two clinical evaluations. Approval for the collection of these data was obtained from the Human Studies Review Board at the Hospital of the University of Pennsylvania. The clinical evaluations were classified as optimal or typical based upon the degree to which test conditions (number of instruments) and performance (number of operators) were controlled. Standard hospital policy requires that all ACT tests be run in duplicate after pre-warming the test tube for 300 sec. All participating operators were clinical perfusionists who completed training in the appropriate technique for performing an ACT.

Optimal—Single Operator/Single Instrument: A single operator performed 107 paired ACT tests using a single Hemochron 801 dual well instrument. These data were collected across all levels of heparinization, and the individual well on the dual well Hemochron was identified for each test result.

Typical—Multiple Operators/Multiple Instruments: Duplicate ACT values were recorded during the standard monitoring of patients on cardiopulmonary bypass (CPB). During CPB, samples were taken under both normothermic and hypothermic conditions with hemodilution to hematocrits in the 20–26% range. Fifty-six paired values were obtained by multiple (six to eight) operators using multiple Hemochron 801 dual well analyzers. All duplicates were obtained from the two wells in a single instrument.

ANALYSES

For all studies (laboratory or clinical) the CV was calculated as 100 times the standard deviation divided by the mean of the data set in question. For pairwise analyses, where CV is not an appropriate statistical value, the average variance (8) was calculated. This is defined, for duplicate samples A and B, as: 100 * (A − B) / [(A + B)/2]. That is, 100 times the difference between duplicates divided by the average of the duplicates. Frequently, the absolute value of this number (absolute variance) was used to maximize the level of variability observed.

RESULTS

LABORATORY EVALUATIONS

The coefficients of variation were tabulated for 40 consecutive lots of normal control plasma (CPL-N) manufactured between January 1997 and December 1998. These plasmas were tested with a total of 10 different lots of celite and eight lots of kaolin ACT test tubes. All lots of CPL-N tested exhibited mean clotting times between 110 and 160 sec. For the celite based

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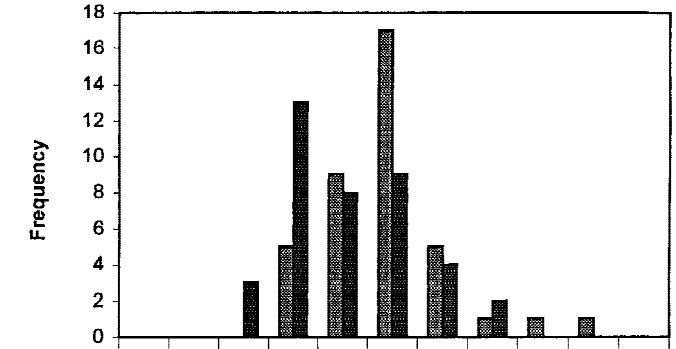
ACT, CVs ranged from 3.5 to 9.7%, average = 5.4% (Figure 1). Only three lots of CPL-N exhibited CVs of greater than 7%. The kaolin ACT tube performance showed an improved precision with a CVs range of 2.2 to 7.8%, average = 4.7% (Figure 1), with no CVs for any of the 40 lots exceeding 8%.

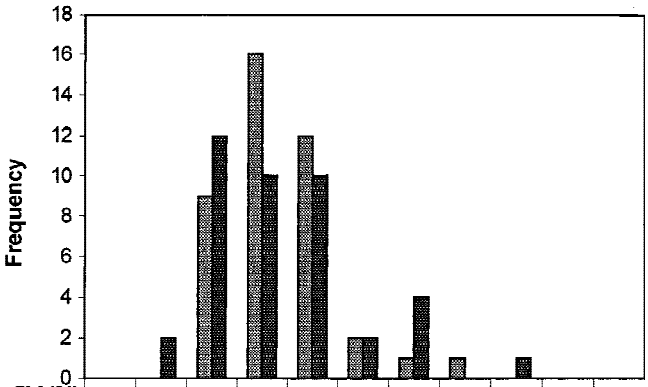
A similar analysis was performed for 40 consecutive lots of abnormal control plasmas (CPLII, Figure 2) that exhibited average clotting times of 270±30 sec. These plasmas, manufactured between February and July 1998, were tested using eight lots of celite ACTs and eight lots of kaolin ACTs. The precision of these control plasmas proved to be better than observed for the normal level controls. An average CV of 4.1 and 4.2% (range 2.1–7.1% and 2.0–10.0%) were obtained for the celite and kaolin activated tests, respectively. Only two lots of CPLII exhibited variabilities exceeding 7% (Figure 2).

Laboratory based reproducibility was also analyzed using freshly collected whole blood from a normal, healthy donor. A total of 16 ACTs, four celite and four kaolin on each of two different instruments, was performed at each heparin level tested. The resulting heparin dose response showed excellent reproducibility at each heparin level (Figure 3). Variability was quantified in two ways (Table 1). First, the absolute variance observed between paired samples (one test on each of two instruments) was analyzed. The over-all absolute variance across all heparin levels is 3.2% for both the celite and kaolin tests. Without calculating the absolute value, the average variance was 0.0 for celite and −2.3% for the kaolin assay. The eight values obtained at each heparin level for each ACT type resulted in CVs ranging from 1.0 to 8.4% (Table 1). The celite assay showed an increase in CV with heparin concentration; whereas, the kaolin test did not.



CV (%)	1	2	3	4	5	6	7	8	9	10	More
FTCA510	0	0	0	5	9	17	5	1	1	1	0
KACT	0	0	3	13	8	9	4	2	0	0	0

Figure 1: ACT reproducibility using normal control plasma. Forty consecutive lots of CPL-N were tested with 10 celite (FTCA510) and 10 kaolin (KACT) ACTs. The CV percentage was calculated for each lot of CPL-N and each tube type. The frequency of each CV value is plotted. The numbers in the figure represent the number of lots found to have a CV percentage in the specified range.



CV (%)	1	2	3	4	5	6	7	8	9	10	More
FTCA510	0	0	9	16	12	2	1	1	0	0	0
KACT	0	2	12	10	10	2	4	0	1	0	0

Figure 2: ACT Reproducibility using normal control plasma. Forty consecutive lots of CPL-II were tested with 10 celite (FTCA510) and 10 kaolin (KACT) ACTs. The CV percentage was calculated for each lot of CPL-II and each tube type. The frequency of each CV value is plotted. The numbers in the figure represent the number of lots found to have a CV percentage in the specified range.

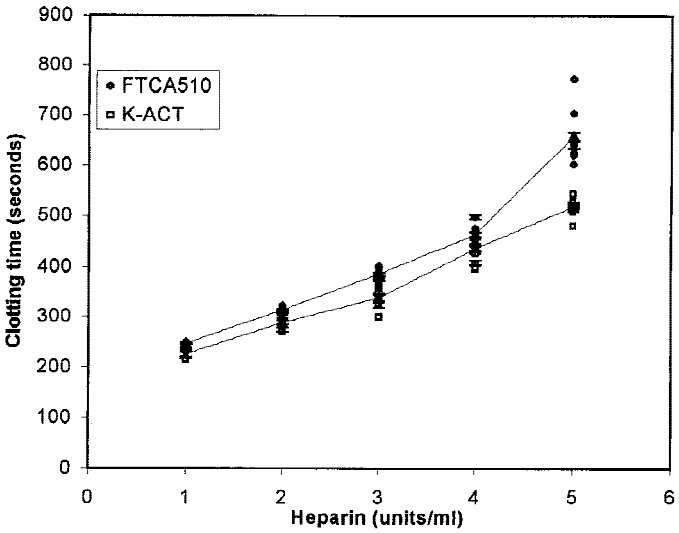


Figure 3: ACT heparin dose response. Heparin, at 1, 2, 3, 4, or 5 units /mL, was added to fresh whole blood samples from a normal, healthy donor. Four celite ACTs and four kaolin ACTs were performed on samples at each heparin level on each of two instruments (N = 16 at each heparin level).

CLINICAL EVALUATIONS

Optimal Conditions: During the clinical evaluation, duplicate celite ACTs were run on patient samples from baseline through high-dose heparin regimens. All tests were performed by a single trained operator (research technician) with values from the top and bottom wells of the single instrument noted.

Table 1: Precision of the celite ACT in a donor heparin dose response assay

	Heparin (units/ml)	Absolute variance (%)	Overall CV (%)
Celite ACT	1	0.6	1.0
	2	1.6	2.5
	3	2.1	3.5
	4	4.9	4.3
	5	5.5	8.4
	Combined	3.2	N/A
Kaolin ACT	1	0.5	4.9
	2	2.3	4.3
	3	5.0	6.6
	4	3.3	5.1
	5	3.7	3.5
	Combined	3.2	N/A

Heparin, at 1, 2, 3, 4, or 5 units/mL, was added to fresh whole blood samples collected from a normal, healthy donor. Four celite ACTs and four kaolin ACTs were performed on samples at each heparin level. The absolute variance for paired duplicates as well as the over-all CV% for the four replicate celite and kaolin tests is presented.

One hundred and seven paired results were collected. The average variance between duplicates was –1.4% (absolute variance = 7.0%, Figure 4). Only one pair showed a clinically important difference in which heparin administration during bypass would be changed by the result of the ACT. There was a small difference in reproducibility between high and low clotting times. The absolute variance for duplicates with an average value between 400 and 1100 sec was 8.9%, for lower ACT pairs (evaluated at both <400 or <250 sec limits) the absolute variance was only 6.0%.

Typical Conditions: Under typical clinical conditions, multiple operators (clinical perfusionists) recorded the duplicate values obtained while patients were on bypass for 56 paired results. Again, only one value showed a clinically important difference. The absolute variance for these data was 5.4% (Figure 5).

DISCUSSION

ACT reproducibility expectations have remained unchallenged for many years, with a stated reference value of approximately 10%. In 1987, during a comparison of the manual ACT, the Hemochron ACT, and the Sonoclot[b] ACT, Sillick and colleagues (5) reported preheparin variabilities ranging from 5.6% (Hemochron) to 7.7% (Sonoclot). After heparin administration, these values rose dramatically to 12.3 and 16.5% for the Hemochron and Sonoclot systems, respectively. Gravlee and co-workers (6) extended these observations with the Hemochron system to include baseline, heparinized, and post-protamine samples. Although these investigators saw reduced variability from that reported by Sillick, they reconfirmed the significant increase in variability observed in heparinized samples (baseline CV = 3.9%, heparin CV = 7.8%, and post-protamine ACT = 3.1%). Most recently, Bennett and Horrow (7) reported duplicate absolute variance values ranging from 6.9 to 12.5%. This study examined a much larger number of samples (683 paired ACTs) than either the Gravlee (138 paired ACTs) or Sillick studies (100 samples per ACT test type). In the Bennett and Horrow report, a 16% incidence of

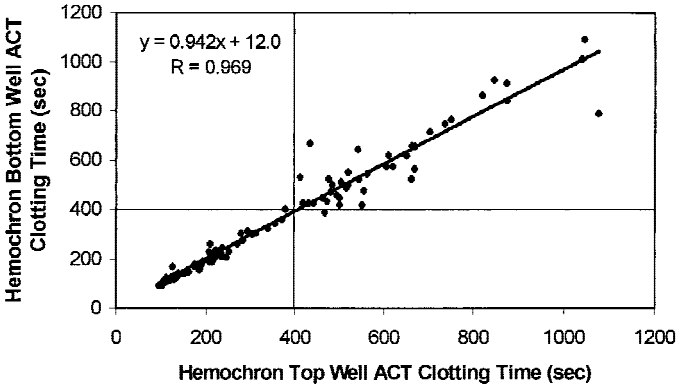


Figure 4: Optimal clinical evaluation. Duplicate ACTs were performed by a single operator for 107 paired patient samples. Records were maintained regarding which instrument well was used for each result. The duplicates showed a high level of reproducibility, with only one clinical difference observed. The horizontal and vertical lines represent the clinical decision point of 400 sec. The one clinical discrepancy is seen as a single datapoint in the lower right quadrant of the graph.

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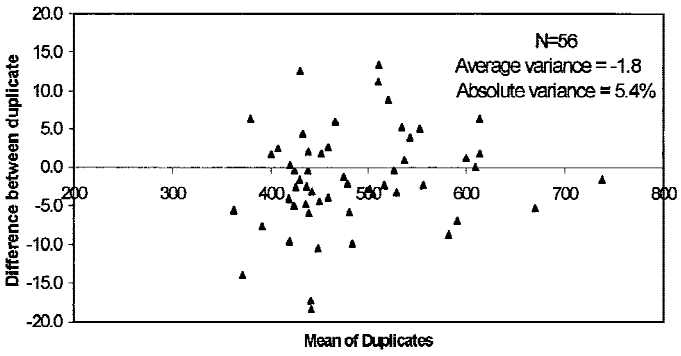

Figure 5: Typical clinical evaluation. Duplicate ACTs were recorded by all perfusionists for 56 patient samplings while on cardiopulmonary bypass. The difference between duplicates is plotted as a function of the average of the duplicates. Although a few samples show large differences (>60 sec), the over-all average absolute difference is only 5.4%.

clinically significant differences was reported based on a 450-sec clinical decision limit.

The data analyzed as part of the present study suggest significantly lower levels of variability in the Hemochron ACT. Reproducibility of control plasma values is frequently used at a clinical site to evaluate the precision of an assay. It is expected that using a lyophilized control will increase the variability of an assay as compared to that observed using replicate analysis of a liquid substrate (e.g., patient or donor blood). This is caused by the variability of the assay (in this study, the ACT) and the additive variability of the reconstitution of the control material. This study revealed that the Hemochron ACT reproducibility, under these conditions, averaged only 5%, half the value anticipated. This high level of precision was verified in an in vitro heparin dose response assay using a single normal donor. Similar results have been obtained in additional single donor heparin dose response analyses (data not shown).

The clinical analyses reviewed for the current study agreed more closely with Gravlee's (6) results than with either Sillick (5) or Bennett and Horrow (7). No major increase in variability was observed in these data after heparin administration. When the datasets from the two clinical studies are combined, the absolute variance for all 163 data pairs was 6.4%. Duplicates averaging less than 250 sec showed an absolute variance of 6.1%, and duplicates greater than 400 sec, 6.7%.

Although this retrospective analysis does indicate differences that might be attributable to single versus multiple operators, an analysis of between-operator variability has not been addressed. Operator differences are known to be an important factor because of the technique-dependent nature of the test tube ACT procedure. For the control plasma data collection, two operators performed five tests each for each lot of CPL. Even with two independent operators, the CVs are in the 4 to 5% range. Although it is likely that the excellent level of precision observed is, in part, attributable to the experience of these operators, these data demonstrate clearly that highly reproducible ACT results are achievable across operators when a standardized technique is employed.

In summary, these data reflect a level of ACT reproducibility that is superior to the historically accepted value of 10%. Advances in technology and operator training are certainly contributing to this improvement in ACT precision. The clinical data collected under typical conditions emphasizes that with appropriate training and operator technique, variances between duplicates of less than 6% are readily achieved. As new ACT test systems become available, precision and variability should be compared to this current clinical standard, which is significantly better than the historical reference value.

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