Observation of a Newly Introduced Bias

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After the introduction of Coulter S-Cal™, a bias became apparent between platelet counts obtained from instruments calibrated with this material and those obtained from a Clay Adams Ultra-Flo®. Statistical methods were used to compare platelet counts obtained from the Coulter S-Plus IV®, Ortho ELT-800®, Clay Adams Ultra-Flo 100®, and phase microscopy. At a P value of 0.01, paired t analysis revealed statistically significant biases between the Ultra-Flo and each of the other methods. Significant biases were also found between phase microscopy and each of the other methods, although these were of a smaller magnitude. The results indicate the necessity for users of multiple platelet counting methods to conduct comprehensive interinstrument evaluations, particularly when altering methods of calibration.

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imens are obtained from a properly calibrated and controlled S-Plus IV. Platelet counts are monitored daily by comparison of two normal ELT-800 platelet counts to the values determined by the Ultra-Flo 100 and phase microscopic examination. A daily interinstrument check is performed by comparing the average of five specimens run in duplicate on the ELT-800 to averages obtained by the S-Plus IVs. Stability of the instrument over a 24-hour period is monitored by periodically comparing the values for two normal specimens to their respective baseline values.

The Ultra-Flo 100, calibrated by the manufacturer, is monitored by three levels of Hematronix Tri-Count® platelet controls and by comparison of two normal platelet counts with the values obtained by phase microscopic examination.

**Methods**

Interinstrument consistency in the normal range was preliminarily assessed by comparison of platelet values obtained by each method. Fifty-four whole blood specimens anti-coagulated with potassium EDTA (Becton-Dickinson Vacutainers®) were run in duplicate on each of the multiparameter instruments (the Ortho and both Coulter instruments) and the Ultra-Flo 100. Phase enumerations on the specimens were performed independently by two technologists. Each technologist made duplicate Unopette® (test 5855, Becton-Dickinson) dilutions of the samples and plated each Unopette on a single Neubauer® hemacytometer. The platelet count for each specimen was determined as the average of the four chamber counts. The mean of the 54 specimens was 244 \( \times 10^9 \) platelets/L with a range of 195-337 \( \times 10^9 \) platelets/L, as determined by phase microscopic examination. For each method pair, the average difference (bias) was determined and a Student's \( t \) value was calculated.

To more fully assess the method differences noted in the above study, linear regression analysis was performed on data collected from one method pair. The ELT, chosen because of its virtual equivalence to either Coulter instrument and its high volume use, and the Ultra-Flo were employed in this second study. A sample of 132 randomly selected specimens that encompassed nearly the entire range of platelet values (64-991 \( \times 10^9 \) platelets/L), was evaluated. Additional specimens were selected to independently evaluate the performance between the two systems at the low (23-117 \( \times 10^9 \) platelets/L) and high (358-991 \( \times 10^9 \) platelets/L) ends of the platelet counting spectrum. A correlation coefficient was also determined for each of the three sets of data.

**Results**

A preliminary study of normal range biases existing between pairs of various platelet counting methods was undertaken. Results of the 54 specimens examined are shown in Table 1. Counts obtained from the multiparameter instruments (ELT-800 and S-Plus IVs) averaged at least 12.8 platelets higher than those obtained by phase microscopic examination. The Ultra-Flo 100 platelet counts averaged 13.8 platelets less than those obtained by phase microscopic examination. As expected, counts obtained from the multiparameter instruments were seen to average at least 26.6 platelets higher than those obtained from the Ultra-Flo 100. At a \( P \) value of 0.01, Student’s \( t \) test results showed that these differences were statistically significant. Differences between paired multiparameter instruments were much smaller, as would be expected because of the method of calibrating these instruments (see “Materials and Methods”).

In addition to \( t \) test analysis of the bias in the normal range, the variability of the individual differences themselves was examined. Each instrument platelet count was compared with the corresponding phase determination and a histogram prepared from the 54 differences obtained. The histograms for all instrument-phase pairs are shown in Figure 1. In each case, the individual differences tended to group about the mean difference, as would be expected in a normal distribution. Extreme values did not appear to greatly influence the mean differences. However, the dispersion of the differences does highlight the fact that a number of specimens would exceed the average difference by a substantial amount. With an average difference of 12.8 \( \times 10^9 \)/L, for example, occasional S-Plus IV counts were observed to exceed corresponding phase counts by 40 \( \times 10^9 \) platelets/L in the normal range.

A second study compared the performance of the ELT-800 with that of the Ultra-Flo over the entire range of platelet values (Fig. 2). Although excellent correlation was found (\( r = 0.98 \) in the overall-determination), linear regression analysis revealed a proportionate bias of at least 10% at all levels, as indicated by the slopes. Linear regres-

Table 1. Instrument Pairs: Average Differences and Student’s \( t \) Values

| Methods       | Average Difference (A-B) | \(| t \)| | Difference Significant |
|---------------|--------------------------|-------|-----------------------|
| S+IVa vs. phase count               | +12.8                     | 5.88  | Yes                   |
| S+IVb vs. phase count               | +17.6                     | 7.57  | Yes                   |
| ELT vs. phase count                | +16.4                     | 6.59  | Yes                   |
| UF vs. phase count                 | -13.8                     | 6.57  | Yes                   |
| S+IVa vs. UF                      | +26.6                     | 16.1  | Yes                   |
| S+IVb vs. UF                      | +51.4                     | 15.4  | Yes                   |
| ELT vs. UF                        | +30.2                     | 15.5  | Yes                   |
| S+IVa vs. ELT                     | -3.6                      | 2.52  | No                    |
| S+IVb vs. ELT                     | +1.2                      | 0.64  | No                    |
| S+IVa vs. S+IVb                   | -4.8                      | 3.12  | Yes                   |

\* \( P = 0.01, n = 54 \), critical value of \( t = 2.67 \).
sion analysis was not performed for the other multiparameter instrument and Ultra-Flo pairs because of the excellent agreement between the ELT and either S-Plus IV indicated by the paired t analysis of normal platelet counts (Table 1).

Discussion

After the introduction of S-Cal, a large bias in the normal range was observed between instruments calibrated with this commercial material and the Ultra-Flo, which was calibrated by the manufacturer and had previously been employed as a reference method in this laboratory. Although the data in Figure 2 reveal excellent correlation between a representative of these instruments (the ELT-800) and the Ultra-Flo, a bias of 27-31 platelets x 10^9/L was noted (Table 1). A similar discrepancy was reported in a CAP survey taken during the same time period (Table 2).^1 (Note that ELT-8/ds, not ELT-800 data was available). This bias is also greater than either the week-to-week coefficient of variation (6.6%)^2 or 90-day coefficient of variation (7.0%)^3 for healthy persons reported by others and thus may be of potential clinical concern. Additionally, it is unacceptable from a laboratory standpoint and can easily be reduced in a number of ways (see below).

The S-Cal calibrated instruments and Ultra-Flo were observed to give approximately equal but opposite biases (absolute values of 12.8-17.6 platelets x 10^9/L in the normal range; 5.2-7.2%, respectively) when compared with manual Unopette phase counts. Short-term bias after calibrating with S-Cal has been noted by others, although the difference was smaller than in the present study.^4 Some have also found a small "insignificant" bias between the Ultra-Flo and phase counts and chose to use that instru-
Inherent differences among various automated platelet counters make necessary careful consideration of calibration methods, especially in laboratories that employ more than one method for routine enumeration of platelets. Performance of multiple phase counts from manual dilutions of whole blood with National Bureau of Standards-certified glassware is the recommended reference method. However, for several years observers have noted the widespread use of a variety of calibration techniques. In this laboratory, S-Cal was recently chosen as an expeditious and relatively accurate method of calibrating the platelet parameter of multiparameter instruments. Because of its waning utility in the face of three other instruments with platelet counting capabilities, the Ultra-Flo was discontinued. Daily phase counts continue to be useful as checks of instrument calibration.

In laboratories that employ more than one method for platelet determination, choice of a calibration method must take into account not only the accuracy, reproducibility, and facility of the method but also its impact on interinstrument variability. Modification of calibration procedures, including cross-calibration of instruments, or reduction of variety of instrumentation within a given institution may be necessary to achieve acceptable interinstrument agreement.

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References