

COMMENTARY

Dr. Bull asserts that results of hematocrit (Hct) measured by centrifugation, whether by macro- or micro- method, deviate from results of Hct obtained by automated hematology instrument (Coulter Counter). On this point we agree. However, it is not logical to argue, as Dr. Bull does, that because they differ, the centrifuged method provides superior results and that the automated method is in error. In fact, Dr. Bull's data (1) clearly show that for specimens with normocytic, normochromic erythrocytes, there is an excellent rectilinear relationship between automated (Coulter) Hct and automated (Coulter) Hb conc. Similarly, I have shown (2) that, for serially diluted blood specimens, there is an excellent rectilinear correlation between automated (Coulter) Hct and automated (Coulter) erythrocyte count (RBC). Furthermore, since there is an exquisitely tight correlation between RBC and Hb Conc. in these serially diluted specimens, it appears that Dr. Bull's data and mine are in perfect concordance. Conversely, my data clearly show that there is not a very close rectilinear correlation between RBC and centrifuged Hct, whether by micro or macro method, in specimens in which erythrocytes were serially diluted in their own plasma before the measurements were made.

Studies of linearity on serial dilution long ago become established as quality assurance criteria in modern laboratory practice, and it might be difficult today to introduce a new test that deviates from linearity as much as does the centrifuged hematocrit. However, because hematocrit became an accepted laboratory procedure more than a century ago, because it is highly reproducible, and because other hematologic measurements were much less precise, linearity studies of hematocrit measurement were not made for almost 70 years. Most of us accepted on faith, as Dr. Bull still does, the validity of the centrifuged Hct values.

Dr. Bull and I agree that the MCV values obtained by automated instruments do not correlate perfectly with the values obtained by

calculation using, in the numerator, the centrifuged Hct value. Dr. Bull prefers to believe that this is because of an artifact in the automated MCV measurement. However, if one examines linearity by plotting MCV vs. RBC in serially diluted specimens, one finds that automated instruments (at least the Coulter Counter---I have not tested other instruments) provide nearly constant MCV values throughout a very wide range of dilutions, whereas the MCV that is calculated using the centrifuged Hct value (whether by macro- or micro- method) is inconstant: it increases or decreases as the RBC increases or decreases, respectively. (These relationships are shown in the following illustrations that are reprinted from reference 2 below.) Dr. Bull may have an alternative explanation for this curious behavior of the manually calculated MCV. However, the simplest and most plausible explanation is that red cells pack more tightly on centrifugation when the RBC (and the hematocrit) is low, and less tightly when the RBC (and the hematocrit) is high. This phenomenon was well-studied in the past, and has been generally recognized for 43 years. It readily explains the non-rectilinearity of the centrifuged Hct, whether by micro or macro method.

In fig. 2 of the article by Crawford, Lau and Bull (ref. 3 in Dr. Bull's commentary, and ref 1 below), the "Automated Hct Error" was plotted on the ordinate against "Manual MCHC" on the abscissa. This graph suggested a rather large error, ranging from -7% to +14%, in the automated Hct measurement. However, the authors erred in failing to simplify these complex variables by removing common factors. In this illustration, they had actually graphed the relationship (automated MCV) X RBC X (1/microhematocrit) = m X (automated MCH) X RBC X (1/microhematocrit), where m represents the slope of the correlation. This is like graphing abc against abd, and then asserting that ab does not equal ab. It is the same kind of error as that illustrated in fig. 6 of our article in *BCMD*. The common factors that they should have removed were RBC X (1/microhematocrit). Upon removal

of these factors, it becomes evident that, in reality, Crawford, Lau and Bull had plotted MCV (automated) on the ordinate vs. MCH (automated) on the abscissa. They demonstrated the expected excellent rectilinear relationship between automated MCV and automated MCH, but they did not demonstrate an “automated hematocrit error,” as they supposed they had. Dr. Bull and I have had extensive private correspondence about this, in which he agreed with my interpretation of this illustration. Graphs do not always portray what they purport to portray. Unwary clinicians and laboratorians are easily misled by inappropriately graphed data. We are all vulnerable to this kind of error.

Of course, automated instruments are calibrated against centrifuged hematocrit values in the normal range. It is critically important that an automated hematology analyzer be properly calibrated against a standard with a known microhematocrit value in the normal range. This calibrates both the automated hematocrit and the MCV. For this reason, it is in the normal range that there is excellent agreement between hematocrit values obtained by centrifugation and those obtained by automated instruments from the calculation $MCV \times RBC$. In severe anemia, the centrifuged Hct value is an underestimation by as much as 30%; in severe polycythemia, the centrifuged Hct value is an overestimation by 5% or more.

The introduction of Coulter Counters placed in our laboratories an extraordinary tool that has enormously improved the quality of all hematology measurements, particularly the primary measurements of RBC, Hb Conc., and MCV, but also of the derived measurements including Hct. I do not denigrate the microhematocrit measurement, but one needs to place it in proper perspective as an essential means for calibrating automated instruments, and nothing more than that.

In the 45 years that I have worked in hematology laboratories, I have never heard that anybody does a “macro” hematocrit by centrifuging for only 20 minutes, as Dr. Bull

seems to think.

Dr. Bull’s recent presentation at the International Hematology Society meeting (ref. 5 in his commentary) was only an attempt to resuscitate the MCHC. Nobody needs it today except as an indicator of quality control within the laboratory. Clearly, the low values found historically in the MCHC were largely artifactual, the result of relatively increased plasma trapping that distorted the centrifuged hematocrit values because of poikilocytosis in severe iron deficiency anemia or thalassemia. Misshapen red cells do not pack tightly on centrifugation.

Some years ago I was disturbed to discover that red cell mass calculations were being made in my laboratory using Coulter Counter hematocrit values. I changed my mind when I saw the excellent results that were being obtained. Empirical observations should not be brushed aside in favor of ancient theories. Rather, theories must be adjusted to accommodate facts.

In the broader context, the issue raised by Dr. Bull is not relevant to the main points of our presentation. It really should not matter greatly whether one uses an automated instrument “hematocrit” or a centrifuged microhematocrit in the calculations. Either should give nearly equivalent results. Although in polycythemic cases one will obtain a slight overestimate of RCM if one uses microhematocrit values in the calculations, and moderate underestimate of RCM in anemic cases, the differences should not be clinically important.

Readers will find the proof of the pudding by tasting it.

REFERENCES

1. Crawford MJ, Lau YR, Bull B. Calibration of Hematology Analyzers. Role of the Microhematocrit. *Arch Pathol Lab Med* 111:324-327,1987.
2. Fairbanks VF. Nonequivalence of Automated and Manual Hematocrit and Erythrocytic Indices. *Am J Clin Pathol* 73:55-61, 1980.

Legends for Figures

Figures 1-3 demonstrate the linearity (or deviation from linearity) of Hct and the constancy (or inconstancy) of MCV for both manual and automated methods using erythrocytes that have been serially diluted in their own plasma. The dilutions spanned a wide range, corresponding to severe anemia and severe polycythemia, including one dilution that would not be attainable in vivo, but which helps to test the linearity of the Hct as a function of erythrocyte count. Most of the Hct measurements were made using macromethod (Wintrobe tubes). However, these observations were confirmed using the microhematocrit method. They were also confirmed using blood from a patient with severe polycythemia vera,

whose erythrocytes were serially diluted in her own plasma. In this case (not illustrated here), the automated (Coulter) MCV ranged 75-77 fL as the erythrocyte count ranged from $1.0\text{-}8.3 \times 10^{12}/\text{L}$, whereas the MCV that was calculated from centrifuged macro Hct ranged from 63-82.5 fL in this same range of RBC. The red cell counts were the same; the striking variability in manual MCV could only have come from non-linearity of the centrifuged Hct.

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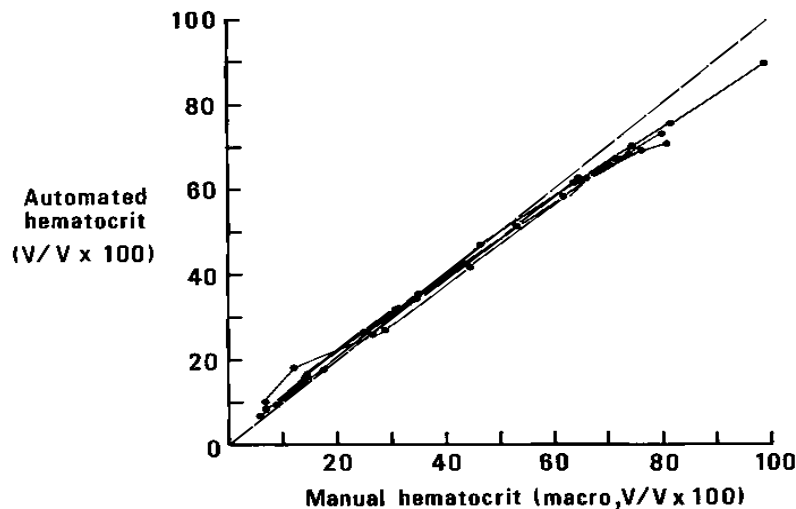


Figure 1. Comparison of automated Hct values with centrifuged Hct values. It shows excellent agreement in the normal range, but significant deviations both above and below the normal range. The interrupted line would represent equality.

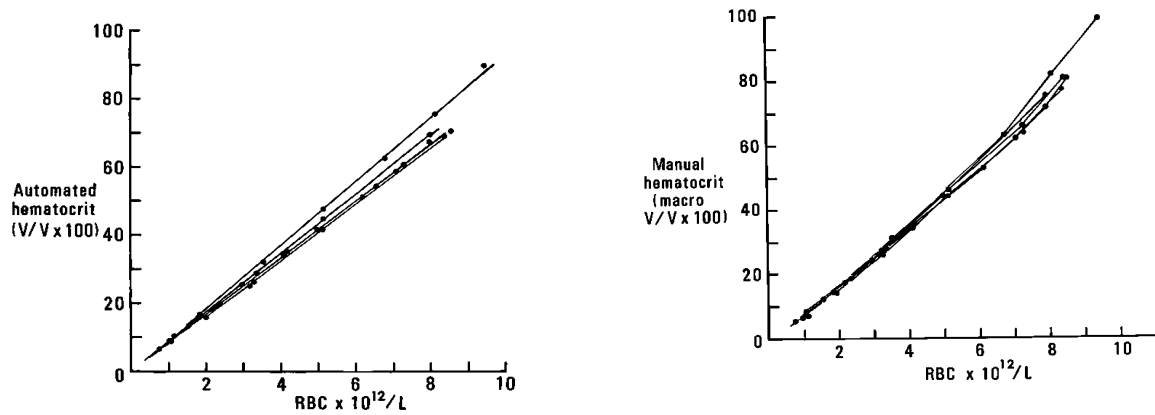


Figure 2. Comparison of the linearity of the automated (Coulter Counter) hematocrit (left panel) and centrifuged Hct (right panel) in relationship to the erythrocyte count. Results with automated Hct are rectilinear; results with centrifuged Hct are not.

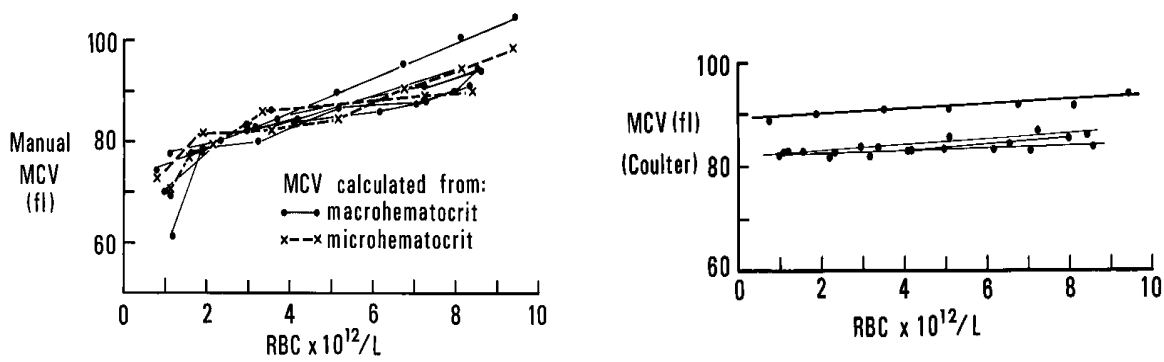


Figure 3. Examination of the constancy of the manual MCV (calculated using centrifuged macrohematocrit and microhematocrit) in the left panel and of the automated MCV in the right panel. The manual MCV is inconstant, varying in direct proportion to the erythrocyte count. If the MCV were constant, the graph would appear as a ladder of horizontal lines, as is nearly true of the automated MCV.

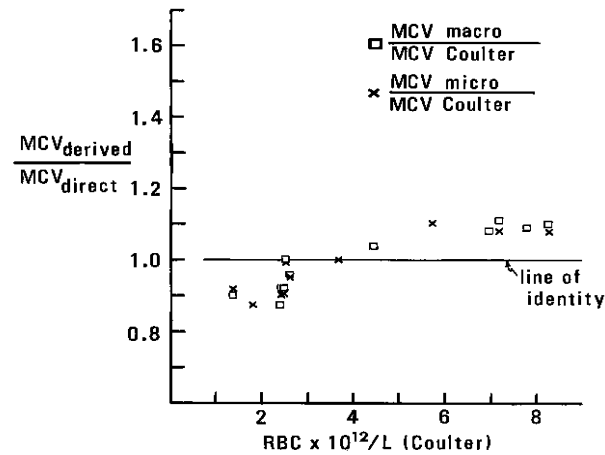


Figure 4. Examination of the ratios of manual MCV (“derived” using centrifuged Hct, both by micro and macro methods) to Coulter Counter MCV (“direct”) as a function of the erythrocyte count for 15 specimens obtained from patients with a variety of disorders including severe anemias and severe polycythemias. (The original data are tabulated in ref. 2) These results were obtained directly from the original specimens without serial dilution. Ideally, the ratio should be 1.0, as shown by the “line of identity.” The ratio deviates from identity by about -10% in anemic cases, and by about +10% in polycythemic cases. These deviations reflect the deviation of centrifuged Hct (macro or micro) from rectilinearity. This is evidence that the phenomena shown in figs 1-3 are not artifacts that resulted from specimen manipulation during serial dilution.

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