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Comparison of Erythrocytic Indices by automated and Manual Methods.

Navapun Charuruks*
Pranee Krailadsiri* Sumpow Punyadilok*

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The manual and automated methods for determining hematocrit (Hct) and mean corpuscular volume (MCV) were compared by using three groups of blood specimens : microcyte (MCV < 80 fl), normocyte (MCV 80-96 fl), and macrocyte (MCV> 96 fl), with each group containing 100 specimens. The average values of Hct by the automated method were 30.2 ± 4.3, 38.1 ± 3.1 and 33.4 ± 3.9% and by the manual method 34.7 ± 5.0, 39.6 ± 3.7, and 36.6 ± 4.4% in all three groups respectively. The average values of MCV by the automated method were 69.8 ± 4.8, 88.3 ± 3.2, and 105.4 ± 4.6 fl, and by the manual method 78.9 ± 5.6, 91.1 ± 3.7, and 112.2 ± 5.5 fl in all three groups respectively. The predicted linearity of Hct and predicated constancy of MCV were evaluated by five randomly selected specimens from each group which had been serially diluted in their own plasma. The automated Hct was a rectilinear function of the erythrocyte count. Whereas the manual Hct value deviated systematically from that of the automated Hct, its correlation with the erythrocyte count was more curvilinear. The automated MCVs were nearly constant throughout the range of dilutions of erythrocytes. The MCVs calculated from the manual Hct values varied strikingly as a function of the latter. This study showed that Hct and MCV by the automated method was more reliable than by the manual method.

Key words : *Manual hematocrit, Automated method, Blood indices.*

Reprint request : Charuruks N, Department of Laboratory Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.
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* Department of Laboratory Medicine, Faculty of Medicine, Chulalongkorn University.

นวพรรณ จารุรักษ์, ปราณี ไกรลาศศิริ, สำเภา ปัญญาดิลก. การเปรียบเทียบค่าดัชนีของเม็ดเลือดแดงที่ได้จากเครื่องมืออัตโนมัติกับค่าที่คำนวณได้จากปริมาณเม็ดเลือดแดงอัดแน่นโดยวิธีปั่น. จุฬาลงกรณ์เวชสาร 2536 พฤษภาคม; 37(5) : 317 - 326

ปริมาณเม็ดเลือดแดงอัดแน่น และค่าดัชนีของเม็ดเลือดแดงที่ได้จากเครื่องมืออัตโนมัติและจากการปั่นด้วยเครื่องปั่นตกตะกอนเม็ดเลือดแดง ถูกนำมาศึกษาเปรียบเทียบกันโดยใช้ตัวอย่างเลือด 3 กลุ่มๆ ละ 100 ตัวอย่าง กลุ่มที่หนึ่งเป็นกลุ่มที่มีค่าเฉลี่ยของปริมาตรของเม็ดเลือดแดงต่ำกว่าปกติ คือ น้อยกว่า 80 fl, กลุ่มที่สองเป็นกลุ่มที่มีค่าเฉลี่ยของปริมาตรของเม็ดเลือดแดงปกติ คือ 80-96 fl, และกลุ่มที่สามเป็นกลุ่มที่มีค่าเฉลี่ยของปริมาตรเม็ดเลือดแดงสูงกว่าปกติ คือมากกว่า 96 fl พบว่าค่าเฉลี่ยของปริมาณเม็ดเลือดแดงอัดแน่นโดยวิธีปั่นมีค่าสูงกว่าค่าเฉลี่ยที่ได้จากเครื่องอัตโนมัติเท่ากับ 30.2 ± 4.3, 38.1 ± 3.1, และ 33.4 ± 3.9% ตามลำดับ และที่ได้จากวิธีปั่นมีค่าเท่ากับ 34.7 ± 5.0, 39.6 ± 3.7, และ 36.6 ± 4.4% ตามลำดับ ส่วนค่าเฉลี่ยของปริมาตรของเม็ดเลือดแดงที่ได้จากเครื่องอัตโนมัติเท่ากับ 69.8 ± 4.8, 88.3 ± 3.2 และ 105.4 ± 4.6 fl ตามลำดับ และที่ได้จากการคำนวณจากวิธีปั่นมีค่าเท่ากับ 78.9 ± 5.6, 91.1 ± 3.7 และ 112.2 ± 5.5 fl ตามลำดับ และเมื่อสุ่มตัวอย่างจากแต่ละกลุ่มมากลุ่มละ 5 ตัวอย่าง เพื่อศึกษาถึงความสัมพันธ์ระหว่างค่าเม็ดเลือดแดงอัดแน่นที่ได้จากวิธีทั้งสอง และค่าเฉลี่ยปริมาตรเม็ดเลือดแดงเปรียบเทียบกับจำนวนเม็ดเลือดแดงที่มีความเข้มข้นต่างๆ กัน ซึ่งเกิดจากการเจือจางแต่ละตัวอย่างด้วยน้ำเลือดของตนเอง พบว่าความสัมพันธ์ระหว่างค่าเม็ดเลือดแดงอัดแน่นกับจำนวนเม็ดเลือดแดงที่ความเข้มข้นต่างๆ กัน โดยการใช้เครื่องอัตโนมัติเป็นเส้นตรงในขณะที่โดยวิธีปั่นเป็นเส้นโค้งกว่า ส่วนความสัมพันธ์ระหว่างค่าเฉลี่ยปริมาตรเม็ดเลือดแดงกับจำนวนเม็ดเลือดแดงที่มีความเข้มข้นต่างกัน โดยการใช้เครื่องอัตโนมัติเกือบเป็นเส้นขนาน ในขณะที่โดยวิธีปั่นมีความแตกต่างกันมากกว่า การศึกษานี้สรุปได้ว่า การหาค่าเม็ดเลือดแดงอัดแน่นและค่าดัชนีของเม็ดเลือดแดงโดยการใช้เครื่องอัตโนมัติมีความน่าเชื่อถือกว่าโดยวิธีปั่น

In 1890, Hedin[1,2] introduced a reliable and reproducible technique for the determination of hematocrit (Hct), the portion of the blood occupied by erythrocytes. Laboratories for hematologic study were carefully standardized and developed for measurement of Hct, hemoglobin concentration (Hb), and erythrocyte counts of venous blood.[3-5] A system for classification of anemia was adopted, based on the erythrocytic indices such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Erythrocytic indices were calculated from Hct, Hb, and erythocyte counts. Hct is expressed as a percentage, or as a ratio in the International System of Units (SI sytem). Hct may be determined on a "macro" scale[3-8] using relatively low-speed centrifugation in tubes 3 mm in diameter, or on a "micro" scale,[3-7] using capillary tubes and high-speed centrifugation. In 1955, Coulter introduced an electronic blood counting machine, which it claimed could provide more accurate results. The value of Hct is derived from direct measurement of red cell size and red cell numbers.[9] Even though electronic cell counting methods are now widely used and believed to permit accurate results, the manual micro method is still preferred because it is cheap and easily performed. Becaused the value of these contributions significantly influences the results of therapy and because of the discovery of discrepancies between the manual and automated hematocrit and erythocytic indices,[1,10] the results obtained with both methods were compared, studied and evaluated.

Materials and Methods

Three hundred blood samples were collected from specimens sent to the laboratory of the Medical Department, Chulalongkorn Hospital, during the period 17 August to 30 September 1992. All samples were anticoagulated with potassium EDTA (1.3 mg/ml) and studied on the same day. The samples were divided into three groups of 100 each : the microcyte group comprising those who had automated MCV of less than 80 fl, the normocyte group comprising those who had automated MCV of 80-96 fl, and the macrocyte group comprising those who had automated MCVs of more than 96 fl.

The study was separated into two parts. Each part was duplicated and the average values were used for evaluation. The first part compared the automated Hct and MCV method with the manual microhematocrit method and calculated MCVs in each group. In the second part, five samples from each group were randomly chosen for serial-dilution studies. Whole blood was then centrifuged at 1,500 x g for 20 minutes at 5°C by refrigerated centrifuge (Omnifuge 2.0 RS). Supernatant plasma was removed. Then packed erythrocytes (PRC) were resuspended in their own plasma in proportion to provide erythrocyte counts ranging from approximately 1 to 9 x $10^6/\mu l$.

Example : for making serial-dilution erythrocyte (providing counts ranging from approximately 1 to 9 x $10^6/\mu l$ for the samples) counts of 5x $10^6/\mu l$ and Hct 40%, the PRC, with Hct being about 80T[11] was made from whole blood by refrigerated centrifuge at 1,500 x g, for 30 min at 5°C. For providing erythrocyte counts ranging from approximately 1 to 9 x $10^6/\mu l$, the PRC-to-plasma ratios were 1:9, 1:4, 3:7, 2:3, 1:1, 3:2, 7:3, 4:1, and 9:1, respectively

In these specimens, the Hb, Hct erytrocyte count, and indices had been determined by Tecnicon HI analyzer, and the results were compared with centrifuged Hct, and calculated MCV. Comparisons of the two methods were made on the same day, within, at most, one or two hours, i.e. the time required for processing the specimens. Calibration for the automated method, Technicon Hl analyzer, was checked several times daily in accordance with the manufacturer's instructions. Using this method, erythrocytes were counted directly electronically; MCV was measured as a function of laser scattering; the Hct value was calculated from the product of MCV multiplied by the erythrocyte count; the Hb was measured by the cyanmethemoglobin method.[12-14]

Centrifuged Hct values were obtained by microhematocrit tubes 75 mm in lenght, and 1 mm im diameter. These were centrifuged at 1,300 x g for 5 min, by Haemofuge A. Erythrocytic indices determined by the Technicon H-1 instrument were employed without further calculation by dividing the centrifuged Hct values by the automated erythrocyte counts. The desirability of calculating MCV from manually determined erythrocyte counts was considered, because manual erythrocyte counts have been noted to be imprecise.[3,5,6,7,10,12,15] Imprecise values would introduce poor results that would make interpretation difficult. Therefore, automated erythrocyte counts were used as the denominator in calculating the manual MCV values.

The data for comparing the results of the manual and automated methods were analyzed by statistical analysis by using the chi-square test, and the upaired student's-t-test.[1,2,6,7,10,12,13,15-27] Factors with p-values <0.05 were considered statistically significant. For the second part, the relationship between the two methods was subjected to analysis by using correlation study, r as the coefficient of linear correlation between two variables. If $r = \pm 1$, then all the points in the scattergram line would form a straight line, i.e., there would be a perfect linear relationship between those two variables. If $r = 0$, there would be no linear relationship.[16,27]

Results

According to the data (Table 1), the first part of this study showed a significant fifference between automated values and manual values ($p<0.05$). The automated Hct and MCV values were 4.5, 1.5, and 3.2%;

and 9.1, 2.8, and 6.8 fl lower than those derived from the manual method in the microcyte, normocyte, macrocyte groups, respectively. These results meant 28% of the microcyte group had normally calculated MCVs and 3% of the normocyte group had been counted as macrocytes. In contrast, the discrepancies between both methods for Hct nad MCV were highest in the microcyte group and were lowest in the normocyte group.

Table 1. Comparision of automated Hct & MCV with manual Procedure.

Indicies / methods	Microcyte (n=100)	Normocyte (n=100)	Macrocyte (n=100)
Hct (%) = x^+ SD			
Automated	30.2 ± 4.3	38.1 ± 3.1	33.4 ± 3.9
Manual	34.7* ± 5.0	39.6* ± 3.7	36.6* ± 4.4
MCV (fl) = x ± SD			
Automated	69.8 ± 4.8	88.3 ± 3.2	105.4 ± 4.6
Manual	78.9* ± 5.6	91.1* ± 3.7	112.2* ± 5.5

* Statistical significance at $p < 0.05$

In the second part of the study, the relationship between automated Hct and the serial dilution of the erythrocyte count was directly proportional and shown as a perfect linear function with $r_i = 1$ in all three groups (Figure 1). While the correlation between the centrifuged Hct and serial diluted erythrocyte count was more curvilinear, with r = 0.9 in all three groups (Figure 2), thus suggesting an exponential relationship, that is, Hct values were disproportionately higher when the erythrocyte count was high, at Hct values between 10 and 50%, erythrocyte counts between 1 and 5 x 10^6/µl in normocytes and Hct between 10 and 40%, and erythrocyte counts between 1 and 4 x 10^6/µl in microcytes and macrocytes, the relationship was nearrly linear. The MCVs were measured directly by Technicon H1 analyzer; they remained nearly constant throughout the range of dilutions of erythrocytes, increasing only a few precentage points with increasing erthrocyte counts (Figure 3), thus, automated MCV values are nearly constant with the serial dilution of erythrocytes. In contrast, the MCVs derived from the centrifuged HCT were strikingly inconstant (Figure 4).

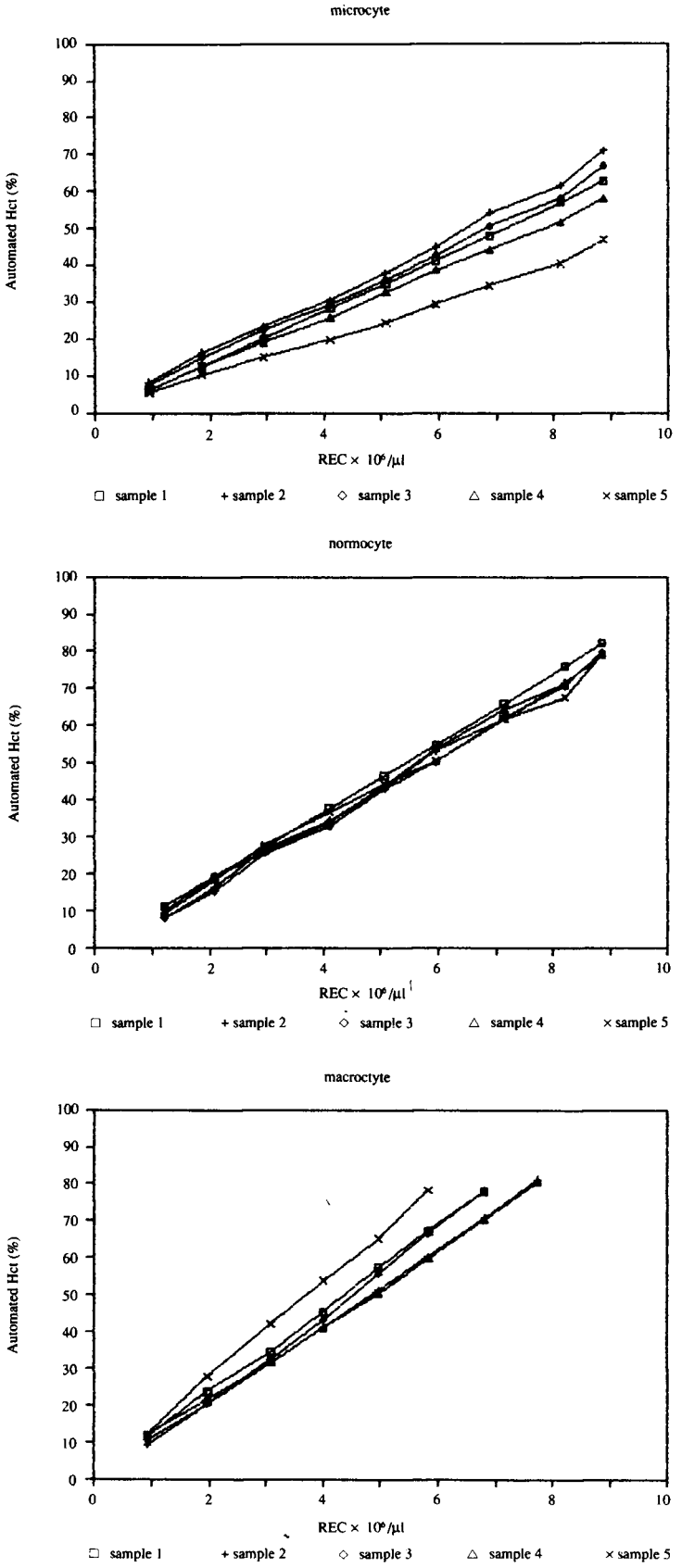

Figure 1. Linearity correlation between automated Hct and serial dilution of Rbc in MICROCYTE, NORMOCYTE, and MACROCYTE

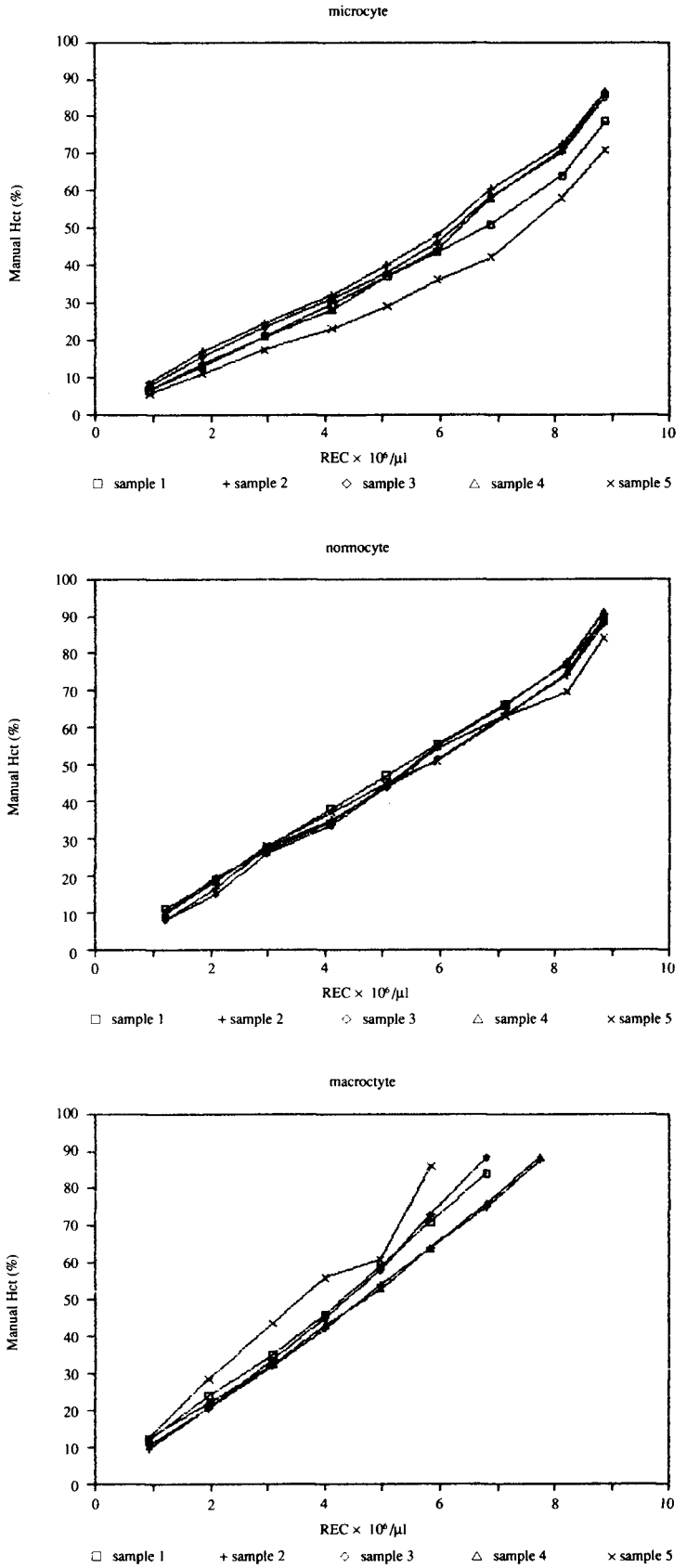

Figure 2. More curvilinear correlation between manual Hct and serial dilution of Rbc in MICROCYTE, NORMOCYTE, and MACROCYTE.

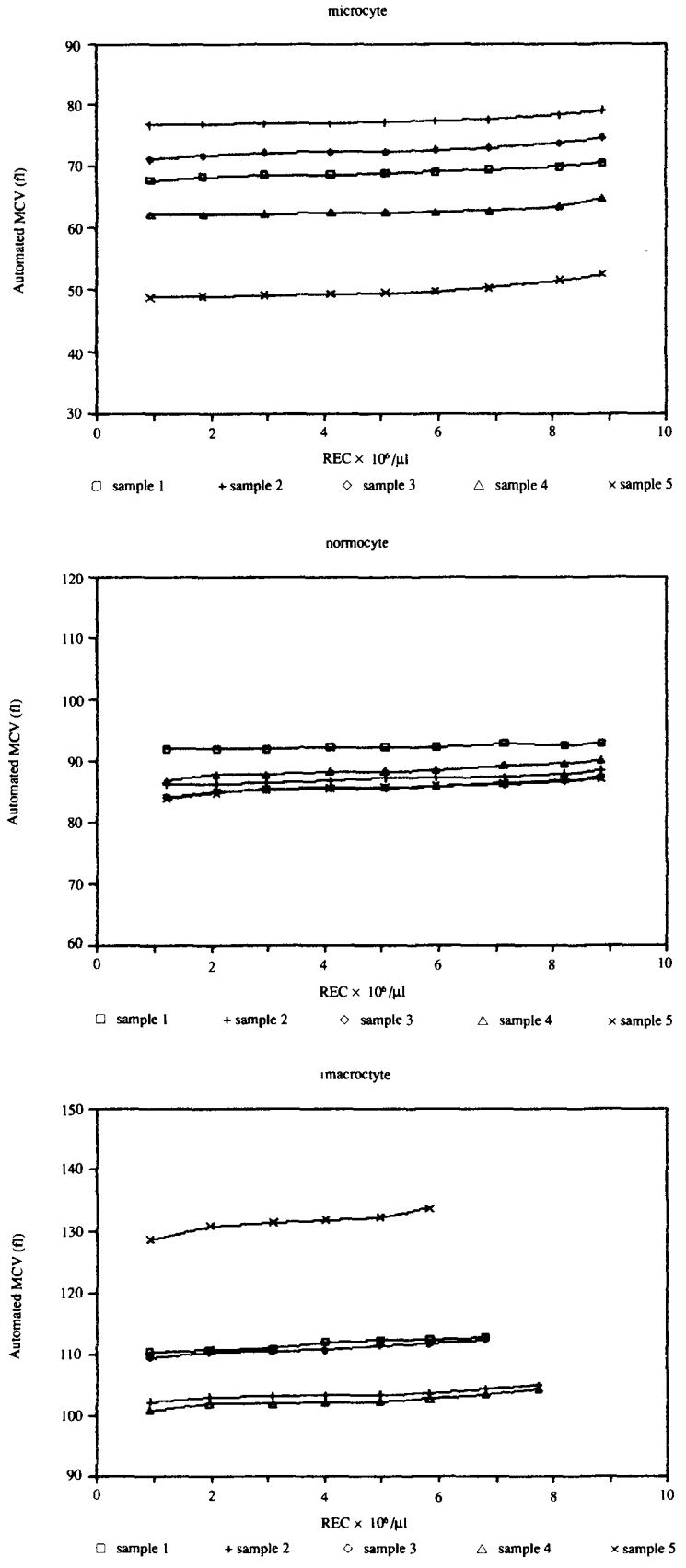

Figure 3. Nearly constant relationships of automated MCV to erythrocyte count in MICROCYTE, NORMOCYTE, and MACROCYTE.

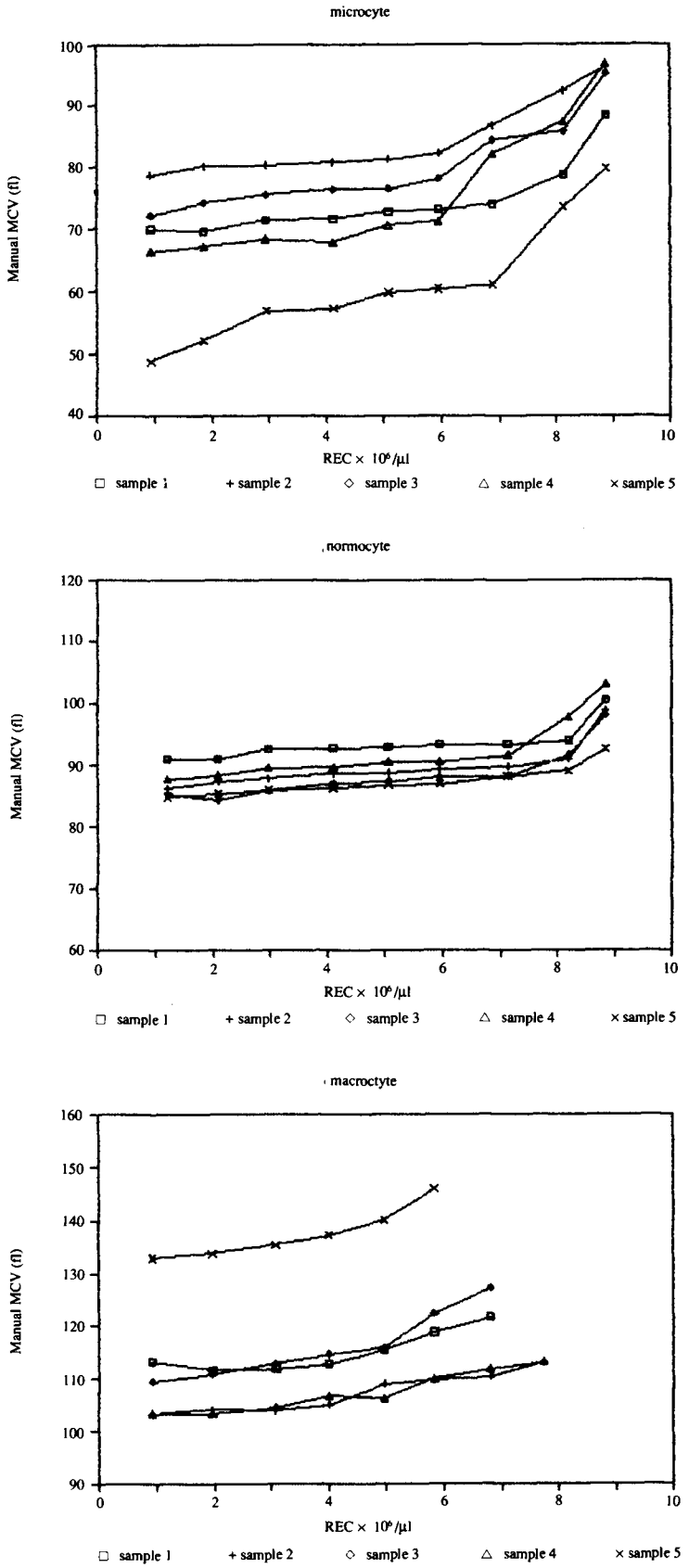

Figure 4. Inconstant relationship of manual MCV to erythrocyte count in MICROCYTE, NORMOCYTE, and MACROCYTE.

Discussion

The results of the study showed that in all three groups the automated Hct and MCV values were lower than those obtained by the centrifuged microhematocrit method. The discrepancy between both methods was highest for microcytes, and lowest for normocytes, i.e. the discrepancy was higher in abnormal red blood cells than in normal ones. England et al.[1] studied the reliability of the hematocrit and found that in the microhematocrit the quantities of plasma trapped between the centrifuged red cells produced a falsely high hematocrit and MCV. They also showed that in normocytes the trapped plasma accounts for approximately 3% of the values; slightly higher values are found in macrocytic anemia, and values up to 5.6% higher in hypochromic anemia, and even 20% higher in sickle cell samples of blood. Owen and Power[25] showed that plasma trapping was non-uniform within the Hct column of the centrifuged blood. Chaplin et al.[18] and Ebaugh et al.[19] showed that the degree of intercellular plasma trapping was porprotional to the apparent Hct value. Two factors influence the trapped plasma value. The first is the intracellular factor, i.e. red bllod cell morphology.[4,6,11] Furth[20] showed that, in patients with hereditary spherocytosis, plasma trapping was relatively more common than in normal subjects. Heseltine et al.[22] found that the discrepancy appeared to be related to erythrocyte water content so that intracellular factor was greater in subjects with a high level of cell water. The secondly is the extracellular factor, i.e. plasma osmolality and erythrocyte count.[1,4,6,11,25] Even the centrifuged method, especially the microhematocrit method, was claimed to reduce trapped plasma better that the macrohematocrit method. The amount of trapped plasma in the red blood cell column of the microhematocrit was also dependent on the centrifugal force and the time of centrifugation.[1,10,25]

Summary

In this study, the correlation of manual and automated Hct and MCV values with serially diluted erythrocytes showed that automated Hct and MCV were moresensitive and reliable that the manual method. The automated results are essentially uninfluence by hemodilution (anemia), hemoconcentration (erythrocytosis), or even the size of red blood cells. However, manual methods are still widely employed throughout the world. including in Thailand. Fortunately, the result of the second part of this study showed that, when the manually obtained Het values between 10 and 50%, with erythrocyle counts between 1 aand 5 x 10^6 µl in normocytes, and between 10 and 40%, with erythrocyte counts between 1 and 4 x 10^6 µl in microcytes and macrocytes, the relationship was nearly linear. This meants that, in most of the specimens, especially abnormal microcytic and maacrocytic erythrocytes which usually occur with anemia, Hct values were lower than 36% and still in good correlation. Thus, the results obtained fromthe manual method could be used with precaution and careful interpretation.

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