

# Is the Packed Cell Volume (PCV) Reliable?

BRIAN S. BULL, KAREN L. HAY

*School of Medicine, Department of Pathology and Human Anatomy, Loma Linda University, Loma Linda, California*

## ABSTRACT

The reliability of the packed cell volume (PCV) has received far less scrutiny than that given to the reliability of hemoglobin and red cell count measurements. The availability of a reference PCV method allowed us to examine 12 different capillary tubes available from scientific supply houses. Our goal was to determine if PCVs obtained in these tubes were of adequate accuracy to allow the PCV to perform its calibration and quality control functions in the clinical laboratory. We confirmed that several sources of error exist in the routine laboratory performance of the PCV. Fortunately, these errors compensate for each other in the usually encountered hematocrit range. The majority of the tubes with internal diameters >1.0 mm, when used with normal blood samples, produced PCV results with biases of less than half a PCV unit. Of the 12 brands of capillary tubes evaluated, 10 were deemed “acceptable” by comparison to reference methods. Using these 10 acceptable tubes, the mean bias at PCV 0.3 was  $0.004 \pm 0.0011$  (95% confidence limits); mean bias at PCV 0.4 was  $0.001 \pm 0.0012$ ; and mean bias at PCV 0.5 was  $-0.002 \pm 0.0014$ . The cards commonly used to read PCV values introduce significantly more irreproducibility. The smallest marked interval (0.01) commonly used on PCV reading cards is 5 to 10 times greater. *Lab. Hematol.* 2001;7:191–196

**KEY WORDS:** Packed cell volume · PCV · Hematocrit · Reference method · Reliability

Correspondence and reprint requests: Brian S. Bull, MD, School of Medicine, Department of Pathology and Human Anatomy, Loma Linda University, Loma Linda, CA 92354 (e-mail: bbull@som.llu.edu).  
Received July 20, 2001; accepted July 24, 2001

## INTRODUCTION

The measurement of the packed cell volume (PCV), otherwise known as the hematocrit, is so useful in any hematologic workup that its presence in hematology test panels is taken for granted. It is also a linchpin of quality control programs in the hematology laboratory. Despite this importance, it has received far less scrutiny from the standpoint of its reliability than have the measurements of hemoglobin or red cell counts.

The trapping of white cells or plasma will erroneously increase the spun hematocrit; this much is obvious. If, however, the vast majority of the plasma escapes from the red cell column during centrifugation and only a few white cells fail to relocate to the buffy coat overlying the red cells—and this does seem to be the case—what other errors can affect reliability of the spun hematocrit? Surprisingly, to the extent that the reliability of the spun hematocrit can be questioned it is because it virtually always underestimates the true packed cell volume of the red cells, even though the errors referred to above would seem to make overestimation more likely.

The reliability of a particular laboratory method can be discussed meaningfully only if a reference method exists against which the particular working method can be compared. A PCV reference method [1] that can be performed in any laboratory capable of performing a reference hemoglobin determination [2,3] has now been described. The existence of this PCV reference method has made it possible to determine the presence and the extent of errors in the spun hematocrit as it is routinely performed in the hematology laboratory. For the sake of clarity, the PCV reference method will be referred to as the PCV; the routine laboratory method will be called the spun microhematocrit.

## MATERIALS AND METHODS

The reference PCV procedure [1] was used for comparison with the spun microhematocrit. The reference PCV method, in brief, consists of removing a few microliters of

Capillary Tubes Used in the Study\*

	Source	Reorder #	Item Name	Specifications (mm)		
B	Allegiance Healthcare, McGaw Park, IL	B4416-1	S/P Microhematocrit capillary tubes (soda lime glass)	Soda lime glass	ID	1.0-1.2
					Length	75
F	Allegiance Healthcare, McGaw Park, IL	B4416-10	S/P Microhematocrit capillary tubes (borosilicate glass)	Borosilicate glass	ID	1.0-1.2
					Length	75
A	Becton Dickinson, Franklin Lakes, NJ	1031	Yankee micro-hematocrit tubes	Soda lime glass	ID	1.10
					Length	75
I	Becton Dickinson, Franklin Lakes, NJ	36-1025	Plastic clad micro-hematocrit tubes	Soda lime glass (mylar-clad)	ID	1.1
					Length	75
E	Drummond Scientific, Broomall, PA	1-000-7500-C	Hemato-Clad tubes (Mylar wrapped)	Soda lime glass (mylar-clad)	ID	1.1-1.2
					Length	75
X	Drummond Scientific, Broomall, PA	1-000-7510	Special collection tube, 75 mm (Reference PCV tube)	Borosilicate glass	ID	1.55 ± 0.85
					Length	75 ± 0.5
D	Drummond Scientific, Broomall, PA	1-000-7520	Capillary tubes for microhematocrits	Soda lime glass	ID	1.1-1.2
					Length	75
L	Drummond Scientific, Broomall, PA	1-000-3200	32 mm Micro-hematocrit tubes	Soda lime glass	ID	0.6
					Length	32
H	Fisher Healthcare, Houston, TX	22-274912	SafeCrit Micro-Hematocrit Tubes (Plastic)	Polycarbonate	ID	0.89
					Length	75
J	Sysmex, Long Grove, IL	—	Platelet Sampling Set (plastic tubes and pipetting bulbs) (nonhematocrit tube)	Plastic	ID	0.9
					Length	75
G	VWR Scientific, West Chester, PA	15401-650	VWRbrand Micro-Hematocrit Capillary tubes	Soda lime glass	ID	1.3-1.5
					Length	75
K	VWR Scientific, West Chester, PA	48300-456	Select capillary tubes	Soda lime glass	ID	1.1-1.2
					Length	75

\*All tubes were plain and contained no anticoagulant.

packed red cells from the middle of a properly centrifuged red cell column and dividing the hemoglobin value measured on this sample of completely packed red cells into the hemoglobin value of the well mixed original whole blood sample. Although it seems counterintuitive, the result of this hemoglobin ratio is the PCV of the original blood sample, as can be seen from the following:

$$MCHC = \frac{Hb}{PCV} \tag{1}$$

Hence,

$$PCV = \frac{Hb}{MCHC} \tag{2}$$

However, because the red blood cells taken from the middle of the properly centrifuged red cell column are fully packed and the PCV of this aliquot is 1.0, the MCHC of this aliquot is therefore identical to the hemoglobin of this same aliquot (from Equation 1).

Hence (from Equation 2),

$$PCV = \frac{Hb \text{ (original well-mixed blood sample)}}{Hb \text{ (fully packed red cell sample)}} \tag{3}$$

Minor corrections for dilution are necessary because the sampled volume for the packed red cell hemoglobin (typically 6.66 μL) is less than that of the standard whole blood hemo-

globin (typically 20 μL). Aside from these manipulations, the reference PCV is simply the ratio of the 2 hemoglobin values. The values that this method yields are as accurate as the reference hemoglobins on which they are based.

Microhematocrit (and 1 nonhematocrit) capillary tubes were obtained from several manufacturers in a variety of materials and sizes, as shown in the Table. Blood samples from hospital inpatients and normal donors were used. Residual patient samples from the hematology laboratory were obtained after all ordered testing was complete. Blood samples from healthy adult donors were drawn after receiving appropriate informed consent. All samples were collected into Vacutainer blood collection tubes anticoagulated with K3EDTA (Becton Dickinson, Franklin Lakes, NJ). Mixing was achieved by at least 12 full inversions either by hand or using a Lab-Tek Aliquot Mixer (Miles Laboratories, Elkhart, IN).

Unless otherwise indicated, duplicate microhematocrit capillary tubes were filled with blood about three-quarters full, and one end of each tube was sealed with S/P Miniseal Capillary Tube Sealant (Allegiance Healthcare, McGaw Park, IL). Tubes were placed in numbered slots of an IEC Model MB microhematocrit centrifuge (International Equipment, Needham Heights, MA) and centrifuged for 5 minutes. The rpm of the centrifuge head was certified to produce approximately 16,000g at the tube periphery by reference to an electronic stroboscope (Strobotac Type 1531; QuadTech,

Maynard, MA). Microhematocrit values were calculated after examining each capillary tube under 78× magnification, using a vernier scale to determine the plasma/air, leukocyte/erythrocyte, and erythrocyte/clay interfaces. The calculated hematocrit values were then compared to the reference PCV values and the acceptability of each type of capillary tube was determined as described in the Reference PCV Procedure [1]. The data were analyzed using Analyse-It for Microsoft Excel, a software package distributed by Analyse-It Software, Leeds, UK.

## RESULTS

The relationship of the underestimation error of the spun PCV compared with the reference PCV throughout the tested range is shown in Figure 1. As can be seen from comparison to the line of identity for the reference PCV, the various spun microhematocrits slightly overestimated the true PCV at hematocrits below approximately 0.3, and consistently underestimated the reference PCV at hematocrits of 0.4 and above. Two tubes—tubes J and L (Table; Figure 1)—were deemed unacceptable because the bias of their regression lines at a PCV of 0.6 exceeded the ICSH specifications of  $\pm 0.01$  [1]. The biases of the other tubes, although not large, were consistent. Underestimation at the high hematocrit levels was most pronounced—approximately 0.02 PCV units, or a little more than 3%—in the capillary tube with the smallest bore. Thus, in the centrifugation of a blood sample, there appear to be forces at work that shrink the length of the red cell column relative to the plasma volume. Whatever the cause of this phenomenon, it is more apparent in longer red blood cell columns (higher hematocrits) and tubes of narrower bore.

A consideration of possible causes for these findings raised the question whether red cells were being sorted according to density in the lower portion of the microhematocrit tube or whether they simply became more dehydrated near the bottom because the  $g$  force was greater there. Figure 2 provides an answer to this question, in the results of an experiment in which 3 large-bore (1.55×75-mm) borosilicate capillary tubes were filled to different heights from each of 30 blood samples. After centrifugation, the midpoint of each red cell column was etched. The tubes with the longest blood column were also etched at the 20% and 85% positions, as measured down from the white cell–red cell interface. Mean cell hemoglobin concentrations (MCHCs) were measured at each of these etch points. As can be seen in Figure 2, all 5 MCHCs show a linear relationship with respect to aspiration position (A) but not with respect to  $g$  force (B). It seems clear that red cells are, in fact, being sorted by density quite effectively during the brief time period before all of the plasma has been squeezed out of the red cell layer.

If the entire length of the red cell column is sampled from tubes completely filled with high-hematocrit blood, the full range of the MCHC distribution can be measured. In Fig-

ure 3, the results from 2 donors [4] are presented. The MCHC is lowest just below the upper meniscus of the red cell column where white cells and trapped plasma are most likely to be found. The MCHC rises more rapidly (the curve is steeper) for the first few samples. It then settles down to a straight line for the midportion of the red cell column as the samples come from progressively deeper layers. Finally, just above the clay seal, the MCHC begins to rise more rapidly again, eventually reaching levels of 38% and 42%. These levels are surprising, for they are quite unphysiological. Red cells with MCHCs this high are too rigid to travel through the microcirculation and when they occur in the circulation are rapidly removed by the spleen.

## DISCUSSION

The determination of the hematocrit by centrifugation is not as simple as it seems to be. It would seem to be little more than the rapid sedimentation, under a high  $g$  force, of solid particles suspended in fluid. The result quantitates the relative proportion of solids (red cells) and liquid (plasma) in the sample. The PCV measurement is much more complicated than this, largely because the red cells are not solid. Rather, they are highly flexible membrane sacs filled with a viscous hemoglobin solution. When sedimented under a high  $g$  force, they first move toward the bottom of the container. They are heavier than plasma because the hemoglobin protein they contain is present in higher concentration than the protein in the suspending plasma. Having reached the top of the developing red cell deposit, individual red cells continue to travel further into the deposit until they reach a level where they are of the same density as the surrounding cells. Clearly, the heavier the cell, the further it must travel through the thickening deposit. The deposit of red cells, meanwhile, becomes less and less fluid as the small amount of remaining plasma is squeezed toward the red cell–plasma interface.

Given that the red cells relocate in the red cell column, eventually forming a density gradient of low to high MCHC, it is not surprising that in normal blood the plasma also relocates upward, virtually all escaping the red cell column. The only measurable plasma that remains below the red cell–plasma interface in a properly centrifuged microhematocrit capillary tube is the small amount that surrounds the few white cells that are entrapped in the upper portion of the red cell column. These white cells are rigid in comparison to the surrounding red cells, and plasma remains behind to fill the nooks and crannies associated with them.

The unwanted white cells below the upper red cell meniscus and the small amount of plasma that accompanies them increase the measured length of the red cell column. Thus the average spun microhematocrit overestimates the reference PCV. This finding is limited to samples of low hematocrit, however. What is entirely unexpected is that this overestimation decreases to zero somewhere in the range of normal

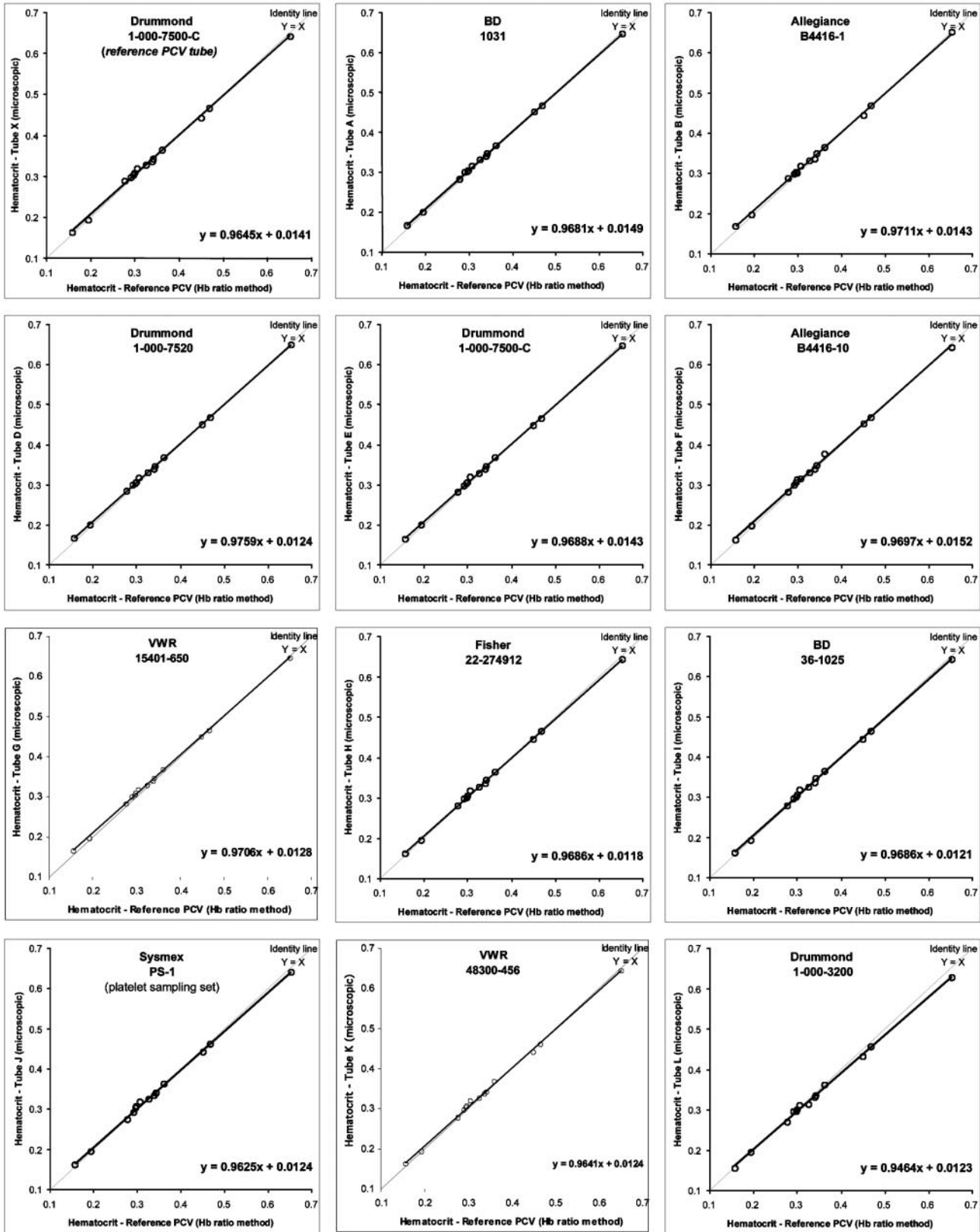
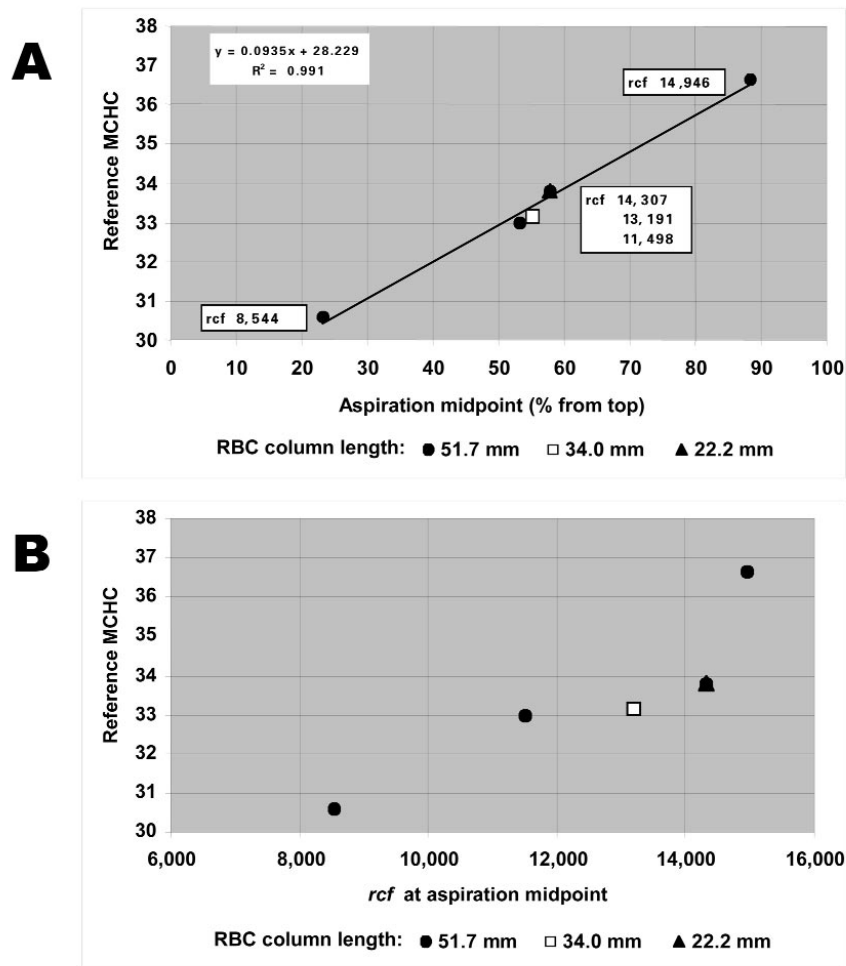


FIGURE 1. Deming regression method comparison plots of spun microhematocrit versus reference packed cell volume (PCV) using 12 different capillary tubes of different materials and dimensions. Hb indicates hemoglobin; BD, Becton Dickinson.



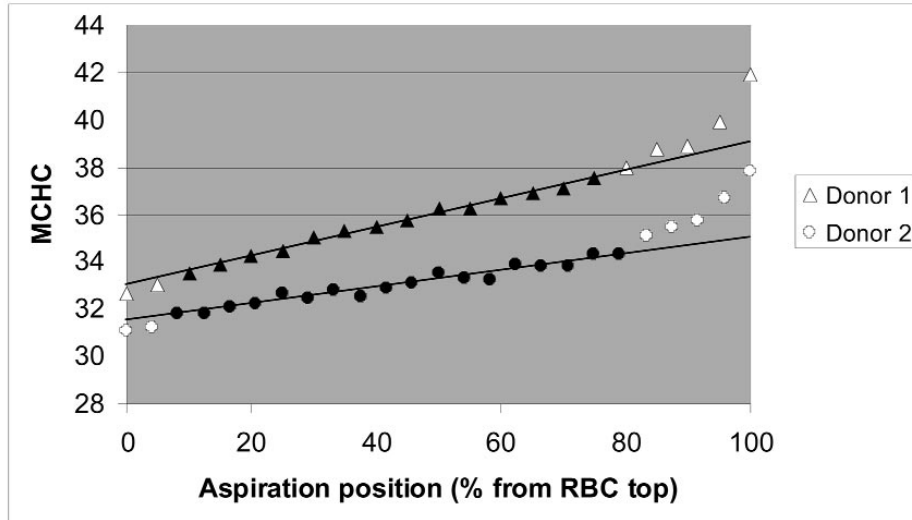
**FIGURE 2.** Average mean cell hemoglobin concentration (MCHC) values at different aspiration positions within the packed red cell column. Three 1.55-mm bore tubes were filled to different heights from each of 30 different blood samples. The fullest tube was etched at 20%, 50%, and 85% depths into the red cell column, whereas the other 2 tubes were etched at only the 50% point. Samples were aspirated starting at these depths, and MCHCs were determined. We conclude that red cells are sorted by density relative to position in column (A) and not by the relative centrifugal force (rcf) to which they are exposed (B). RBC indicates red blood cell.

hematocrits and that, in high hematocrit samples, the spun microhematocrit consistently underestimates the reference PCV. Clearly, there is more going on here than the lengthening of the red cell column by the inclusion of a few white cells and some accompanying plasma.

The “more” that is occurring concerns those red cells, referred to earlier, that are worming their way through the thickening red cell deposit so as to come to rest at a depth corresponding to their MCHC. That they can do this at all is surprising, but do it they must, else the experiment reported in Figure 2 would have turned out quite differently. Given that some of the heavier MCHC cells that reach the plasma–red cell interface in the later stages of centrifugation must travel 3 to 4 cm through the red cell column, it would be surprising if they survived the process completely unscathed. Indeed, some of them do sustain damage, and it is

this damage that leads to the unexpected PCV underestimation already noted. However, the red cell damage is subtle and is not sufficient to produce lysis. Any lysis would be evident simply by remixing and recentrifuging the contents of a microhematocrit tube. What happens is one stage short of that. In the membrane of some of the cells, pores open. These are of sufficient size to allow water molecules to escape. The pores are not large enough to allow hemoglobin to exit. As the water escapes, the MCHC rises to very high levels and the cells rapidly make their way to the very bottom of the hematocrit tube. The water that they have lost escapes with the plasma. The red cell column is artifactually shortened as a result.

Nor is this the only way in which the red cells may be damaged as they worm their way downward. Those that travel along the inside of the glass capillary, between the glass wall and the outer layer of the red cell column, experience



**FIGURE 3.** Mean cell hemoglobin concentration (MCHC) distribution curves from 2 normal donors demonstrating sorting of erythrocytes according to density (MCHC) during centrifugation for 10 minutes at 10,000g. Rapidly increasing MCHCs near the bottom of the tube represent dehydrated cells, damaged during the sorting process by cell-cell and cell-glass interactions. RBC indicates red blood cell.

major pH shifts as well as potential damage from friction. Some of these cells also become dehydrated and therefore more dense. As soon as their density increases over that of their neighbors they will be forced to relocate toward the bottom of the tube by the high  $g$  forces acting on the red cell column. The combination of these two effects is sufficient to account for the complex relationship between the spun microhematocrit and the reference PCV documented in Figure 1. These dehydrated cells accumulate at the very bottom of the spun microhematocrit tube. Their presence is indicated by the very rapid rise in MCHC at the bottom of the tube [4] shown in Figure 3. The cells have been photographed [4], and it has been previously confirmed that they can be successfully rehydrated if suspended in plasma at 1g for several minutes. The number of these cells is a function of the length they are forced to travel through the red cell column and this, in turn, is directly a function of the hematocrit. The higher the hematocrit, the longer the red cell column and the larger the number of dehydrated cells produced. In very-small-bore tubes where the area of the inner tube surface is large relative to the quantity of blood present, the additional damage caused by wall contact further increases the number of desiccated cells.

## SUMMARY

The reassuring news is that, although complex processes affect red cells during the performance of a spun microhematocrit, the errors introduced are both positive and negative. Some errors increase the column length, and others decrease it. In the usually encountered range of hematocrits (from 0.3

to 0.5), the errors are fortuitously balanced. As a result, spun microhematocrits in this range yield biases that are within acceptable limits. Across the 10 acceptable tubes evaluated, the mean bias at PCV 0.3 was  $0.004 \pm 0.0011$  (95% confidence limits); mean bias at PCV 0.4 was  $0.001 \pm 0.0012$ ; and mean bias at PCV 0.5 was  $-0.002 \pm 0.0014$ . The smallest marked interval (0.01) commonly used on PCV reading cards is 5 to 10 times greater.

## ACKNOWLEDGMENT

This study was funded in part by a grant from the B-K Foundation, San Bernardino, California.

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