The Effects of Over-anticoagulated Blood on Hematocrit Values by the Microcentrifuge Method

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OBJECTIVE: To determine equivalency of hematocrit results by three methods.

DESIGN: A total of 101 whole blood samples in EDTA tubes were analyzed in this repeated measures study.

SETTING: East Carolina University's clinical laboratory science program, Greenville NC.

PARTICIPANTS: The blood specimens were from adult patients at Nash General Hospital in Rocky Mount NC who had a CBC performed.

MAIN OUTCOME MEASURE: Hematocrit values from a whole blood sample with EDTA anticoagulant performed by a Sysmex XE-2100 and by microcentrifuge with two different types of capillary tubes (i.e., heparinized and non-heparinized) filled from the EDTA tubes.

RESULTS: The hematocrit means of the total sample for the three methods were 36.2%, 35.4%, and 35.6% for the Sysmex XE-2100, non-heparinized capillary tubes, and heparinized capillary tubes, respectively. Pearson correlation coefficient (pairwise) analyses produced significant r-values at an alpha of .01 for all three method comparisons.

CONCLUSIONS: Based on statistically significant Pearson (pairwise) correlation coefficients, the hematocrit values by all three methods can be considered relatively equivalent. The differences between methods are quite small and would be clinically insignificant, thus likely not altering clinical decisions. Though this study was conducted under somewhat ideal conditions relative to the blood specimens selected, the results indicate that the additional dilution produced in a heparinized capillary tube when being filled from an EDTA-anticoagulated tube is not sufficient to produce clinically different microhematocrit results as compared to using the recommended non-heparinized capillary tube.

ABBREVIATIONS: Aut = automated; CBC = complete blood count; CT = capillary tube; EDTA = ethylenediaminetetraacetic acid; Hct = hematocrit; Hep = heparinized capillary tube; NonHep = non-heparinized (i.e., no anticoagulant) capillary tube; RBC = red blood cell; RPM = revolutions per minute; WBC = white blood cell.

INDEX TERMS: capillary tube; hematocrit; microhematocrit; microhematocrit centrifuge.

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The spun microhematocrit is a method which is often used in screening patients for anemia in physician office laboratories, clinics, and blood donation centers, and as a back-up method in some hospital clinical laboratories. It is simple to perform, inexpensive, and can provide results in about five minutes. In 1993, the FDA added the spun microhematocrit to the list of CLIA-waived tests, thus allowing non-laboratory personnel to perform this procedure.[1] Personnel such as nurses, patient care technicians, medical assistants, and various other non-laboratory personnel are often the ones performing waived tests in point-of-care situations.

There are heparinized capillary tubes available for fingerstick blood samples and non-heparinized tubes for anticoagulated blood samples. Non-laboratory personnel may not always appreciate the different types of capillary tubes and their associated use. It is possible that personnel performing a spun microhematocrit could mistakenly use a heparinized tube with an anticoagulated blood sample. The question then is whether the heparin lining of the capillary tube produces a significant lowering of the hematocrit value due to the additional dilution from an already diluted EDTA-anticoagulated blood sample. A study to answer this question, which used 30 healthy adults, compared hematocrit values by the spun microhematocrit method using heparinized versus non-heparinized capillary tubes, both filled from an EDTA-anticoagulated Vacutainer® tube. Hematocrit values ranged from 35% to 49%. Based on a correlation coefficient of r=.99, the researchers concluded that results from the two types of capillary tubes showed no significant difference in hematocrit result regardless of which type of capillary tube was used with an EDTA specimen.[2] The purpose of the study described herein was to determine if these results could be replicated with a larger sample size which included both well and ill adults, and to expand the methods compared to include an automated hematocrit.

Some factors can affect spun microhematocrit readings, including improper sealing of the capillary tube resulting in blood loss from the tube, not mixing the specimen, the time and speed of centrifugation outside the standard five minutes at 10,000 to 15,000 RPM, incorrect reading with the hematocrit reader, excessive trapped plasma in the packed red cells such as from neonate hyperviscosity syndrome, dehydration of the patient, capillary tube size outside the usual 75mm with 1.1 mm diameter, oxygenation of the blood sample, and whether the patient is sedentary or active before being drawn.[3-9] Both increased anticoagulant and prolonged sitting of red blood cells in an EDTA tube can falsely lower a microhematocrit result due to shrinkage of the RBCs. Poikilocytes such as sickle cells and disorders which affect MCV are also cited as a cause of error.[3] These factors were incorporated into specimen eligibility criteria for the study as described below.

MATERIALS AND METHODS

Before beginning this study, the protocol and design were approved by East Carolina University's institutional review

Table 1. Rejection criteria for blood specimens

Based on visual inspection of the specimen and test(s) order collection time:
< 1.0 ml blood in the EDTA tube

> 1.0 hours since time of collection

Presence of hemolysis, lipemia, icterus, or clots

Based on CBC results from the Sysmex-2100:
Hematocrit > 55.0%

Hemoglobin > 18.0 or < 7.0 g/dl

WBC count > 30,000 or < 2,000/ul

MCHC > 36%

Instrument report had any flag(s) noted

A manual differential and morphology was performed for any other reason

board for human subject research. The hematocrit values on each sample were recorded by a unique identification number with no personal or identifying information recorded for the patients. All data was kept in a locked file cabinet except when in use by one of the researchers. All results are reported only in an anonymous, aggregate format.

Specimens

Patients of Nash General Hospital who had venous blood drawn for a CBC during a three-week period in November 2006 provided the potential blood specimens for the study. Only specimens from patients 19 years of age or older were accepted for the study. Each specimen was drawn in a three ml Vacutainer® tube with dry K2 EDTA anticoagulant by laboratory phlebotomy staff as part of the early morning test orders. The rejection criteria for the study specimens are displayed in Table 1. A total of 101 specimens were accepted for inclusion in the study.

Methods

Prior to beginning the specimen analyses, proper quality control for the microcentrifuge method was performed including timer and RPM verifications. Appropriate controls were run on the Sysmex XE-2100 each day that specimens were collected. The capillary tubes used were all made by Drummond, 75 mm height and 1.1 mm diameter, and plastic and mylar wrapped for safety. It should be noted that the Sysmex XE-2100 performs a measured hematocrit based on cumulative RBC pulse height, which is different than some other instruments that calculate the hematocrit based on the MCV and RBC count.

The hematocrit from the Sysmex XE-2100 was recorded as the automated method result for each accepted specimen. These specimens were next prepared for manual analysis by microcentrifuge. Each EDTA tube was inverted five times for adequate mixing. Two heparinized capillary tubes and two non-heparinized capillary tubes were filled until two-thirds full with blood sample, and one end was sealed with Critoseal. Tubes were placed in a Damon/IEC Division microhematocrit centrifuge in opposite slots with the sealed ends toward the outside and centrifuged for five minutes at 13,000 rpms. Once the microhematocrit centrifuge had completely stopped, without use of the brake, the hematocrit was read within 10 minutes as the percent of whole blood occupied by packed RBCs using a Damon/IEC Division Microcapillary Reader. In reading the hematocrit value, care was placed on excluding the buffy coat (i.e., layer of platelets and leukocytes) and reading the nonheparinized tubes and corresponding heparinized tubes in pairs to avoid sample confusion. The readings for each type of capillary tube were performed in duplicate, read once by two researchers, and averages were taken. The duplicate readings for each type of capillary tube had to agree within one percent to be acceptable.

Data analysis

All data entry and statistical analyses were performed in SPSS-PC+ version 15.0. The data include hematocrit values for each of the three methods as well as calculated variables representing the difference between each method with each of the other two methods. Descriptive statistics were calculated for all three methods (automated, non-heparinized capillary tubes, heparinized capillary tubes) as well as for method differences. Pearson (pairwise) correlation coefficients were calculated by bivariate analyses and scatterplots produced comparing each method with each of the other two methods, along with a repeated one-way analysis of variance (ANOVA) for all three methods. All analyses were evaluated for significance at an alpha of .01. For the two types of capillary tubes, analyses were performed only on the hematocrit averages for the duplicate readings.

RESULTS

The hematocrit values for all three methods ranged from 20.0% to 53.8%, with a mean of 35.7% for all three methods combined. Descriptive statistics for each method are shown in Table 2. Comparisons of methods by scatterplots are in Figures 1 through 3. All three scatterplots show only minimal differing of results between methods with the hematocrits of the two types of capillary tubes agreeing the most closely.

Review of the descriptive statistics for each method as well as for differences in means between methods indicates that the three methods produced similar results with differences

Table 2. Descriptive statistics by methods (N=101)

Hematocrit		Capillary tube	
(in %)	Automated	Heparinized	Non-heparinized
Mean	36.16	35.61	35.40
Median	36.00	35.00	35.00
S.D.	6.02	5.44	5.47

that would not be clinically significant for patient care. Over half of the samples produced hematocrit values with zero difference when comparing results of the two types of capillary tubes, though only two samples had zero differences when the automated results were compared to each type of capillary tube. For 68% of the samples, the automated hematocrit was higher than the microhematocrit centrifugation values from either of the two types of capillary tubes. The two capillary tubes had a smaller difference in means than did either capillary tube compared to the automated method (Table 2). All Pearson (pairwise) correlation coefficients comparing two methods were statistically significant at <.01 as follows: 1. for automated vs. heparinized capillary tubes, r(99)=.980, p<.005; 2. for automated vs. non-heparinized capillary tubes, r(99)=.981, p<.005; and 3. for heparinized vs. non-heparinized capillary tubes, r(99)=.996, p<.005.

When the repeated measures one-way ANOVA was performed, the variability about the means was found to be significantly different between the methods with an F(2,100)=27.8, p<.005. Though the differences in hematocrit between methods appear not to be clinically significant, there was probably enough variability about the means to be statistically significant, particularly with a large sample where small differences can cause statistical significance. So, though the results vary together in a positive relationship, there may be relatively large differences between methods for some specimens in this study.

DISCUSSION

As in previous research studies,[2] a statistically significant correlation (p<.005) between the hematocrit results by the two types of capillary tubes was found when filled with whole blood from an EDTA-anticoagulated Vacutainer® tube. Using the Sysmex XE-2100 hematology analyzer, statistically significant correlations (both p<.005) were also found with automated hematocrit results when compared to each of the two types of capillary tubes. Based on repeated measures one-way ANOVA, a significant difference between the three methods based on variability about the means (p<.005) was also found. Upon review of the differences in hematocrit values between the two types of capillary tubes and between the automated method versus each type of capillary tube, the differences were statistically significant based on the large sample size as used in this study but were still minimal enough not to alter the direction of patient evaluation relative to anemia screening by hematocrit. The differences between the methods appear clinically insignificant with the closest agreement being between the hematocrit values produced by the two types of capillary tubes when filled from an EDTA tube.

Interestingly, it has long been held that automated hematocrit results will be one percent to three percent less than the microhematocrit results when abnormal cells such as sickle cells, macrocytes, hypochromic cells, and spherocytes are present because of the effect of trapped plasma in the microcentrifuge method.[3] The results from this study, though, found for 68% of the specimens, the automated hematocrits were higher than the microhematocrits with either type of capillary tube. This may be due to the fact that we did not

Figure 1. Automated hematocrit vs. non-heparinized hematocrit in percentages

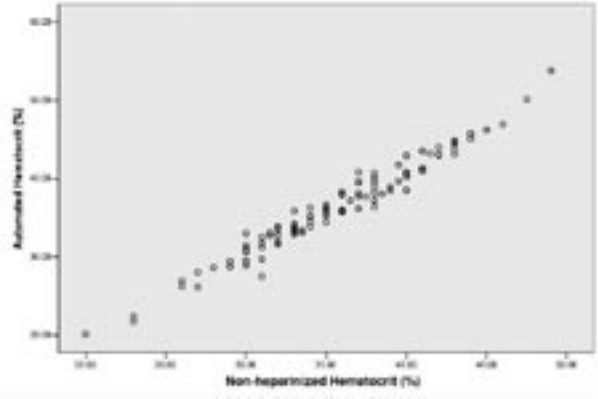
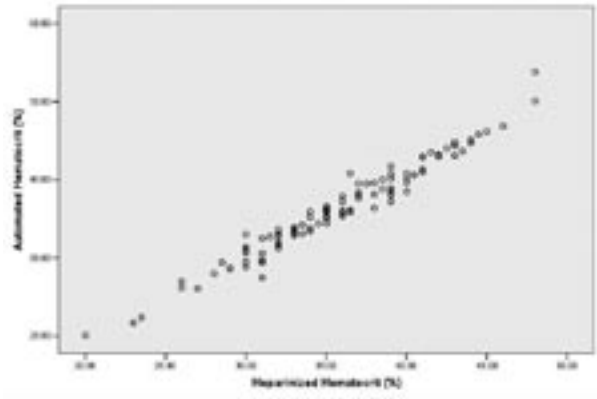


Figure 2. Automated hematocrit vs. heparinized hematocrit in percentages



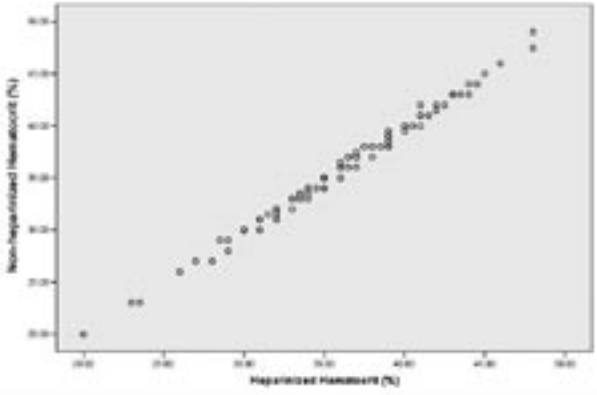
accept specimens for this study that had flags, as well as some other extreme results on the automated report which may have eliminated blood samples with abnormal cells. Also, the difference between automated and microcentrifuge hematocrit results stated above are more applicable to analyzers that calculate the hematocrit,[3] whereas the analyzer used in this study measures the hematocrit directly.

CONCLUSIONS

The results of this study reinforce previous research[2] and support the belief that it does not make a clinical difference in the hematocrit result if non-laboratory personnel in point-of-care testing situations were to use a heparinized, as opposed to the recommended non-heparinized, capillary tube to fill it from an EDTA-anticoagulated tube and then perform a microhematocrit. In essence, use of the non-recommended capillary tube would not produce a clinically significant difference in results such that patient evaluation for anemia by hematocrit would not be affected by the procedural error.

The study conclusions are limited by the study design for specimen acceptance which created an ideal situation not reflective of busy clinical laboratories. Non-laboratory personnel performing microhematocrits will not be able to screen samples as was done in this study. These conclusions are further limited to use of the same materials and methods employed for this study. Additional research should investigate this topic but in an actual clinic or physician office laboratory staffed by non-laboratory personnel to see if similar results are obtained under true working conditions.

Figure 3. Non-heparinized hematocrit vs. heparinized hematocrit in percentages



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