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Short-Term Stability of Hematologic Parameters in Frozen Whole Blood

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Abstract

Background: Complete blood counts (CBCs) are commonly obtained in large multicenter studies. We assessed the stability of 10 parameters after short-term (up to 30 days) frozen storage.

Methods: We compared CBC measurements from fresh samples (n = 53) with samples stored for up to 30 days at −70 °C. We calculated the CVs and intraclass correlation coefficients.

Results: Mean values of most parameters, with the exception of hemoglobin and platelet count, were significantly different by 15 days of storage. White blood cell count (CV, 38.3%; 95% CI, 31.3%–46.2%) and red cell distribution width (CV, 37.7%; 95% CI, 34.1%–41.3%) were the most variable. After 30 days, only hemoglobin remained stable and reliable (CV, 0.8%; 95% CI, 0.4%–1.3%).

Conclusions: Hemoglobin remained stable in frozen blood samples stored for up to 30 days at −70 °C and may be reliably used in research studies using short-term frozen specimens. Other CBC parameters measured in stored blood are not sufficiently reliable for research or patient care.

A complete blood count (CBC)[4] is one of the most important and frequently ordered clinical laboratory tests (1). Hematologic parameters from the CBC are used to screen for a variety of conditions ranging from anemia to infections to thrombocytopenia (2). Certain components, particularly white blood cell count (WBC), platelet count (PLT), and hemoglobin (HGB) are associated with a variety of chronic conditions, including cancer, kidney disease, and cardiovascular disease (3–6). The stability and analytic variability of these parameters can have implications for studies evaluating the association of these parameters with risk of clinical outcomes.

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To minimize methodologic contributions to analytical variability, multicenter studies often rely on central laboratories for sample processing and specimen testing. Immediate shipping of fresh specimens is not always feasible because of budgetary or logistical concerns; therefore, frozen samples may be the sole specimen source. However, the stability of hematologic parameters in frozen whole blood samples has not been previously characterized. Prior studies have mainly focused on the short-term stability of blood specimens stored at 4 °C and have observed stability of most CBC parameters up to 24 h, with greater stability demonstrated for certain parameters, including WBC, PLT, hematocrit (HCT), mean corpuscular hemoglobin (MCH), and HGB up to 3 days (7–9).

The objectives of our study were to evaluate the analytical stability of CBC components using frozen EDTA whole blood specimens stored up to 30 days at −70 °C compared with fresh samples.

MATERIALS AND METHODS

Study population

To characterize the analytical stability of the CBC parameters using stored whole blood collected in EDTA tubes, we obtained excess blood from clinical patients (n = 53) at the University of Minnesota Medical Center (Minneapolis, MN). These tests were conducted for quality assurance purposes and were determined to be exempt from Institutional Review Board approval by the University of Minnesota.

Measurements

Deidentified excess fresh venous blood samples collected in EDTA tubes were each aliquoted into 3 cryovials. CBC analyses were conducted at the following intervals: (*a*) immediate testing, (*b*) frozen immediately at −70 °C and analyzed 15 days later, and (*c*) frozen immediately at −70 °C and analyzed 30 days later. CBC parameters [WBC, red blood cell count, HGB, HCT, mean corpuscular volume (MCV), MCH, MCH concentration, PLT, and mean platelet volume (MPV)] were measured using the Sysmex XS-1000i analyzer between August 2016 and September 2016. The laboratory is certified by CLIA and accredited by the College of American Pathologists.

Statistical analysis

CVs capturing the analytic variability associated with short-term frozen storage and delayed processing (CV_A) were calculated using the root mean squared approach (10, 11). We used 2-way mixed model absolute difference intraclass correlation coefficients (12) to compare the paired measurements. Bland–Altman plots (13) and locally weighted scatterplot smoothing fits were used to visualize systemic bias and variability in comparisons of measurements in frozen stored samples compared with fresh samples. All analyses were conducted using Stata version 15.1 (StataCorp) (14).

RESULTS

The Sysmex XS-1000i analyzer yielded reportable values for all CBC parameters when fresh venous whole blood specimens were analyzed within 48 h of collection. With the exception of MPV, reportable values for most parameters were obtained, even after 30 days of frozen storage at −70 °C (see Table 1 in the Data Supplement that accompanies the online version of this article at http://www.jalm.org/content/vol4/issue3). However, after 15 days of frozen storage, more than half of the specimens no longer had a reportable MPV. Furthermore, with the exception of HGB and PLT, mean values of other CBC parameters were significantly different by 15 days (Table 1), with systematic biases observed for all parameters except HGB and PLT, and some indication of a greater bias at higher WBC, HCT, and MCV (see Fig. 1 in the online Data Supplement). After 15 days, measurements of the WBC and red cell distribution width had the highest CVs, both >30%, while HGB was the most reliable, with a CV of 1.2% (95% CI, 0.6%–1.8%). After 30 days of frozen storage, only mean HGB and PLT did not differ significantly from measurements in fresh samples, with HGB being the most stable among the hematologic parameters (Table 1).

DISCUSSION

Although the hematology analyzer reported values for most parameters after 30 days of frozen storage, the mean values of most parameters significantly differed after 15 days. Our data demonstrate that HGB, and, to a more limited extent, PLT, remained stable and could be reliably measured in whole blood specimens after 30 days of frozen storage. Few previous studies have evaluated the stability of CBC parameters in whole blood samples stored at −70 °C, although there have been reports focused on the short-term biological variability of these parameters (15) and the preservation of certain characteristics of the frozen cells, including DNA integrity (16).

Multicenter research studies often use certified central laboratories and conduct laboratory testing in stored biospecimens that have been shipped from different clinical centers. Our findings support the accuracy and use of HGB measurements in whole blood specimens stored at −70 °C for up to 30 days. Despite the lack of significant bias in the mean PLT, the high variability we observed suggests measurements of this parameter in frozen samples may be of only limited utility.No other CBC parameters were sufficiently stable or reliable to be used for research purposes, suggesting delayed measurement of these analytes does not accurately reflect the physiologic state of the study participant.

Strengths of our study include being able to determine the stability of CBC parameters in frozen clinical samples at 2 durations of storage. Measurements were completed by a laboratory that serves as the central laboratory for large multicenter NIH-funded trials and cohort studies.

Our study has some limitations. The clinical samples were processed using asimple freeze–thaw protocol. Whether alternative handling methods or addition of a cryoprotectant such as DMSO could improve the preservation and stability of these components was not assessed.

Furthermore, we are unable to differentiate the effects of storage from the freeze–thaw. However, our study does reflect the usual processing of samples by a central laboratory.

In summary, we have highlighted the limitations of using stored whole blood samples in research studies for assessment of most CBC parameters. HGB was the exception; we found that HGB can be measured with sufficient reliability in frozen whole blood samples stored at −70 °C for up to 30 days, suggesting that HGB measured in stored whole blood samples can be used for research purposes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS:

CBC	complete blood count
WBC	white blood cell count
PLT	platelet count
HGB	hemoglobin
HCT	hematocrit
MCH	mean corpuscular hemoglobin
MCV	mean corpuscular volume
MPV	mean platelet volume

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IMPACT STATEMENT

Complete blood counts are commonly obtained in multicenter studies utilizing central laboratories; however, the stability of these hematologic parameters when measured in stored samples has not been previously characterized. We compared measurements of hematologic parameters in fresh samples vs samples stored at −70 °C for up to 30 days. Most hematologic parameters were not stable in frozen samples; the exception was hemoglobin, which may be reliably used in research studies utilizing short-term frozen specimens.

Table 1.

Short-term stability of hematologic parameters in frozen stored clinical whole blood samples (n = 53).

		Baseline	Day 15 (frozen sample)			Day 30 (frozen sample)		
Parameter	n	Mean (SD)	Mean (SD)	CV_A, %	ICC	Mean (SD)	CV_A, %	ICC
WBC, 10^9/L	52	7.7 (5.2)	4.8 (3.4)[b]	38.8 (31.3 to 46.2)	0.68 (0.03 to 0.88)	5.3 (4.0)[b]	32.1 (26.0 to 38.3)	0.79 (0.11 to 0.93)
RBC, 10^{12}/L	53	3.1 (0.7)	2.5 (0.7)[c]	16.0 (12.6 to 19.4)	0.58 (−0.08 to 0.84)	2.5 (0.7)[c]	16.4 (14.1 to 18.7)	0.68 (−0.06 to 0.91)
HGB, g/dL	53	9.4 (2.1)	9.3 (2.1)	1.2 (0.6 to 1.8)	0.99 (0.98 to 0.99)	9.4 (2.1)	0.8 (0.4 to 1.3)	1.00 (0.99 to 1.00)
RBC, 10^{12}/L	53	3.1 (0.7)	2.5 (0.7)[c]	16.0 (12.6 to 19.4)	0.58 (−0.08 to 0.84)	2.5 (0.7)[c])	16.4 (14.1 to 18.7)	0.68 (−0.06 to 0.91)
HCT, %	53	29.1 (6.6)	25.6 (7.5)[a]	11.7 (8.1 to 15.2)	0.69 (0.31 to 0.85)	25.1 (8.3)[b]	13.7 (11.2 to 16.3)	0.76 (0.17 to 0.91)
MCV, fL	53	93.8 (6.7)	102.3 (9.1)[c]	6.8 (5.5 to 8.1)	0.31 (−0.06 to 0.59)	100.0 (10.3)[c]	6.7 (5.4 to 8.0)	0.30 (0.02 to 0.53)
MCH, pg	53	30.5 (2.9)	38.5 (10.2)[c]	15.0 (12.0 to 18.0)	0.06 (−0.11 to 0.25)	38.5 (6.2)[c]	15.8 (13.6 to 18.1)	0.18 (−0.09 to 0.47)
MCHC, g/dL	53	32.4 (1.4)	37.9 (10.0)[c]	10.9 (7.8 to 14.1)	0.00 (−0.20 to 0.23)	38.8 (7.0)[c]	13.3 (10.8 to 15.8)	0.10 (−0.08 to 0.30)
PLT, 10^9/L	53	187.1 (127.4)	202.0 (131.1)	15.2 (9.7 to 20.6)	0.95 (0.91 to 0.97)	236.8 (146.2)	24.2 (15.5 to 33.0)	0.58 (0.35 to 0.74)
RDW, %	49	15.4 (2.4)	26.7 (3.9)[c]	37.7 (34.1 to 41.3)	0.03 (−0.03 to 0.12)	31.0 (13.5)[c]	44.0 (39.1 to 49.0)	0.00 (−0.10 to 0.14)
MPV, fL	16	10.1 (0.8)	12.2 (0.7)[b]	13.6 (11.5 to 15.8)	−0.28 (−0.57 to 0.11)	11.8 (1.0)[c]	11.3 (8.6 to 14.1)	0.21 (−0.11 to 0.54)

ICC, intraclass correlation coefficient; RBC, red blood cell count; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width.

[a] $P < 0.05$ compared with baseline mean.

[b] $P < 0.01$ compared with baseline mean.

[c] $P < 0.001$ compared with baseline mean.