Evaluation of a New Reagent for Preserving Fresh Blood Samples and Its Potential Usefulness for Internal Quality Controls of Multichannel Hematology Analyzers

Wolfram Springer, MD,[1] Wolfgang Prohaska, MD,[2] Jörg Neukammer, PhD,[3] Andreas Höpe, PhD,[3] and Alexander von Ruecker, MD, PhD[1]

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Abstract

We describe a new, easy-to-use reagent, Cyto-Chex (Streck Laboratories, Omaha, Neb), that preserves fresh whole blood in a non–cross-linking, nonformalin manner. Target values assigned to fresh blood were essentially met after preservation and storage of up to 31 days. Respective mean analytic inaccuracies and short-term intra-assay coefficients of variation (n = 30) were as follows: WBCs, 6.7% and 1.99%; RBCs, 0.7% and 0.76%; hemoglobin, –1.8% and 0.79%; hematocrit, –0.3% and 0.75%; mean corpuscular volume, –1.0% and 0.78%; and platelets, 6.9% and 3.12%. Linearity of dilution-sensitive analytes was satisfactory over a wide range of dilutions after preservation of blood samples. Ten independent laboratories using 10 different instruments determined day-to-day interassay imprecision during four 7-day periods after blood preservation. Mean interassay coefficients of variation for participating laboratories were WBC, 1.92%; RBC, 1.00%; hemoglobin, 1.29%; hematocrit, 2.00%; and platelets 3.29%. Cyto-Chex enables long-term monitoring of instrument accuracy and precision with retained blood specimens of healthy persons. Blood from patient cohorts with various hematologic disorders and with a wide range of numeric abnormalities and/or parameter aberrations can be preserved satisfactorily with this reagent. The reanalysis of preserved patient blood samples is an important adjunct to the use of commercial control material in quality control programs of multichannel hematology analyzers.

The use of instrument-specific, stabilized blood samples as reference material for the internal quality control of multichannel hematology analyzers is practiced in many clinical laboratories. This approach is not without drawbacks. For one, fresh blood is a better sensor of instrument malfunction than stabilized blood samples. Furthermore, most commercial control materials ignore the fact that patients with hematologic disorders may require controls with specific characteristics.

Since multichannel automated analyzers were introduced in clinical hematology laboratories more than 25 years ago, determination of the CBC count, ie, the WBC and RBC counts, hemoglobin level, the hematocrit, the mean corpuscular volume (MCV), and the platelet count, can be routinely performed in large numbers on a daily basis. Because the CBC is one of the most frequently required laboratory parameters, quality control in hematology is essential for providing proper patient care. Although external and internal quality assessment has been permanently improved, an ideal quality control system for multichannel hematology analyzers is not available.[1]

Conventional methods of analytic quality control using accuracy-based target values and stable control or reference materials as in clinical chemistry are, strictly speaking, available only for measuring the hemoglobin concentration, for which an internationally accepted reference method[2,3] and stable control materials exist. Widely accepted reference methods also exist for the hematocrit,[4,5] WBC, and RBC,[6] but not for the enumeration of platelets. Furthermore, these reference methods were established for the use of fresh blood with its known limited stability.[7]

Multichannel hematology analyzers are usually calibrated using stabilized cell suspensions or nonbiologic surrogate materials (eg, latex particles). These materials do not match the properties of their fresh blood counterparts. The aforementioned

reference methods do not apply to these surrogate calibrants. Target values are assigned to such calibrants indirectly by using fresh blood that has been evaluated by reference methods as a *primary* standard and then by comparing the fresh blood calibrant with stable surrogate calibrant suspensions.[8]

Cross-linking cell preservatives can substantially change the physical (eg, density, flexibility, shape) and functional (eg, rheologic) properties of fresh blood. Any malfunction in the sensing compartments of a hematology analyzer may, therefore, remain undetected when using stabilized whole blood suspensions.[7,8] In addition, instruments based on different physical principles may respond differently to the stabilized cells; hence, the assigned values are not necessarily interchangeable between instruments. Furthermore, instrument manufacturers optimize their technology to ensure accurate performance with fresh blood from *healthy subjects,* and performance claims are generally restricted to such normal specimens.[8]

Performance Goals

Performance goals, which should be applied for the evaluation of a new blood-preserving reagent and its use in multichannel hematology analyzers, were taken from document H26-A, recently published by the National Committee for Clinical Laboratory Standards (NCCLS).[9] This document specifies reasonable limits for analytic precision and accuracy. Analytic short-term variation or imprecision should not exceed 25% of the within-person diurnal variation. The ideal limits for analytic accuracy were set at 3 times the short-term variation data. These performance goals are summarized in ▮Table 1▮ together with the practical precision and accuracy limits that indicate the present state-of-the-art in instrument technology.[9,10]

We evaluated the blood preservative Cyto-Chex (Streck Laboratories, Omaha, Neb; local distributor: Günter Keul GmbH, Steinfurt, and Vaupel GmbH, Hanau, Germany). This easy-to-use presumably urea-based reagent preserves EDTA-blood in a non–cross-linking, nonformalin manner. Previous studies have shown that Cyto-Chex can be used advantageously to preserve WBCs in peripheral blood samples without reducing the activity of important antigenic sites, such as CD4, CD8 and CD3[11] (confirmed by personal observations); as a result, it is being marketed as a preservative for immunophenotyping. We investigated whether Cyto-Chex can be used for the internal quality control of multichannel hematology analyzers and whether the NCCLS performance goals for instrument precision and accuracy are met by Cyto-Chex–treated, fresh blood control material. We assigned target values to fresh blood in accordance with reference methods. From our results, we conclude that Cyto-Chex–preserved blood specimens are suitable for quality control of hematology analyzers. Compared with conventional blood control materials, Cyto-Chex–preserved blood has the advantage of being better adjustable to special patient cohorts with various hematologic disorders. The use of retained patient specimens for periodic reanalysis in hematology quality control has been advocated as an adjunct to the use of commercial control material for many years.[12]

Materials and Methods

Materials

Cyto-Chex, a liquid reagent belonging to a family of presumably urea-based, non–cross-linking, non-formalin

▮Table 1▮
Population Mean Values, Within-Person Diurnal Changes, Ideal and State-of-the-Art (Practical) Limits for Analytic Imprecision and Inaccuracy

	Population Mean*		Coefficient of Variation (%)				
Analyte	Traditional Units	SI Units	Diurnal Changes*	Ideal Analytical Imprecision†	Ideal Analytical Inaccuracy‡	Practical Imprecision Limits*	Practical Inaccuracy Limits‡
WBC count	7,000/μL	7.0×10^9/L	14	3.50	10.50	1.68	5.04
RBC count	4.8×10^6/μL	4.8×10^{12}/L	3.5	0.88	2.63	1.02	3.06
Hemoglobin	14.2 g/dL	142.0 g/L	3.0	0.75	2.25	0.93	2.79
Hematocrit	42.5%	0.425	3.7	0.93	2.78	1.48	4.44
Mean corpuscular volume	89.2 μm^3	89.2 fL	0.5	0.13	0.38	0.95	2.85
Platelet count	257×10^3/μL	257×10^9/L	5.0	1.25	3.75	2.65	7.95

SI = Système International.
* Modified from NCCLS H26–A[9] and Richardson Jones et al.[10]
† Ideal analytic short-term imprecision is set at 25% of the within-person diurnal variation.
‡ Inaccuracy limits are set at 3 times the imprecision limits (see text).

fixatives that can be stored at room temperature for up to 12 months, was supplied by Streck Laboratories. When not specified, other reagents and chemicals were supplied by E Merck (Darmstadt, Germany).

Methods

Blood Samples, Preservation With Cyto-Chex

Fresh venous blood samples were obtained from healthy persons with normal CBC counts who came for routine check-ups or from healthy blood donors. If not stated differently, blood was collected by syringe in K_2-EDTA–containing tubes (final concentration, 3.7–5.4 µmol; 1.5–2.2 mg/mL). Samples with visible hemolysis or microclots were rejected. For preservation, fresh whole blood samples were diluted (1:1) with Cyto-Chex as specified by the manufacturer and stored at 6°C. For analysis, tubes with Cyto-Chex–preserved blood were brought to room temperature in 10 to 20 minutes, mixed thoroughly by hand inversion (>25 times), and opened on the days indicated. During trials over several days, tubes were closed firmly after use and re-refrigerated until the next day.

Accuracy, Target Value Assignment

Internationally accepted and published reference methods were applied for the assignment of target values to undiluted fresh (ie, <2 hours old) blood samples from 2 healthy blood donors. The hemoglobin concentration and the hematocrit were determined as described by the NCCLS standards H15-A2[2] and H7-A2,[4] respectively. We determined target values for WBC and RBC counts by a reference method published by the International Council for Standardization in Haematology (ICSH)[6] using a single channel, semiautomated, electronic analyzer based on aperture impedance counting (Coulter ZM, Coulter, Krefeld, Germany). Target values for the platelet count were measured by flow cytometry in combination with fluorescein isothiocyanate–labeled, platelet-specific antibodies and the erythrocyte/fluorescein isothiocyanate–platelet ratio.[13] The number of replicate measurements performed was in accordance with the recommendations of the ICSH.[6,14] Ten replicate measurements were performed in all other cases.

Analytical Inaccuracy (Bias) and Short-Term Intra-Assay Imprecision

The 2 fresh blood samples to which target values had been assigned were analyzed in our reference laboratory by 3 different multichannel hematology analyzers: a Technicon H-1 (Bayer Diagnostics, Munich, Germany), an Abbott CD3500 (Abbott Diagnostics Division, Mountain View, Calif), and a TOA-Sysmex E 5000 (Digitana, Hamburg, Germany). All instruments had recently been serviced and calibrated. With each instrument, 10 replicate measurements of all CBC analytes from undiluted fresh blood samples were performed within 4 hours after blood collection. In addition, 10 measurements of CBC analytes were repeated with each instrument on Cyto-Chex–preserved (ie, diluted) blood samples after storage for 5, 10, 18, and 25 days. To evaluate the analytic inaccuracy, target values were set at 100%, and each CBC analyte value measured in the 3 instruments was (after correction for dilution) expressed as percentage of its corresponding target value. The mean (percentage) value ±SD or coefficient of variation (CV) for all measurements and each CBC analyte on a specific day was then formed ($n = 3 \times 10$), demonstrating analytic inaccuracy and intra-assay imprecision.

Day-to-Day Intralaboratory Imprecision and Interlaboratory Comparisons of Normalized CBC Analyte Measurements

Ten independent laboratories participated in this trial, using 10 different blood cell count analyzers (CD3500, Abbott Diagnostics, Abbott Park, Ill; COBAS ARGO 5Diff and HELIOS ABC, Roche Diagnostic Systems, Branchburg, NJ; STKS and MAXM, Coulter, Miami, Fla; Technicon H-1, Bayer Diagnostics, Tarrytown, NY; E-5000, K-1000, K-4500, Sysmex, Long Grove, Ill) and a reference instrument for blood cell counting developed at the German Federal Institute for Physics and Metrology,[15] listed as instruments 1–10 in **Figure 1**, respectively. Each laboratory and instrument analyzed 1 individual blood sample. Samples of fresh blood were collected in K_2-EDTA–containing tubes from healthy persons as indicated. All CBC analytes of the undiluted fresh blood were first measured 3 times in duplicate. Next, the blood samples were preserved with Cyto-Chex (1:1) and stored at 6°C as described. Tubes were opened after 5, 10, 18, and 25 days, and CBC analytes were measured daily in triplicate for 1 week.

The day-to-day intralaboratory imprecision of instrument measurements (expressed as CV) for a 7-day period was determined for each laboratory and each CBC analyte and summarized by forming the average CV from all laboratories (n = 10).

To estimate the extent of interlaboratory variation and accuracy, CBC analyte measurements after Cyto-Chex preservation were first normalized for the fresh blood value, arbitrarily set at 100%. Next, all normalized values from all laboratories during a 7-day measuring period ($n = 10 \times 7$) were summarized, and the overall mean value and SD and CV of each CBC analyte were determined. Also, the interlaboratory variation for each analyte was determined daily by forming the CV of normalized sample values (n = 10) for a defined day after preservation. These daily interlaboratory

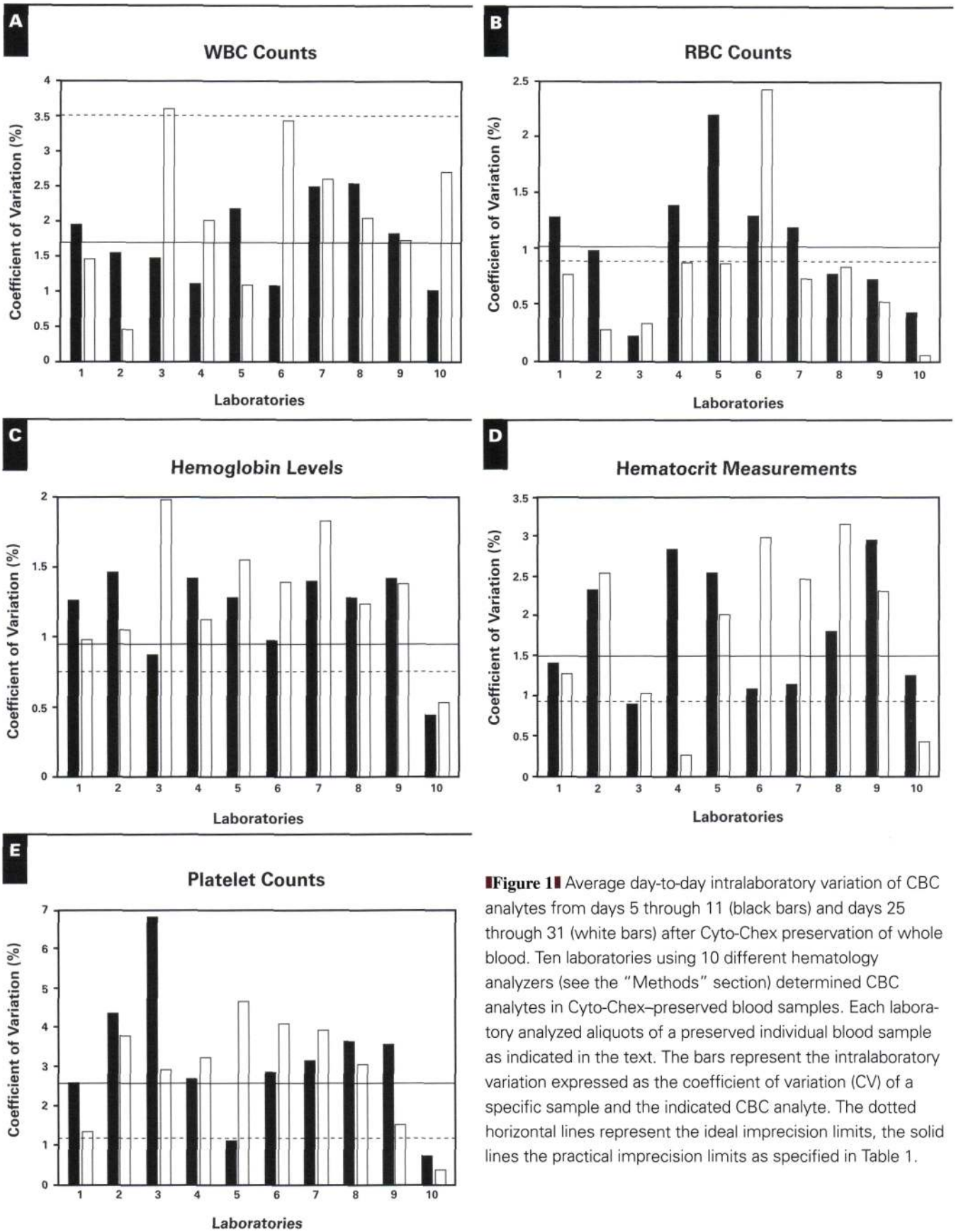

Figure 1 Average day-to-day intralaboratory variation of CBC analytes from days 5 through 11 (black bars) and days 25 through 31 (white bars) after Cyto-Chex preservation of whole blood. Ten laboratories using 10 different hematology analyzers (see the "Methods" section) determined CBC analytes in Cyto-Chex–preserved blood samples. Each laboratory analyzed aliquots of a preserved individual blood sample as indicated in the text. The bars represent the intralaboratory variation expressed as the coefficient of variation (CV) of a specific sample and the indicated CBC analyte. The dotted horizontal lines represent the ideal imprecision limits, the solid lines the practical imprecision limits as specified in Table 1.

CVs of each analyte were then averaged over a 7-day period and defined as day-by-day interlaboratory variation (expressed as CV).

Linearity

For linearity measurements, fresh whole blood containing K_2-EDTA from 1 healthy donor was acquired from the local blood transfusion service. After centrifugation (500*g*, 10 minutes) in appropriate glassware, whole blood components were separated, and, subsequently, cellular blood components were reunited to obtain an enriched whole blood preparation: WBC count, 56,800/μL (56.8×10^9/L); RBC count, 7.32×10^6/μL (7.32×10^{12}/L); and platelet count, 845×10^3/μL (845×10^9/L). The hemoglobin and hematocrit in this sample were 22.1 g/dL (221 g/L) and 66.8% (0.668), respectively. To determine linearity, the cell-free plasma fraction was added for serial dilutions up to 1:64. Each resulting sample (n = 10) was analyzed in triplicate on 3 analyzers (Technicon H-1, Abbott CD3500, TOA-Sysmex E 5000). The samples were then treated with Cyto-Chex 1:1, and CBC analytes were again measured 7 and 20 days after preservation, as described. Correction for Cyto-Chex dilution was carried out in all samples. Results were converted to graph form for visual appraisal in accordance with the recommendations of the ICSH.[14]

Data Analysis

The results are expressed as mean, SD, or CV and were compared with the performance goals shown in Table 1. We compared the means of the groups by using multivariate analysis of variance (MANOVA) followed by the Scheffé test and the Student *t* test, as appropriate. The Passing-Bablok nonparametric linear regression analysis was performed to calculate instrument linearity before and after preservation of fresh blood.[16] The level of significance was set at $P<.05$. MANOVA was calculated by using the statistical software SPSS 7.5.2 for Windows (SPSS, Chicago, Ill).

Results

Linearity

Inspection of plots of CBC analyte values against the indicated sample dilution range (see Methods) demonstrated a satisfactory linearity for all analytes sensitive to dilution (ie, WBC, RBC, hemoglobin, hematocrit, platelet count) in preserved and unpreserved blood samples and in all hematology analyzers tested. The results from samples preserved for 7 and for 20 days were similar. Data analysis (shown for the WBC and platelet counts in **Table 2**) confirmed highly satisfactory linearity over the range of dilution before and after preservation, with correlation coefficients above 0.986.

Analytic Inaccuracy, Intra-Assay Short-Term Imprecision

Fresh blood was first measured by reference methods and by routine hematology analyzers. When comparing the CBC analyte values reached by both methods, we found that the accuracy limits shown in Table 1 were met by all instruments and in all cases. **Table 3** shows the means and CVs of all CBC analyte measurements. The individual instruments did not show significant performance differences. This indicates that the hematology analyzers used in this study were functioning properly.

Cyto-Chex preservation and storage of whole blood up to 31 days had a variable effect on each CBC analyte. CBC analyte measurements fluctuated moderately in each analyzer (data not shown) and varied from one measuring day to the next. These fluctuations were not always insignificant compared with the target or fresh blood instrument

Table 2
Instrument Linearity

	Technicon H-1			Abbott CD3500			TOA-Sysmex E 5000		
		After Preservation			After Preservation			After Preservation	
Analyte*	Fresh Blood	7 d	20 d	Fresh Blood	7 d	20 d	Fresh Blood	7 d	20 d
WBC									
r	0.999	0.991	0.986	0.999	0.993	0.990	0.999	0.995	0.989
Slope	0.976	0.954	0.968	1.008	1.057	1.052	1.001	1.040	1.015
y intercept ($\times 10^9$)	1.125	0.378	0.529	–0.412	–0.305	0.104	0.741	–0.158	0.349
Platelet count									
r	0.999	0.996	0.997	0.999	0.990	0.996	0.998	0.994	0.994
Slope	1.000	1.103	1.037	0.982	1.005	1.017	0.985	1.049	1.000
y intercept ($\times 10^9$)	0.500	–9.226	–8.045	14.167	–8.600	–11.667	12.724	–7.313	–1.500

* See the "Methods" section for CBC analyte values and manufacturer information.

Table 3
Analytic Inaccuracy and Intra-Assay (Short-Term) Imprecision for Normal Range CBC Before and After Cyto-Chex* Preservation and Storage

Analyte/Unit of Measure	Fresh Whole Blood†		Days After Cyto-Chex Preservation (Mean Percentage)‡				
	Target Values	Routine Instruments (Mean Percentage)§	5	10	18	25	Mean
WBC count, /μL (× 10^9/L)							
Sample 1	3,460 (3.46)	102.7	105.5‖	106.9‖	105.9‖	107.8‖	106.7
Sample 2	5,820 (5.82)	CV 1.34	1.32	2.77	1.83	2.04	1.99
RBC count, × 10^6/μL (× 10^{12}/L)							
Sample 1	4.83 (4.83)	100.6	101.3¶	100.2	99.4‖	101.7‖	100.7
Sample 2	4.31 (4.31)	CV 0.63	0.72	0.73	0.91	0.68	0.76
Hemoglobin, g/dL (g/L)							
Sample 1	14.69 (146.9)	99.1	97.5‖	98.4¶	98.0‖	98.8	98.2
Sample 2	13.38 (133.8)	CV 0.68	0.82	0.76	0.74	0.82	0.79
Hematocrit, % (L/L)							
Sample 1	43.1 (0.431)	98.9	100.6‖	98.2¶	99.7‖	100.3‖	99.7
Sample 2	38.2 (0.382)	CV 0.74	0.67	0.83	0.79	0.69	0.75
MCV, μm^3 (fL)							
Sample 1	89.2 (89.2)	98.7	99.2	98.3	99.4¶	99.1	99.0
Sample 2	88.7 (88.7)	CV 0.71	0.83	0.85	0.79	0.64	0.78
Platelet count, × 10^3/μL (× 10^9/L)							
Sample 1	198.7 (198.7)	102.3	106.6‖	106.0‖	108.1‖	106.4‖	106.9
Sample 2	208.4 (208.4)	CV 3.66	2.74	3.01	3.81	2.92	3.12

CV = coefficient of variation; MCV = mean corpuscular volume.
* Streck Laboratories, Omaha, Neb.
† Target values were determined by reference methods and arbitrarily set at 100%. Other measurements (n = 30) were determined by using a Technicon H-1, an Abbott CD3500, and a TOA-Sysmex E-5000 multichannel hematology analyzer as described in the "Methods" section.
‡ Values were corrected for dilution and show the percentage of the corresponding target value.
§ Values show the percentage of the corresponding target value.
‖ $P < .01$, significantly different from fresh blood instrument value (multivariate analysis of variance and Scheffé procedure).
¶ $P < .05$, significantly different from fresh blood instrument value (multivariate analysis of variance and Scheffé procedure).

value, but mostly no explicit trend (ie, increase or decrease) of values could be detected. Some significant ($P < .05$) differences also were noted between the 3 different analyzers, but these variations were highly inconsistent between days (MANOVA). Only the platelet count and the WBC increased significantly ($P < .05$) and unambiguously by about 3% to 4% after preservation treatment in all instruments, but no storage effect could be observed. The average effect of Cyto-Chex preservation and storage on CBC analyte measurements in the 3 hematology analyzers is shown in Table 3. The accuracy limits shown in Table 1 were adequately met by all instruments. The average analytic inaccuracies were as follows: WBC count, 6.7%; RBC count, 0.7%; hemoglobin, –1.8%; hematocrit, –0.3%; MCV, –1.0%; and platelet count, 6.9%.

The average short-term intra-assay variations of CBC analytes (expressed as CV in Table 3) observed with hematology analyzers did not differ substantially in fresh or Cyto-Chex–preserved blood specimens. Intra-assay variations in the individual instruments were usually somewhat lower. Minor fluctuations in intra-assay precision were observed after preservation in each instrument (data not shown). These fluctuations were inconsistent and, thus, may not have been caused by Cyto-Chex preservation or length of storage, but could have been generated by instrument drift. The criteria for practical or ideal short-term analytic imprecision specified in Table 1 were met in almost all cases except for platelet measurements.

Day-to-Day Intralaboratory Imprecision and Interlaboratory Comparisons of Normalized CBC Analyte Measurements

Ten independent laboratories using 10 different hematology analyzers evaluated Cyto-Chex blood preservation (see the "Methods" section). Each laboratory analyzed 1 individual fresh blood sample and 4 aliquots of preserved samples from the same specimen. All CBC analytes were first measured in fresh blood, then daily for four 7-day periods in the 4 preserved blood samples as indicated. Figure 1 illustrates the intralaboratory variation of CBC analyte measurements in the independent laboratories during the first and fourth 7-day periods after sample preservation. As can be seen in Figure 1 and **Table 4**, changes in the intralaboratory CVs varied for each CBC analyte in each laboratory. CVs also varied between the different laboratories during each 7-day period and between the 4 different periods. No significant changes in day-to-day intralaboratory precision were observed after blood preservation, and no clear trends could be determined during storage time. The performance goals set for short-term precision (Table 1) were frequently

Table 4
Intralaboratory Day-to-Day Variability and Estimate of Interlaboratory Accuracy and Variation of CBC Analytes in Cyto-Chex*–Preserved Blood†

Analyte/Unit of Measure	Fresh Blood Value‡	Days After Cyto-Chex Preservation§				
		5–11	10–16	18–24	25–31	Mean
WBC count, × 10^9/L	7.43 (5.09–12.6)					
Overall mean		101.7 ± 3.53	103.2 ± 3.44‖	106.9 ± 4.65‖	108.1 ± 4.63‖	105.0 ± 4.25
Intralaboratory CV		1.74 (1.10–2.56)	1.70 (0.53–3.72)	2.11 (0.91–3.59)	2.14 (0.46–3.6)	1.92 (0.46–3.72)
Interlaboratory CV		3.39 (2.81–4.11)	3.21 (2.46–3.91)	4.46 (2.99–5.53)	4.45 (3.15–6.49)	3.88 (2.46–6.30)
RBC count, × 10^{12}/L	4.71 (4.17–5.04)					
Overall mean		100.8 ± 2.06	101.6 ± 2.59‖	101.2 ± 2.72	101.3 ± 2.40¶	101.2 ± 2.47
Intralaboratory CV		1.06 (0.22–2.20)	1.02 (0.46–2.15)	1.11 (0.25–3.24)	0.84 (0.07–2.43)	1.00 (0.07–3.24)
Interlaboratory CV		1.94 (1.63–2.34)	2.50 (1.65–3.09)	2.69 (2.28–3.30)	2.36 (1.96–3.13)	2.37 (1.63–3.30)
Hemoglobin, g/L	142.2 (127.0–156.0)					
Overall mean		101.1 ± 1.89	101.9 ± 2.67‖	103.0 ± 2.16‖	102.6 ± 2.11‖	102.2 ± 2.26
Intralaboratory CV		1.18 (0.45–1.46)	1.47 (0.57–4.01)	1.22 (0.37–3.05)	1.31 (0.53–1.98)	1.29 (0.37–4.01)
Interlaboratory CV		1.80 (1.57–1.97)	2.53 (1.94–2.88)	2.08 (1.75–3.33)	2.10 (1.32–2.93)	2.13 (1.32–3.33)
Hematocrit, L/L	0.415 (0.380–0.445)					
Overall mean		100.5 ± 3.08	100.2 ± 3.13	100.8 ± 3.24	100.5 ± 2.95	100.5 ± 3.12
Intralaboratory CV		1.82 (0.90–2.95)	1.97 (0.48–4.21)	2.35 (0.63–4.29)	1.84 (0.27–3.16)	2.00 (0.27–4.29)
Interlaboratory CV		2.88 (2.02–4.42)	2.91 (2.19–3.74)	2.97 (2.36–4.41)	2.86 (2.24–3.38)	2.91 (2.02–4.42)
Platelet count, × 10^9/L	258 (196–328)					
Overall mean		102.0 ± 6.62	105.3 ± 6.81‖	106.6 ± 6.07‖	105.3 ± 5.75‖	104.8 ± 6.61
Intralaboratory CV		3.18 (0.75–6.82)	3.47 (0.75–6.33)	3.59 (1.40–7.20)	2.92 (0.39–4.67)	3.29 (0.39–7.20)
Interlaboratory CV		6.30 (5.31–8.37)	6.33 (5.44–7.77)	5.56 (3.53–7.54)	5.32 (3.47–6.82)	5.88 (3.47–8.37)

CV = coefficient of variation.
* Streck Laboratories, Omaha, Neb.
† Ten laboratories used 10 different analyzers, each measured 1 individual blood sample as indicated in the "Methods" section. Conversions from Système International units to traditional units are as follows: WBCs (/mL), divide by 0.001; RBCs (× 10^6/mL), divide by 1.0; hemoglobin (g/dL), divide by 10; hematocrit (%), divide by 0.01; platelets, divide by 1.0.
‡ Fresh blood values were arbitrarily set at 100%. Data are given as mean of all samples (range).
§ CBC analyte values of all preserved blood samples (n = 10) were (after correction for dilution) expressed as the percentage of their corresponding fresh blood value, and the mean ± SD of all normalized sample values was calculated for the indicated 7-day periods. The average day-to-day intralaboratory and interlaboratory variability in normalized CBC analytes (including ranges), both expressed as CV, were determined for the days indicated and summarized as described in the "Methods" section (see also Figure 1).
‖ $P < .01$, significantly different from fresh blood instrument value (multivariate analysis of variance and Scheffé procedure).
¶ $P < .05$, significantly different from fresh blood instrument value (multivariate analysis of variance and Scheffé procedure).

not met by the hematology analyzers (ie, laboratories) that took part in the study.

To estimate the extent of interlaboratory accuracy and variation in CBC analyte measurements, we normalized the values for each laboratory for the fresh blood sample values by setting them at 100% (see the "Methods" section). All normalized values from all laboratories during a defined 7-day period after preservation were then summarized. The overall means ± SD of each CBC analyte during different 7-day periods are shown in Table 4. Compared with fresh blood, we found significant ($P < .05$) overall increases in most CBC analytes, although these were usually quite moderate and highly inconsistent during the different 7-day periods. Only in the case of the platelet count and the WBC could a small but significant ($P < .05$) 1-step increase be determined 2 to 3 weeks after preservation. No clear storage effect could be observed. Interestingly, the overall mean values indicate that, typically, the mean differences between fresh and preserved blood samples during 7-day periods do not exceed the short-term accuracy limits set (Table 1).

The interlaboratory variation (expressed as CV) of each CBC analyte on a defined day after preservation was then calculated from the normalized values of all participating laboratories. Subsequently, the CVs were averaged on a day-to-day basis for each 7-day study period. No clear systematic changes in interlaboratory variation were detectable on a day-by-day (not shown) or week-by-week basis after blood preservation (Table 4). Significant differences between days within a 7-day period were calculated by MANOVA. These were based on differences between laboratories and were not found to be consistent with any specific laboratory. The observation that the overall variation of all CBC analyte measurements (expressed as SD in Table 4) and the average day-to-day interlaboratory variation are both similar and low underlines the fact that the preserved blood samples did not change essentially within the 7-day periods.

Discussion

The use of whole blood control materials with assigned target values for the internal quality control of multichannel hematology analyzers is recommended and may soon be mandatory in hematology laboratories in Germany.[17] Alternative internal quality control methods for hematology analyzers include the random duplicates' method[12] and the

use of RBC indices for control of analyzer drift (Bull method),[18] which is an accepted and widespread hematologic quality control method in North America. The Bull method is recommended only in laboratories analyzing more than 100 specimens per day.[19] Ideally, 2 methods should be combined to confirm the other's adequacy.[1]

The use of untreated whole blood as control material has one main drawback, namely, its instability. Preserved whole blood preparations offer the advantage of a longer shelf-life. However, form and function of the stabilized cells generally are modified by the preserving chemicals. Adenosine triphosphate depletion and change in the membrane lipids of RBCs may lead to dramatic changes in rigidity and volume.[20] Furthermore, fixation of fresh blood cells may generate difficulties in RBC lysis, possibly causing RBCs to be counted as WBCs, and deviations in hemoglobin, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration measurements. What increases the complexity is the fact that some hematology analyzers do not directly measure variables (eg, the hematocrit), but derive these from other variables (ie, the MCV) that have been measured with a different method. Therefore, changes found, for instance, with manual hematocrit methods, eg, cell shrinkage due to preservation or osmotic changes, can be missed in automated analyzers.

Despite the potential disadvantages of preserved whole blood, certain performance criteria must be set to evaluate the usefulness of a preserving reagent for whole blood. We decided that the performance goals recently specified for multichannel hematology analyzers by the NCCLS standard H26-A for short-term analytic accuracy and precision (Table 1) would be appropriate for these evaluations.[9] The performance of instruments using fresh whole blood specimens or comparably preserved blood specimens should be within the accuracy and precision limits specified by the NCCLS H26-A standard. It was the primary aim of our study to compare numeric changes of CBC analytes that occur after blood preservation with the performance limits proposed by the NCCLS.

Our results show that Cyto-Chex, a reagent presumably belonging to a family of urea-based, non–cross-linking, nonformalin fixatives, can preserve whole blood samples for at least 31 days. The overall performance characteristics of the 3 hematology analyzers in our laboratory were satisfactory when Cyto-Chex–preserved whole-blood samples were compared with fresh blood samples. Storage of preserved samples in our laboratory for longer periods (up to 3 months) was not studied systematically but seems unproblematic (data not shown).

Linearity

All instruments showed a highly satisfactory linearity of CBC analytes over a wide range of dilutions before and after preservation of fresh blood samples, with excellent correlation between preserved and fresh blood samples. Our dilution experiments indicate that Cyto-Chex enables laboratories to produce a wide range of blood control materials by removing plasma from or adding specific blood components to normal whole blood samples before preservation.

Accuracy

Measurements of CBC analytes in preserved blood samples showed moderately fluctuating changes in all instruments from one measuring day to the next. These fluctuations were not always insignificant compared with target or fresh blood values (Table 3), but no clear trend (ie, increase or decrease) could be found. Only the platelet and WBC counts in all instruments showed a minor but significant 3% to 4% increase after preservation; however, no relevant storage effects were observed. Nevertheless, the short-term analytic and practical accuracy limits specified by the NCCLS and shown in Table 1 were met satisfactorily.

Precision

Intra-assay short-term variations of CBC analyte measurements did not differ substantially before or after preservation (Table 3). The moderate changes observed were inconsistent and, thus, probably were not caused by preservation or storage of blood samples but by instrument drift or minor variations in preanalytic handling. In short, our results show that Cyto-Chex preservation and storage of whole blood can induce slight fluctuations in CBC analyte measurements, but these changes are adequately within the performance limits specified by the NCCLS.

Ten laboratories, all using a different hematology analyzer, also were engaged in evaluating Cyto-Chex preservation. Day-to-day intralaboratory variations (Figure 1 and Table 4) were very inconsistent. CVs varied between laboratories and from week to week in most laboratories. The performance profiles of the participating laboratories were quite different (Figure 1), and individual laboratory instruments often did not meet the NCCLS criteria for short-term imprecision (Table 1). But the NCCLS criteria were not primarily meant to be used to judge long-term day-to-day intralaboratory variation. Tolerance limits for long-term intralaboratory precision specified by the American Pathologists Quality Assurance Service[21] for different analyte concentrations were met in all cases in which the NCCLS criteria were not fulfilled.

Interlaboratory comparisons of normalized CBC analyte measurements (Table 4) revealed that significant increases of some CBC analytes occurred after preservation, but these increases were highly variable within the different 7-day periods. Only the platelet and WBC counts showed consistent minor increases (approximately 3%–5%) that became

significant ($P < .05$) after 10 to 16 days of blood preservation, but no clear storage effects were found. These minor increases were similar to those described in Table 3 and may be due to the stabilization of fragile cells or platelets after preservation. Highly satisfactory was the fact that the mean differences between fresh blood and preserved blood analyte measurements usually did not exceed the NCCLS short-term accuracy limits (Table 1).

Normalization of measurements also enabled the determination of interlaboratory variation on a daily and 7-day basis after blood preservation. Interlaboratory variations differed moderately from day to day during each 7-day period and from week to week. No systematic changes or trends could be detected. Both day to day and overall interlaboratory variation were almost always similar. This finding underlines the fact that no unusual effects occurred during the four 7-day periods.

The NCCLS document, H26-A, does not define performance goals for the long-term interlaboratory precision of normalized CBC analytes, and the short-term intralaboratory precision limits shown in Table 1 were not met. The German Medical Association has proposed precision limits for interlaboratory comparisons that are set 3 times higher than the short-term intralaboratory precision limits.[17] Therefore, the accuracy limits shown in Table 1, which were formed by multiplying the precision limits by 3, may serve as useful guidelines for judging interlaboratory precision. Table 4 shows that these performance criteria for interlaboratory precision were typically met by instruments that analyzed Cyto-Chex–preserved blood.

Finally, some limitations of Cyto-Chex should be noted. Cyto-Chex, like all preservatives, can alter cell properties. As our results show, such changes do not interfere with cell counting or with CBC analyte measurements in any of the hematology analyzers tested, which was our primary concern. On the other hand, instruments that possess highly sophisticated technology to determine the WBC differential count can produce inaccurate results. We have observed distinct changes in the differential cell count that varied with time in most, but not all, hematology analyzers that have appropriate differentiating facilities (data not shown). The differential count of blood samples from patients with the same disorder also was often altered in an unpredictable way after preservation. Therefore, we find it essential to check the effect of Cyto-Chex preservation before using it for the WBC differential count. Similarly, instrument flags and scatter plots proved highly variable and inconsistent after Cyto-Chex preservation (data not shown) and should be used with caution.

Furthermore, we have observed that Cyto-Chex preservation of abnormal blood samples (mostly from patients with lymphomas and sepsis) can cause immediate, highly inconsistent, and significant numeric changes in the WBC and platelet count that are possibly due to the stabilization of fragile cells (data not shown). One should be aware that such changes can occur with approximately 25% of abnormal blood samples. Predictions on how an individual abnormal blood sample will react after preservation have not proved possible.

Our results show that Cyto-Chex preserves whole blood up to 31 days (and presumably longer), essentially without influencing the results of CBC analyte measurements, ie, the accuracy and precision limits specified by national or international professional committees were adequately met. The results obtained with various hematology analyzers based on different physical measuring principles (ie, impedance and optical counting) and manufactured by different companies accord well. According to our experience, Cyto-Chex is an easy-to-use reagent that enables laboratories to monitor instrument accuracy and precision with retained blood specimens, not only from healthy individuals, but also from patient cohorts with various hematologic disorders and with a wide range of numeric abnormalities or other parameter aberrations. We conclude that the analysis of preserved patient blood samples is an important adjunct to the use of commercial control material in quality control programs of hematology analyzers.

From the [1]Department of Clinical Biochemistry, University of Bonn, Bonn, Germany; the [2]Institute of Laboratory and Transfusion Medicine, Heart and Diabetes Center North Rhine Westphalia, Ruhr-University Bochum, Bad Oeynhausen, Germany; and [3]Physikalisch-Technische Bundesanstalt, Berlin-Charlottenburg, Germany.

Address reprint requests to Dr Springer: Dept of Clinical Biochemistry, University of Bonn, Sigmund-Freud-Strasse 25, 53105 Bonn, Germany.

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