Research Article

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User verification of Abbott Alinity HQ hematology analyzer

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Abstract

Objectives: This study aims to evaluate the performance characteristics of the Alinity HQ hematology analyzer in a routine laboratory setting.

Methods: In the study, precision (short-term and long-term precision), accuracy (method comparison with Abbott Cell Dyn Ruby and estimation of bias), confirmation of a background (Limit of Blank, LoB), and carry-over were used to evaluate the performance of Alinity HQ as recommended by ICSH, CLSI guidelines EP15-A3, EP09, EP17A2, and H26-A2. Acceptance criteria were based on manufacturer technical specifications and the EFLM Biological Variation Database.

Results: According to the short-term precision results, except for mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC), all measurements exhibited coefficient variations (CV) lower than their verification limits. Basophil, eosinophil, and monocyte counts, as well as mean corpuscular hemoglobin (MCH), MCHC, and red cell distribution width standard deviation (RDW-SD), did not meet the allowable imprecision criteria for the long-term precision study. The estimated bias for all analytes was within verification limits. However, the method comparison study showed concentration-dependent variations for MCHC, MCH, and mean platelet volume (MPV) parameters. Furthermore, the correlation of parameters between Alinity HQ and Cell Dyn Ruby ranged from 0.46 to 1.00. The LoB and carry-over studies demonstrated satisfactory performance for the Alinity HQ analyzer.

Conclusions: Although some parameters had higher CVs than expected and concentration-dependent bias, the overall analytical performance of Alinity HQ was found to be satisfactory. Alinity HQ is an accurate, highly precise analyzer with good analytical performance, suitable for high-volume laboratories.

Keywords: method verification; hematology analyzer; CLSI H26-A2; ICSH guideline; CLSI EP15-A3

Introduction

Hematology analyzers are becoming more technologically advanced and are now available for complete blood counts (CBCs) with high sample throughput and reduced turnaround time. They are based on different technologies, such as electrical particle counting (impedance), optical and fluorescence flow cytometry, and digital imaging-based principles [1]. The recently introduced Abbott Alinity HQ module, which uses optical and fluorescence flow cytometry principles, is a new generation, multi-parameter, and high-throughput automated hematology analyzer for CBCs [2].

Manufacturers design devices that aim to minimize interventions while maintaining precision and optimally utilizing their potential. Considering the various technologies different manufacturers use, each analyzer has certain performance characteristics that can be highly sample-dependent. Manufacturers validate their analyzers according to global and regional requirement guidelines. The most informative way is to select the optimum analyzer for laboratory verification studies with relevant samples before a new diagnostic analyzer is put into routine use [3]. Nevertheless, it is a requirement to evaluate the performance of a particular analyzer under laboratory conditions where the device will be run using the samples of a specific targeted population and operated by laboratory personnel.

International standards, such as the International Council for Standardization in Hematology (ICSH) guidelines [4], the Clinical Laboratory Standards Institute (CLSI) guidelines [5–8], and the ISO 15189 standards [9], state that every laboratory should verify the performance of their hematology analyzers. The verification methods described by these guidelines include precision, accuracy, comparability, carry-over,

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background (limit of blank [LoB]), and linearity throughout the expected range of results [4–10]. However, the assessment of analytical performance on different levels is usually not detailed, and interpretation is the responsibility of the laboratory professionals. It is up to the laboratory specialist or laboratory procedures to decide which standard to follow or which verification limit to use [10].

This study aimed to verify the performance characteristics of the Alinity HQ hematology analyzer compared with the previously used Abbott Cell Dyn Ruby in a routine laboratory setting according to the CLSI H26-A2, CLSI EP15-A3, CLSI EP09, CLSI EP17, and ICSH guidelines [4–8]. We analyzed precision (short-term and long-term), accuracy (method comparison and estimation of bias as a measure of trueness), confirmation of a LoB, and carry-over to offer guidance on the verification of the automated hematology analyzer.

Materials and methods

Study protocol

The Ethics Committee of Başkent University approved the study with the decision numbered KA21/333, dated October 14, 2022.

Specimen collection and preparation

The samples used in this evaluation were sourced from the outpatient and inpatient departments of Başkent University Hospital. For routine CBC analyses, all samples were collected using K_2-ethylenediaminetetraacetic acid Vacusera blood collection tubes (Disera, İzmir, Turkey). Samples with visible hemolysis, coagulation, or blood clots were excluded, with the samples selected according to normal and abnormal hematological profiles of the subjects. The whole-blood samples were stored at room temperature (18–26 °C) until analysis, as required by the CLSI H26A guideline [5].

Complete blood count analysis

Alinity HQ (Abbott Laboratories, Diagnostics Division, Hematology, Santa Clara, CA, USA) is a new high-sample throughput hematology analyzer. The Alinity HQ uses optical principles for all measurements. Hemoglobin levels are determined using spectrophotometry. Alinity HQ utilizes advanced multi-angle polarized scatter separation (MAPSS™) technology, using seven light detectors positioned at different angles and a fluorescence detector. The analyzer employs a combination of photometry, optical flow cytometry, fluorescence analysis, and advanced software algorithms to enumerate cells and report 29 different parameters. Nucleated cells are measured using a fluorescent nuclear dye reagent, staining the nuclei for MAPSS. Nucleated cells are reported as six-part white blood cell (WBC) differentials, including immature granulocytes and nucleated red blood cell (RBC) counts [11]. Each Alinity HQ module has two unique measurement units, the “left block” and the “right block.” In routine operation, patient samples are sequentially pipetted into these blocks to enable high throughput. Here, for a more comprehensive assessment, the precision of each block was evaluated separately. In addition to calibration and quality control (QC), carry-over should also be assessed on the two incubation blocks [12].

The CELL-DYN Ruby (Abbott Laboratories, Diagnostic Division, Abbott Park, IL, USA) provides 22 blood count parameters, including a five-part WBC differential. The system utilizes the Multiangle Polarized Scatter Separation technology and laser flow cytometry. The CELL-DYN Ruby also provides an integral reticulocyte analysis and nuclear optical count [13].

Performance evaluation

Precision studies: The precision study was performed in two stages: short-term precision and long-term precision, as recommended by the ICSH and CLSI guidelines, EP15-A3 and H26-A2, respectively [4–6]. Imprecision was reported in terms of CV%. All precision studies were performed for both incubation blocks.

Short-term precision: Repeatability and within-laboratory imprecision studies were performed as short-term precision evaluations. Repeatability was calculated by measuring (1) three different levels of patient samples and (2) three-level internal quality control (IQC) materials (low, medium, and high). Within-laboratory imprecision was calculated using only IQC materials.

Repeatability (CVr) and within-laboratory precision (CVwl) studies for IQC materials were performed according to the CLSI EP15-A3 guideline by measuring three IQC samples per day, five replicates on the left block and five on the right for five days. As per the guidelines, the precision calculation can be summarized as follows: outliers were removed from the dataset using Grubbs’ test, and one-way analysis of variance (ANOVA) was applied. The variance components obtained from ANOVA, V_B and V_W, were utilized to calculate repeatability, between-run standard deviations, and CV% values. Subsequently, actual manufacturer claims were used to determine verification limits for the precision study. Ten different patient samples were analyzed in 10 replicates according to the ICSH guidelines for patient sample repeatability, with mean, standard deviation (SD), and CV% values calculated for these samples.

We also proposed a novel plot for evaluating the precision study results in accordance with the EP15-A3 guideline. In this representation, CV% values are plotted on the x-axis, while repeatability and within-lab CVs are illustrated on the y-axis. Different colors are used to differentiate the various QC sample levels. Manufacturer claims are represented by solid-colored bars, and extended verification limits – sourced from the precision study results – are depicted in transparent colors. A black bar is used to highlight the acquired CV% values. The code associated with this plot is available on GitHub (https://github.com/ditopcu/EP15A3_Precision_Plot).

Long-term precision: A long-term QC precision study as a within-batch precision was carried out for 30 days in alignment with the ICHS guideline. During this period, a three-level QC study was conducted three times a day for both incubation blocks (90 runs per block) at 8-h intervals in accordance with the laboratory’s routine practice.

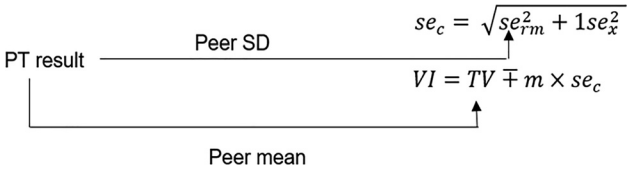
Accuracy studies

Method comparison: Initially, patient samples of different levels were tested using the new Abbott Alinity HQ analyzer and the routinely used

Abbott Cell Dyn Ruby analyzer. The results were then evaluated using Passing–Bablok regression analysis and Bland–Altman plots. To ensure a comprehensive comparison of various CBC parameters, we utilized a minimum of 120 patient samples. These samples were specifically selected from diverse departments, including but not limited to normal, hematological, oncological, adult, and pediatric units. Due to inherent workload and cost constraints, we maintained the sample size at around 120 for method comparison according to the CLSI EP09 guideline [7].

During the study, IQC materials from Abbott Diagnostics (IL, USA) were assessed every 8 h, and monthly proficiency testing (PT) was conducted using RANDOX quality controls (County Antrim, United Kingdom) to ensure the analytical performance of the Abbott Cell Dyn Ruby analyzer. All necessary corrective actions were performed according to the laboratory policy for IQC results, and all results were evaluated as acceptable for proficiency testing.

Estimation of the bias: The estimation of bias was performed using PT samples in bias studies, as they are prioritized over IQC samples according to the CLSI guideline. However, the study was designed to span three days due to constraints with the PT samples, allowing for only three replicates in the left incubation block. In accordance with CLSI EP15-A3 [6], four RANDOX quality control PT samples were used to evaluate whether the results obtained by the laboratory were within the verification limits. Grand means x were determined, and standard error of means (s_{ex}) were calculated using the s_r and s_{wl} values obtained from the precision study. Peer results from the PT were used as the target value (TV), and the standard error of the target value (se_{rm}) was calculated using peer-group standard deviation and number of peers. Using se_x and se_{rm} combined, standard error (se_c) of mean and TV were calculated. The multiplier factor (m) was calculated for four samples, and verification intervals (VI) were determined. The calculation steps are given in the following equations:where m is multiplication factor; PT is proficiency testing; SD is standard deviation; se_c is combined standard error; se_{rm} is standard error of the target value; se_x is standard error of means; TV is target value, and VI is verification interval.



Evaluation of limit of blank: The LoB is determined by analyzing multiple sample replicates with no analyte present and then computing the average result and the standard deviation. The determination of the LoB for WBCs, RBCs, and platelets (PLTs) was conducted in accordance with the CLSI EP17 guideline, utilizing 60 blank samples and employing the following formula [8]:

$$\mathrm{LoB} = M_B + C_p \times \mathrm{SD}_b$$

$$C_p = \frac{1.645}{1 - \left(\frac{1}{4(B-K)}\right)}$$

where LoB is limit of blank; M_B is mean of blank samples; SD_b is standard deviation of blank samples; C_p is multiplier; B is total number of blank results; and K is number of blank samples.

Carry-over: Carry-over was assessed by analyzing three pairs of samples [4, 5]. The data yield from a high-concentration sample followed by a low concentration could be evaluated by running sample A (high sample) three times (A1, A2, and A3), followed by sample B (low sample) three times (B1, B2, and B3). The percentage of carry-over for RBC, PLT, and WBC parameters was calculated as follows:

$$\mathrm{Carryover\%} = \left[(\mathrm{B1} - \mathrm{B3}) / (\mathrm{A3} - \mathrm{B3}) \right] \times 100$$

Statistical evaluation

All statistical analyses were performed using R statistical software 4.3.1. In this study, we utilized up-to-date imprecision and bias targets sourced from the biological variation (BV) due to its more rigorous criteria instead of adhering to the clinical decision levels outlined in CLSI H26-A2. The precision study conducted with patient samples was evaluated according to BV imprecision limits sourced from the EFLM Biological Variation database [14]. Repeatability and within-laboratory precision results for IQC materials were evaluated according to the manufacturer's claims. Finally, long-term precision results were assessed using the BV imprecision data. All limits related to precision and accuracy studies are given in Supplementary Table 1. The method comparison results were not normally distributed, and Spearman correlation coefficients were thus calculated for method comparisons. A degree of agreement between the same parameters analyzed with the two hematology analyzers was evaluated using the non-parametric Passing–Bablok regression method. The mean percentage differences were calculated from the Bland–Altman plots and were used to assess the agreement between the methods. The minimum allowable bias from the BV database was used as the limit of agreement [14].

Results

Precision study results

Short-term precision

Repeatability and within-laboratory imprecision

Based on the repeatability results (Table 1) regarding patient samples, it was observed that for the RBC measurements, the CV% values for all levels in both incubation blocks were consistently lower than the optimal analytical allowable imprecision ($\mathrm{CV_A}$) of 0.7 %, which was calculated in accordance with BV data. For the PLT measurements, the CV% for the low level was less than the minimum $\mathrm{CV_A}$ determined via BV data at 5.7 %. Moreover, the CV values were under the optimum $\mathrm{CV_A}$ of 1.9 % for the medium and high levels in the two incubation blocks. Finally, the WBC measurements showed that the CV values for all levels in both incubation blocks remained generally below the minimum $\mathrm{CV_A}$ as defined by BV data. An exception was seen in the left block readings, where the low-level CV value slightly exceeded the minimum $\mathrm{CV_A}$ threshold.

The results for the evaluation of repeatability CV (CVr) and within-laboratory CV (CVwl) values obtained according to manufacturer claims are given in Table 2 and Figure 1. As

Table 1: Repeatability study results conducted with patient samples.

Test	Level	Left block		Right block	
		Mean	CV, %	Mean	CV, %
WBC, $\times10^9$/L	Low	1.55	2.84	1.77	2.31
	Normal	4.6	1.75	5	1.62
	High	18.16	0.94	18.11	1.44
RBC, $\times10^{12}$/L	Low	2.88	0.386	2.88	0.216
	Normal	5.15	0.468	4.06	0.54
	High	6.87	0.282	6.28	0.62
PLT, $\times10^9$/L	Low	36.1	4.01	35,7	4.64
	Normal	230	1.58	213	1.66
	High	725	0.92	857	0.885

CV, coefficient of variation; WBC, white blood cell.

Table 2 shows, for both blocks, the CVwl values in level 1 and level 2 MCV measurements are above the verification limit calculated according to the CV values given by the manufacturer. Moreover, CVwl values in level 1 and level 2 MCHC QC measurements of the left block were above the compliance limit calculated according to the CV values given by the manufacturer. All other measurement results were within calculated limits.

Long-term precision

When the long-term precision data were analyzed, the CV values obtained for the basophil count at all levels and for eosinophil

Table 2: Repeatability and within-laboratory study results conducted with internal quality control (IQC) materials provided by the manufacturer.

	QC level	Manufacturer claim		Left block					Right block				
		CVr, %	CVwl, %	Mean	Actual CVr, %	Limit CVr, %	Actual CVwl, %	Limit CVwl, %	Mean	Actual CVr, %	Limit CVr, %	Actual CVwl, %	Limit CVwl, %
WBC, $\times10^9$/L	Level 1	4.46	4.46	3.2	2.33	5.97	2.56	5.84	3.12	1.66	5.97	1.66	5.84
	Level 2	2.5	2.5	7.19	1.28	3.35	1.28	3.28	7.04	1.95	3.35	1.95	3.28
	Level 3	2.5	2.5	16	1.09	3.35	1.35	3.28	1.57	1.18	3.35	1.52	3.28
Neutrophils, $\times10^9$/L	Level 1	5	5	1.42	4.86	6.7	4.91	6.55	1.37	4.43	6.7	4.58	6.55
	Level 2	5	5	3.28	3.22	6.7	3.22	6.55	3.24	3.46	6.7	3.46	6.55
	Level 3	5	5	8.02	2.67	6.7	2.67	6.55	7.88	2.79	6.7	2.92	6.55
Lymphocytes, $\times10^9$/L	Level 1	10.9	10.9	0.938	4.39	14.6	4.39	14.2	0.926	4.04	14.6	4.04	14.2
	Level 2	5	5	1.9	2.23	6.7	2.79	6.55	1.86	2.82	6.7	2.82	6.55
	Level 3	5	5	3.36	2.32	6.7	2.32	6.55	3.31	2.47	6.7	2.56	6.55
Monocytes, $\times10^9$/L	Level 1	17.6	17.6	0.393	9.07	23.6	9.53	23.1	0.379	9.53	23.6	9.53	23.1
	Level 2	10	10	0.91	9.32	13.4	9.32	13.1	0.896	8.16	13.4	8.74	13.1
	Level 3	10	10	2	6.81	13.4	6.81	13.1	1.89	7.23	13.4	7.23	13.1
Basophils, $\times10^9$/L	Level 1	50	50	0.043	26.3	67	27.9	65.5	0.036	31.6	67	33.8	65.5
	Level 2	50	50	0.094	27	67	27	65.5	0.087	24.3	67	24.5	65.5
	Level 3	20	20	0.182	16.3	26.8	17.3	26.2	0.176	15.6	26.8	15.9	26.2
Eosinophils, $\times10^9$/L	Level 1	50	50	0.069	12.4	67	12.4	65.5	0.064	17.1	67	17.1	65.5
	Level 2	29.4	29.4	0.17	6.84	39.4	6.84	38.5	0.166	14.1	39.4	14.1	38.5
	Level 3	12	12	0.462	6.18	16.1	6.43	15.7	0.458	5.91	16.1	5.91	15.7
RBC, $\times10^{12}$/L	Level 1	2.08	2.08	2.77	0.923	2.79	0.947	2.73	2.8	0.83	2.79	0.83	2.73
	Level 2	1.5	1.5	4.09	0.507	2.01	0.507	1.96	4.11	0.747	2.01	0.747	1.96
	Level 3	1.5	1.5	5.13	0.596	2.01	0.839	1.96	5.15	0.728	2.01	0.772	1.96
Hemoglobin, g/dL	Level 1	2.11	2.11	7.74	0.686	2.82	0.686	2.76	7.74	0.564	2.82	0.585	2.76
	Level 2	1.36	1.36	12	0.506	1.82	0.506	1.78	12	0.569	1.82	0.569	1.78
	Level 3	1	1	17	0.468	1.34	0.468	1.31	17	0.766	1.34	0.766	1.31
Hematocrit, %	Level 1	3.73	3.73	22.8	0.906	5	1.85	4.89	23	1.09	5	1.64	4.89
	Level 2	2.44	2.44	34.9	1.65	3.27	2.13	3.2	35.2	1.1	3.27	1.81	3.2
	Level 3	1.5	1.5	48.6	0.919	2.01	1.36	1.96	48.9	1.05	2.01	1.22	1.96
MCV, fL	Level 1	1	1	82.2	0.625	1.34	1.54	1.31	82.3	0.585	1.34	1.5	1.31
	Level 2	1	1	85.4	1.07	1.34	1.67	1.31	85.5	1	1.34	1.58	1.31
	Level 3	1	1	94.7	0.682	1.34	0.993	1.31	94.9	0.62	1.34	0.911	1.31
MCH, pg	Level 1	1.5	1.5	27.9	0.957	2.01	0.985	1.96	27.6	0.963	2.01	0.963	1.96
	Level 2	1.5	1.5	29.4	0.845	2.01	0.845	1.96	29.1	0.712	2.01	0.712	1.96
	Level 3	1.5	1.5	33	0.833	2.01	0.977	1.96	32.9	0.927	2.01	0.944	1.96
MCHC, g/L	Level 1	1.5	1.5	34	0.818	2.01	1.98	1.96	33.6	1.22	2.01	1.9	1.96
	Level 2	1.5	1.5	34.4	1.64	2.01	2.19	1.96	34	1.02	2.01	1.79	1.96
	Level 3	1.5	1.5	34.9	1.08	2.01	1.38	1.96	34.7	1.18	2.01	1.19	1.96

Table 2: (continued)

	QC level	Manufacturer claim		Left block					Right block				
		CVr, %	CVwl, %	Mean	Actual CVr, %	Limit CVr, %	Actual CVwl, %	Limit CVwl, %	Mean	Actual CVr, %	Limit CVr, %	Actual CVwl, %	Limit CVwl, %
RDW-SD, fL	Level 1	1.5	1.5	16	0.745	2.01	0.952	1.96	16	0.715	2,01	0.9	1.96
	Level 2	1	1	17.2	0.432	1.34	0.559	1.31	17.2	0.484	1.34	0.526	1.31
	Level 3	1.5	1.5	15.6	0.315	2.01	0.495	1.96	15.6	0.366	2.01	0.408	1.96
PLT, $\times 10^9$/L	Level 1	4	4	68.3	2.21	5.36	2.4	5.24	69.5	2.82	5.36	3.18	5.24
	Level 2	4	4	215	1.37	5.36	1.37	5.24	219	1.46	5.36	1.46	5.24
	Level 3	4	4	480	1.29	5,36	1.81	5.24	488	1.6	5.36	1.97	5.24
MPV, fL	Level 1	4.24	4.24	7.05	1.23	5.69	1.23	5.56	7.07	1.4	5.69	1.49	5.56
	Level 2	4.24	4.24	7.05	0.677	5.69	0.71	5.56	7.08	0.431	5.68	0.536	5.55
	Level 3	4.24	4.24	7.03	0.452	5.69	0.505	5.56	7.07	0.379	5.69	0.394	5.56

CV, coefficient of variation; CVr, repeatability CV; CVwl, with-in laboratory CV.

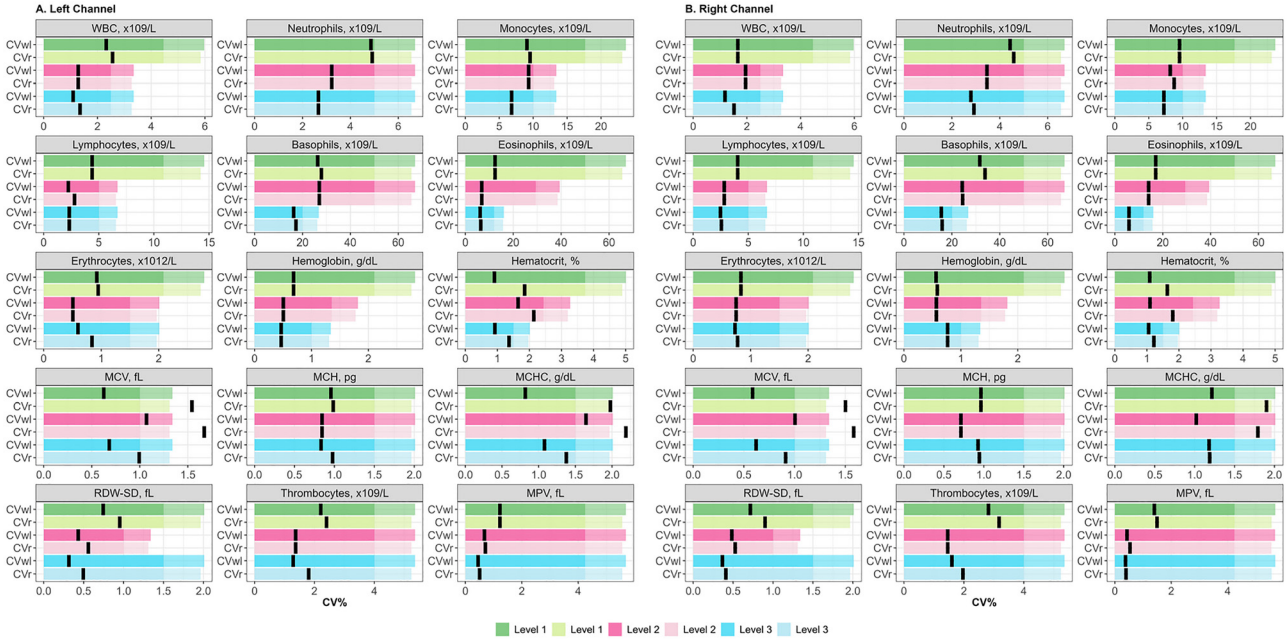


Figure 1: Internal quality control (IQC) precision study results. Black line represents actual coefficient of variation (CV) values. CV% values are plotted on the x-axis, with repeatability and within-lab CVs depicted on the y-axis. Various colors distinguish different QC sample levels. Solid bars represent manufacturer claims, while transparent bars indicate extended verification limits obtained from the precision study. A black bar highlights acquired CV% values. Black line represents actual CV values. Green, pink and blue bars represent level 1, level 2 and level 3 QC materials respectively. Dark colors indicate manufacturer claims and transparent colors indicate limits calculated according to the CLSI EP15A3. CV, coefficient of variation; CVr, repeatability CV; CVwl, within-laboratory CV.

and monocyte counts at level 1 were above the minimum CV_A value determined according to BV data. Similarly, the MCH, MCHC, and RDW-SD parameters also exhibited values above the minimum permitted CV_A for all levels and blocks. In terms of MCV values, the level-1 CV for both blocks and level-2 CV for the right block were higher than CV_A. In addition, level three CV values for RBC and hematocrit measurements were above the minimum permitted CV_A (Table 3). All other measurement results were found to follow the BV claims.

Accuracy study results

Method comparison

In the patient result comparison, the correlation coefficients varied from 0.46 to 1.00, as shown in Table 4. According to the Passing–Bablok regression analysis results, the slope confidence interval included 1, and the intercept confidence interval included 0 only for the eosinophil and monocyte

Table 3: Intermediate precision for 30 days using manufacturer internal quality control (IQC) materials.

	Left block						Right block					
	Low level		Normal level		High level		Low level		Normal level		High level	
	Mean	CV, %	Mean	CV, %	Mean	CV, %	Mean	CV, %	Mean	CV, %	Mean	CV, %
WBC, $\times10^9$/L	2.99	2.49	6.79	2.30	15.80	1.35	2.98	2.58	6.76	1.74	15.87	1.38
Neutrophils, $\times10^9$/L	1.37	4.01	3.41	2.81	8.35	2.17	1.37	4.26	3.39	2.81	8.39	2.47
Monocytes, $\times10^9$/L	0.32	12.05	0.71	9.20	1.70	9.98	0.31	12.20	0.71	8.69	1.69	9.05
Lymphocytes, $\times10^9$/L	0.89	4.86	1.67	4.93	3.29	3.53	0.88	5.16	1.65	3.82	3.30	3.06
Basophils, $\times10^9$/L	0.03	35.36	0.08	27.96	0.16	24.30	0.03	44.71	0.08	31.32	0.15	21.47
Eosinophils, $\times10^9$/L	0.07	16.48	0.20	8.96	0.50	5.08	0.07	16.98	0.20	9.21	0.50	5.46
RBC, $\times10^{12}$/L	2.86	1.38	4.12	1.86	4.97	3.19	2.85	1.26	4.12	1.63	4.96	2.80
Hemoglobin, g/dL	7.99	1.55	11.80	0.94	17.28	1.84	8.00	1.58	11.84	1.22	17.32	1.94
Hematocrit, %	25.12	1.28	36.78	1.74	50.33	3.10	25.14	1.23	36.81	1.58	50.28	2.66
MCV, fL	87.98	0.64	89.21	0.54	101.21	0.52	88.08	0.70	89.34	0.65	101.29	0.58
MCH, pg	27.97	1.81	28.62	1.46	34.76	2.45	28.05	1.64	28.74	0.94	34.91	2.15
MCHC, g/L	31.79	1.46	32.07	1.28	34.35	2.28	31.84	1.43	32.17	1.02	34.47	2.00
RDW-SD, fL	12.33	2.59	12.14	2.14	11.07	1.54	12.33	2.65	12.17	2.17	11.07	1.49
PLT, $\times10^9$/L	72.46	3.44	227.17	1.99	462.77	2.35	72.64	3.26	226.99	2.05	461.79	2.82
MPV, fL	8.95	0.80	8.96	0.55	8.98	0.54	8.95	0.72	8.96	0.60	8.98	0.61

CV, coefficient of variation.

tests (Figure 2). The confidence intervals for the other tests were extremely close to the desired intervals, except for the MCHC and basophil tests. When the Bland–Altman plots were analyzed, a concentration-dependent pattern was observed in the MCHC, MCH, and MPV parameters, including the calculation shown in Figure 3.

Table 4: Method comparison results.

Test	n	Range		Spearman correlation	Passing–Bablok regression analysis		Bland–Altman	Limit of agreement[a], %
		Abbott Rubby	Abbott Alinity HQ	R	Slope, CI	Intercept, CI	Mean % diff. (mean absolute diff.)	
WBC, $\times10^9$/L	138	0.29–14.6	0.27–13.9	1.00	0.96 (0.95–0.97)	0.07 (0.00–0.15)	2.8 (0.20)	7.4
Neutrophils, $\times10^9$/L	137	0.019–12.1	0.004–11.4	1.00	0.96 (0.94–0.97)	0.03 (0.00–0.08)	4.4 (0.15)	10.3
Monocytes, $\times10^9$/L	137	0.047–1.18	0.030–1.22	0.92	1.04 (0.99–1.09)	−0.01 (−0.04 to 0.01)	−2.0 (−0.02)	9.7
Lymphocytes, $\times10^9$/L	138	0.037–5.41	0.055–5.26	0.99	0.97 (0.95–0.98)	0.00 (−0.03 to 0.02)	2.8 (0.06)	9.4
Basophils, $\times10^9$/L	120	0.007–0.14	0.001–0.082	0.46	0.66 (0.50–0.83)	−0.02 (−0.03 to −0.01)	87.1 (0.04)	10.9
Eosinophils, $\times10^9$/L	129	0.003–0.67	0.003–0.67	0.96	0.99 (0.95–1.03)	0.00 (−0.01 to 0.00)	3.2 (0.00)	25.2
Erythrocytes, $\times10^{12}$/L	137	2.06–6.35	2.00–5.94	0.99	0.91 (0.89–0.92)	0.18 (0.11–0.26)	5.2 (0.23)	2.6
Hemoglobin, g/dL	137	4.90–17.7	5.15–17.4	1.00	0.96 (0.94–0.97)	0.49 (0.35–0.62)	0.3 (0.06)	2.4
Hematocrit, %	138	16.0–53.6	15.3–50.8	0.96	0.95 (0.91–0.98)	0.86 (−0.33 to 2.07)	3.0 (1.11)	2.2
MCV, fL	142	70.5–105	65.4–110	0.93	1.25 (1.18–1.33)	−20.14 (−26.74 to −13.83)	−2.1 (−2.02)	1.4
MCH, pg	137	22.1–36.0	23.1–37.4	0.97	1.00 (0.95–1.04)	1.54 (0.40–2.76)	−4.8 (−1.41)	1.7
MCHC, g/L	139	30.0–36.0	32.2–35.8	0.59	0.50 (0.42–0.60)	17.28 (14.13–20.02)	−2.6 (−0.85)	0.6
RDW-SD, fL	140	9.66–20.2	10.5–23.1	0.91	1.00 (0.93–1.08)	1.18 (0.24–2.03)	−9.5 (−1.26)	1.7
PLT, $\times10^9$/L	137	36.8–597	33.7–665	0.98	1.04 (1.01–1.07)	−4.87 (−12.52 to 0.37)	−1.5 (−5.01)	7.6
MPV, fL	136	4.85–10.0	5.65–10.4	0.90	0.81 (0.75–0.89)	2.04 (1.56–2.47)	−10.3 (−0.72)	2.8

CI, confidence interval; Diff., difference. [a]The minimum allowable bias from BV database.

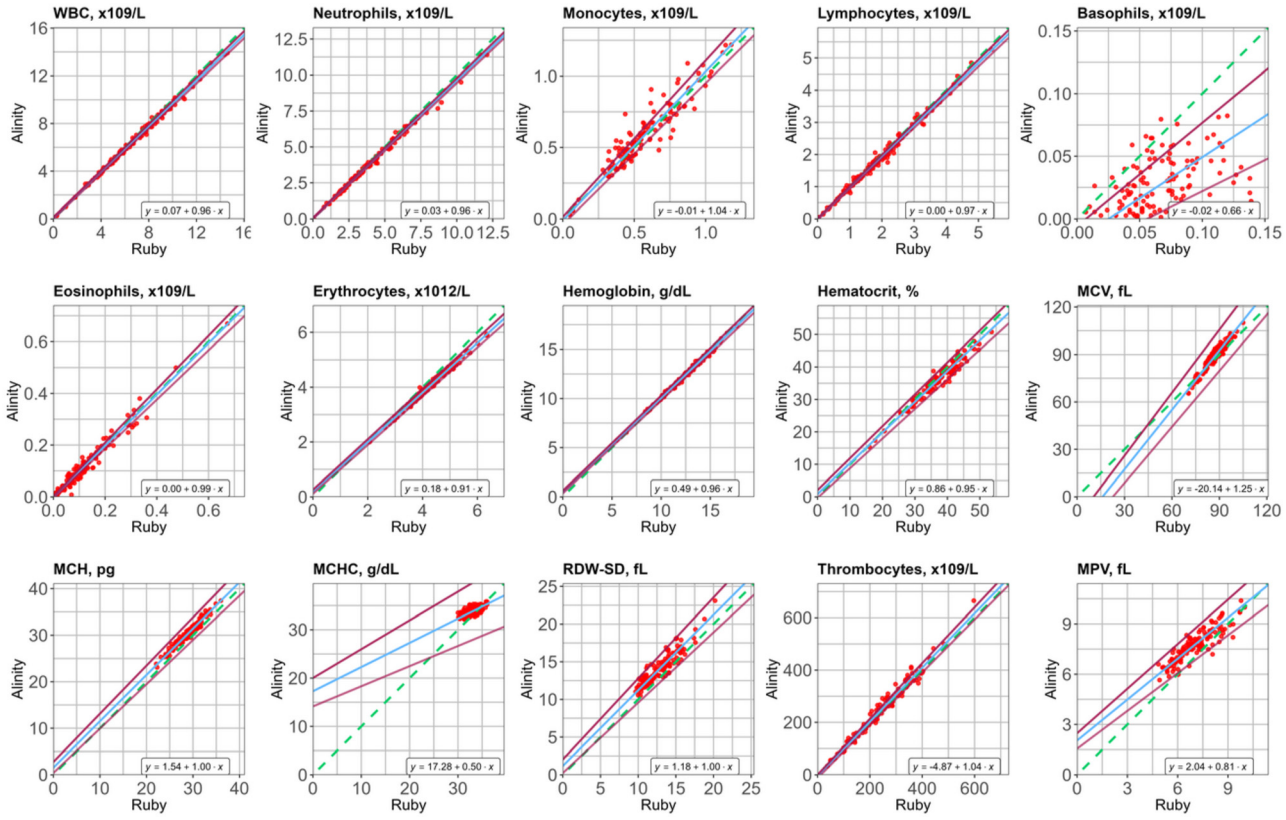


Figure 2: Passing–Bablok regression analysis. Identity lines (y=x) are dashed green, confidence intervals are claret and regression lines are blue.

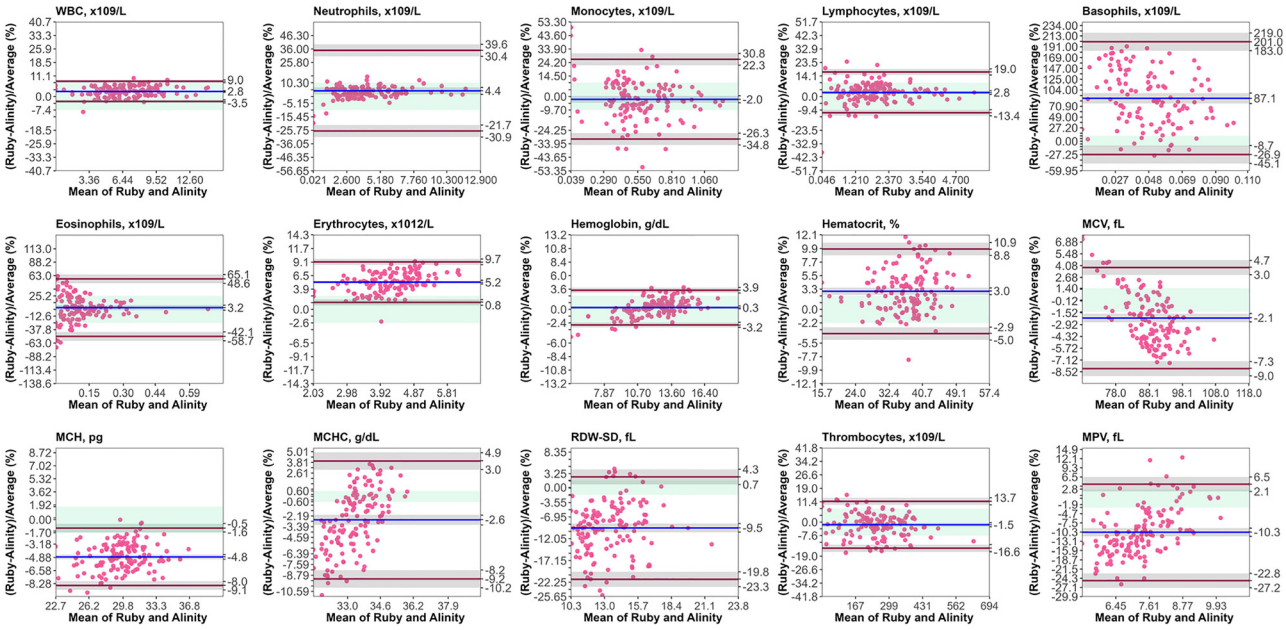


Figure 3: Bland–Altman plots for method comparison study. y=0 lines are dashed green, mean differences (%) are blue, confidence intervals are solid gray. Second y axis represents CIs. Green area indicates the limit of agreement (minimum allowable bias 24.17 %).

Evaluation of the estimation of bias

The results of all parameters were within the verification ranges, except for the low-level QC results of PLT, as shown in Table 5.

Limit of blank

The LoB was calculated as 0.086×10^9/L for WBCs, 0.002×10^{12}/L for RBCs, and 0.331×10^9/L for PLTs, below the manufacturer's claims, as shown in Supplementary Table 2.

Table 5: Estimation of bias according to the CLSI EP15-A3.

Test	Grand mean (x)[a]	se_x	Proficiency testing				Verification interval
			Sample	se_{rm}	$m.se_c$	Target value (peer mean)	
WBC, $\times 10^9$/L	6.00	0.061	1	0.040	0.24	6.00	5.77–6.24
	2.87	0.023	2	0.018	0.089	2.86	2.77–2.94
	9.12	0.041	3	0.054	0.18	8.99	8.81–9.18
	14.00	0.11	4	0.090	0.41	14.3	13.9–14.7
RBC, $\times 10^{12}$/L	5.16	0.018	1	0.026	0.083	5.12	5.04–5.20
	2.01	0.012	2	0.010	0.045	2.00	1.96–2.05
	4.52	0.014	3	0.022	0.070	4.50	4.43–4.56
	3.88	0.009	4	0.018	0.053	3.87	3.81–3.92
Hemoglobin, g/dL	15.70	0.035	1	0.058	0.18	15.7	15.5–15.9
	6.21	0.024	2	0.022	0.093	6.24	6.15–6.34
	13.60	0.027	3	0.049	0.15	13.6	13.4–13.7
	12.40	0.027	4	0.044	0.14	12.4	12.2–1.5
Hematocrit, %	37.50	0.28	1	0.077	1.12	37.5	36.4–38.7
	15.30	0.19	2	0.12	0.71	15.0	14.3–15.7
	32.00	0.33	3	0.24	1.27	31.4	30.1–32.7
	30.50	0.33	4	0.25	1.29	30.2	28.9–31.5
MCV, fL	72.60	0.57	1	0.54	2.30	73.1	70.8–75.4
	75.90	0.57	2	0.49	2.21	75.6	73.4–77.8
	71.00	0.57	3	0.44	2.18	69.5	67.4–71.7
	78.60	0.57	4	0.53	2.25	78.0	75.8–80.3
MCH, pg	30.50	0.11	1	0.18	0.56	30.5	30.0–31.1
	30.80	0.11	2	0.17	0.55	31.3	30.7–31.8
	30.20	0.11	3	0.17	0.54	30.2	29.6–30.7
	31.90	0.14	4	0.19	0.62	32.0	31.3–32.6
MCHC, g/L	42.00	0.21	1	0.35	1.12	41.8	40.7–42.9
	40.70	0.21	2	0.31	1.01	41.1	40.1–42.1
	42.60	0.21	3	0.34	1.06	43.2	42.1–44.3
	40.60	0.21	4	0.33	1.04	40.8	39.8–41.8
RDW-SD, fL	15.00	0.028	1	0.20	0.55	15.2	14.6–15.7
	16.70	0.040	2	0.19	0.52	16.9	16.4–17.4
	16.50	0.068	3	0.18	0.53	16.7	16.2–17.3
	16.70	0.040	4	0.21	0.58	17.2	16.6–17.7
PLT, $\times 10^9$/L	92.80	0.99	1	1.90	5.59	85.3	79.7–90.9
	64.10	0.73	2	1.36	4.03	66.0	62.0–70.1
	242.00	1.43	3	4.81	13.0	234	221–247
	491.00	4.30	4	10.4	29.4	477	448–506
MPV, fL	5.60	0.016	1	0.064	0.18	5.53	5.35–5.71
	5.87	0.016	2	0.065	0.17	5.98	5.81–6.16
	5.43	0.016	3	0.059	0.16	5.47	5.31–5.63
	5.43	0.016	4	0.056	0.15	5.36	5.21–5.52

m, multiplication factor; se_c, combined standard error; se_{rm}, standard error of the target value; se_x, standard error of means. [a]Grand mean must be between verification interval.

Carry-over

The carry-over results for all three parameters were between 0 and 1 %, below the manufacturer's claims, as shown in Supplementary Table 3.

Discussion

In this study, we assessed the analytical performance characteristics of the Abbott Alinity HQ automated hematology system. We concurrently compared the Alinity HQ with the Cell Dyn Ruby hematology analyzer, the latter of which is routinely used in our hospital laboratory. Our short-term precision results for IQC materials, patient samples, and bias estimation were within an acceptable range. However, the long-term precision results did not meet the BV-based acceptability criteria.

We conducted an extended repeatability study using both patient samples and IQC material for short-term precision. Based on the repeatability results with patient samples, the CV values for RBC measurement in both incubation blocks fell below the optimal CV_A calculated according to BV data. In addition, the CV for PLT measurement was below the minimum CV_A based on BV data. In terms of WBC measurement, the CV values in both incubation blocks were below the BV-calculated minimum CV_A, with the left incubation block slightly exceeding this value. These results suggest that the analyzer demonstrated high performance for repeatability. Determining the repeatability was important to determining the test quality of the analyzer and confirming the good repeatability of analyzing samples with minimum differences.

In the short-term precision study conducted using IQC materials, CVr and CVwl values were calculated for both incubation blocks. The CV values obtained were below the limits claimed by the manufacturer for almost all tests. The CVwl values in level-1 and level-2 MCV QC measurements for both blocks and the MCHC at level-1 and level-2 QC measurements of the left block were above the compliance limit calculated according to the CV values given by the manufacturer. This was an interesting finding; both incubation blocks had similarly higher CVs for the same tests.

When the long-term precision data were analyzed, the CV values obtained for basophils at all levels and the eosinophil and monocyte counts at level-1 samples were above the minimum allowable CV_A. Similarly, the MCH, MCHC, and RDW-SD parameters also exhibited values above the minimum permitted CV_A for all levels and blocks. Furthermore, the CV values obtained in the RBC, hematocrit, and MCV measurements were above the minimum allowable CV_A. The poor performance of the long-term precision results compared with the short-term precision results was thought to be due to the relatively low stability of the hematology IQC materials and the use of a new IQC bottle, albeit with the same lot, approximately every 7 days during the one-month period.

In comparing the Alinity HQ analyzer with the Cell Dyn Ruby, the correlation coefficients varied from 0.46 to 1.00. For the eosinophil and monocyte tests, the Passing–Bablok results indicated that the slope confidence interval included 1, and the intercept confidence interval included 0. However, except for the MCHC and basophil tests, the confidence intervals were extremely close to the desired intervals. Another interesting finding from the Bland–Altman plots was the concentration-dependent pattern observed in the MCHC and MCH parameters. It was thought that the differences in the measurement methods of both devices may have caused this pattern. The lower level of correlation among the various parameters, such as differential monocyte, eosinophil, basophil counts, and calculated parameters (e.g., RBC indices and PLT counts) in the thrombocytopenic range, is documented in most analyzers [9–12]. Khodaiji et al. [15] observed that the correlation of results between ELite 580 and LH 780 was $r^2 \geq 0.92$ except for MCHC (0.35), MPV (0.88) and basophils (0.06). They also found satisfying estimated bias results for all WBC differential counts except for basophils in the same study.

According to the published studies, the variability is usually greater than intra-patient variation when comparing two hematology devices because analyzers are independent analytical systems [3, 4, 16]. The use of various methods of determination is one of the reasons for the variability, as well as the use of calibrators and quality control materials from multiple manufacturers. Reflecting on this, we believe that these factors may have come into play in our study. We surmise that the reason for the incompatible results in the method comparison in this study may be related to how the Alinity HQ employs a combination of photometry, optical flow cytometry, fluorescence analysis, and advanced software algorithms. In contrast, Cell Dyn Ruby's limited technology uses a less sensitive optical method.

Due to the technological differences between the Alinity HQ and Cell Dyn Ruby analyzers, bias estimation was performed to evaluate the accuracy, in addition to the method comparison study. Our study included assessing results obtained from the precision study for trueness estimation. We evaluated the statistical significance of the actual bias as per EP15-A3 guidelines, for which we followed a step-wise approach toward finding the verification interval of the

target value, which had a 95 % probability of true difference. For all parameters, the estimated bias in this study was within the Vis, except for the low-level QC results for PLT count.

According to the relevant guidelines and previous studies, a LoB check of the hematology analyzer is important. The manufacturer defines the limit of background electronic noise; for optimal conditions, the background counts should be zero. This is especially important in body fluids with extremely low cell counts, such as cerebrospinal fluid [4, 5, 17]. Here, the LoB was calculated as 0.086×10^9/L for WBCs, 0.002×10^{12}/L for RBCs, and 0.331×10^9/L for PLTs, with all below the manufacturer's claims.

The carry-over values in this analytical performance study were better than those recommended by the Alinity HQ manufacturer; the carry-over for WBC and PLT must be 1 % and for RBC≤0.5 %. These data showed that there was no transfer of material from one sample to the next, and the influence of contamination was close to zero. Many published studies recommend additional washing between one sample and the next to avoid a background count carry-over [18–20].

A key strength of our study is that while the CLSI H26 guideline specifies targets only for WBC, RBC, hemoglobin, hematocrit, and PLT parameters, we expanded the performance evaluation to include both repeatability and bias for WBC subgroups and RBC indices. Another strength was the innovative precision plot, which succinctly represents CV values, facilitating the evaluation of obtained CVs and enabling users to quickly identify any out-of-range values.

The one limitation of the study is that bias and LoB studies were performed in a single incubation block due to the limited amount of both patient and EQC samples. However, the precision studies performed in both incubation blocks enabled us to demonstrate the agreement between them.

Conclusions

In conclusion, despite some measurements not meeting expected requirements, the performance evaluation and comparative analyses of the automated Alinity HQ hematology system indicated an acceptable analytical performance. Furthermore, the laboratory results were comparable to those of the established laboratory practice analyzers. Alinity HQ can be useful for all laboratories, mainly those with a large number of normal and abnormal samples. Our research also showed that conducting long-term precision for hematology analyzers can be challenging compared with when dealing with chemistry analyzers.

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References

1. Bruegel M, George TI, Feng B, Allen TR, Bracco D, Zahniser DJ, et al. Multicenter evaluation of the cobas m 511 integrated hematology analyzer. Int J Lab Hematol 2018;40:672–82.
2. Woo KS, Jeong IH, An GD, Lim HH, Han JY. Performance evaluation of new Abbott Alinity HQ hematology analyzer. Int J Lab Hematol 2019;41: 657–63.
3. Clinical Laboratory Standards Institute. H20-A2: reference Leukocyte (WBC) differential count (proportional) and evaluation of instrumental methods, 2nd ed. Wayne, PA: Clinical Laboratory Standards Institute; 2007.
4. Briggs C, Culp N, Davis B, d'Onofrio G, Zini G, Machin SJ. ICSH guidelines for the evaluation of blood cell analysers including those used for differential leucocyte and reticulocyte counting. Int J Lab Hematol 2014; 36:613–27.
5. Clinical and Laboratory Standards Institute (CLSI). H26-A2: validation, verification and quality assurance of automated hematology analyzers, 2nd ed. Wayne (PA): CLSI document; 2010.
6. Clinical and Laboratory Standards Institute (CLSI). EP15-A3: user verification of precision and estimation of bias; approved guideline, 3rd ed. Wayne (PA): CLSI Document; 2014.
7. Clinical and Laboratory Standards Institute (CLSI). EP09c: measurement procedure comparison and bias estimation using patient samples, 3rd ed. Wayne (PA): CLSI Document; 2018.
8. Clinical and Laboratory Standards Institute (CLSI). EP17-A2: evaluation of detection capability for clinical laboratory measurement procedures, 2nd ed. Wayne (PA): CLSI Document; 2012.
9. ISO. Medical laboratories – particular requirements for quality and competence ISO 15189. Geneva: ISO; 2012.
10. Vis JY, Huisman A. Verification and quality control of routine hematology analyzers. Int J Lab Hematol 2016;38:100–9.
11. Van der Beken Y, Van Dalem A, Van Moer G, Segers E, Damiaens S, Hoffmann J, et al. Performance evaluation of the prototype Abbott Alinity hq hematology analyzerInt. J Lab Hematol 2019;41: 448–55.
12. Lakos G. Calibration and QC guidance for the Abbott Alinity HQ hematology analyzer. In: Diagnostics. Santa Clara, CA: Abbott; 2018; 1–7 pp.
13. Lehto T, Hedberg P. Performance evaluation of Abbott CELL-DYN Ruby for routine use. Int J Lab Hematol 2008;30:400–7.

14. Aarsand AK, Fernandez-Calle P, Webster C, Coskun A, Gonzales-Lao E, Diaz-Garzon J, et al. The EFLM biological variation database. https://biologicalvariation.eu/ [Accessed August 2023].
15. Khodaiji S, Mansukhani D. Performance evaluation of fully automated 5-Part differential hematology analyzer ELite 580 (ERBA). Indian J Hematol Blood Transfus 2022;38:99–105.
16. Becker PH, Fenneteau O, Da Costa L. Performance evaluation of the Sysmex XN-1000 hematology analyzer in assessment of the white blood cell count differential inpediatric specimens. Int J Lab Hematol 2016;38:54–63.
17. Verbrugge SE, Huisman A. Verification and standardization of blood cell counters for routine clinical laboratory tests. Clin Lab Med 2015;35:183–96.
18. Nakul-Aquaronne D, Sudaka-Sammarcelli I, Ferrero- Vacher C, Starck B, Bayle J. Evaluation of the Sysmex Xe-2100 hematology analyzer in hospital use. J Clin Lab Anal 2003;17:113–23.
19. Maciel TS, Comar SR, Beltrame MP. Performance evaluation of the Sysmex® XE-2100D automated hematologyanalyzer. J Bras Patol Med Lab 2014;50:26–35.
20. Kaplan SS, Johnson K, Wolfe N, Brown W, Keeney M, Gray-Statchuk L, et al. Performance characteristics of the Coulter LH 500 hematology analyzer. Lab Hematol 2004;10:76–87.

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