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Performance of digital morphology analyzer Medica EasyCell assistant

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Abstract

Objectives: The EasyCell assistant (Medica, Bedford, MA, USA) is one of the state-of-the-art digital morphology analyzers. We explored the performance of EasyCell assistant in comparison with manual microscopic review and Pentra DX Nexus (Horiba ABX Diagnostics, Montpellier, France).

Methods: In a total of 225 samples (100 normal and 125 abnormal samples), white blood cell (WBC) differentials and platelet (PLT) count estimation by EasyCell assistant were compared with the results by manual microscopic review and Pentra DX Nexus. The manual microscopic review was performed according to the Clinical and Laboratory Standards Institute guidelines (H20-A2).

Results: WBC differentials between pre-classification by EasyCell assistant and manual counting showed moderate correlations for neutrophils ($r=0.58$), lymphocytes ($r=0.69$), and eosinophils ($r=0.51$) in all samples. After user verification, they showed mostly high to very high correlations for neutrophils ($r=0.74$), lymphocytes ($r=0.78$), eosinophils ($r=0.88$), and other cells ($r=0.91$). PLT count by EasyCell assistant highly correlated with that by Pentra DX Nexus ($r=0.82$).

Conclusions: The performance of EasyCell assistant for WBC differentials and PLT count seems to be acceptable even in abnormal samples with improvement after user verification. The EasyCell assistant, with its reliable

performance on WBC differentials and PLT count, would help optimize the workflow of hematology laboratories with reduced workload of manual microscopic review.

Keywords: comparison; digital morphology; EasyCell assistant; manual microscopic review; performance.

Introduction

Complete blood count (CBC) with white blood cells (WBCs) differentials is one of the most frequently requested tests in clinical hematology laboratories [1]. Advances in automated hematology analyzers have improved their analytical performances greatly, and as a result, the proportion of manual microscopic review (MMR) has been diminished 10–15 percent or less in many clinical settings [2]. Although the MMR is an unavoidable, important process in resolving the ambiguities of flags from automated hematology analyzers, it is labor-intensive, time-consuming, technically-demanding, and vulnerable to intra and inter-observer variability [1, 3, 4].

New ways for cell analysis using digital image processing techniques have been developed to provide reliable data [5]. Compared with MMR, an automated digital morphology (DM) analyzer system, in combination with a fully-automated hematology platform, would provide a more rapid and standardized differential result [6]. It would help decrease variability with subjectivity and allow for increased reproducibility between samples and operators [7, 8]. It would provide greater time savings by the less-trained laboratory personnel; in addition, it would enable digital archiving and retrieval of blood films and allows remote review of blood films [9]. Previous studies on clinical applications of automated DM analyzers have shown potential benefits and some limitations [10–17].

The EasyCell assistant (Medica, Bedford, MA, USA) is a fully automated DM analyzer, which is co-operable with Pentra DX Nexus (DX, Horiba ABX Diagnostics, Montpellier, France) including automated slide making/staining device; thus, it allows a single sample placed on an automation line to produce CBC results, smear preparation, and digital cell location. Alike the widely used CellaVision DM96 system

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(DM96, CellaVision, Lund, Sweden) and Sysmex DI-60 system (DI-60, Sysmex, Kobe, Japan), EasyCell assistant can pre-classify WBC differentials and estimate platelets (PLT) count on peripheral blood smears [13]. The International Council for Standardization in Hematology (ICSH) guidelines recommend evaluating all performance characteristics when a new DM analyzer is launched [18]; however, no peer-reviewed literature is available on EasyCell assistant [19]. In this study, we wanted to explore the performance of EasyCell assistant with regard to WBC differentials and PLT count, in comparison with Pentra DX Nexus and MMR.

Materials and methods

Study samples

This *in vitro* evaluation study was conducted in Konkuk University Medical Center (KUMC), Seoul, Korea, using a total of 225 samples (100 normal and 125 abnormal samples). These samples were either leftover blood samples or umbilical cord blood (CB) samples that would have been discarded. This study required neither study-specific intervention nor additional blood collection, and the study protocol was designed following the criteria of the Declaration of Helsinki; therefore, the study protocol of this study was exempted from the approval of the Institutional Review Board of KUMC (KUH1200063) before collecting the first sample. Written informed consent was obtained from the mother of each neonate for the use of CB samples and was waived for the use of leftover blood samples.

Normal samples were obtained from healthy individuals who showed unremarkable findings in routine physical check-up. Abnormal samples consisted of 42 samples from CB and 83 samples from the patients with abnormal CBC findings and/or hematologic disorders (Table 1). The samples were obtained from the antecubital veins of participants in K₃-EDTA-containing vacuette (Greiner Bio-One GmbH, Frickenhausen, Germany). CB samples (3 mL) were obtained directly from umbilical veins of neonates using syringes and transferred immediately into K₃-EDTA containing vacuette (Greiner Bio-One GmbH). These samples were analyzed using Pentra DX Nexus – an automated CBC analyzer with integrated slide-maker and stainer (SPS evolution) – within 4 h after collection. The slides were prepared and stained automatically using SPS evolution and Wright Giemsa, respectively. Slides were reviewed according to the Clinical and Laboratory Standards Institute (CLSI) H20-A2 guidelines [3]. Two hematology experts scanned the slides at low magnification using light microscopy and counted 200 cells each on each slide at 200× magnification; an additional slide was processed if the counted cells on each slide were less than 200 cells [3]. The average values of the results obtained from two experts were used for the evaluation. The data from EasyCell assistant (pre-classification and verification), Pentra DX Nexus, and MMR were compared one another.

EasyCell assistant

The EasyCell assistant consists of an optic unit consisting of a microscope, camera, up to 30 position slide carousels with the capability of

Table 1: Study population and counted cells per slide of samples by EasyCell assistant.

Sample	Counted cells per slide, median (IQR)
Total (n=225)	220 (182–220)
Normal samples (n=100)	220 (220–220)
Abnormal samples (n=125)	213 (103–220)
Cord blood (n=42)	219 (148–220)
CBC abnormality in routine work-up (n=20)	220 (220–220)
AML (n=18)	115 (62–211)
Malignant lymphoma (n=10)	172 (57–220)
PCM (n=7)	216 (120–220)
ALL (n=6)	95 (76–157)
MPN (n=6)	130 (44–162)
Others ^a (n=16)	NA
Abnormal samples with leukopenia ^b (n=45)	172 (78–220)
Mild (n=34)	220 (134–220)
Moderate (n=4)	110 (83–152)
Severe (n=7)	29 (14–58)

^aOthers include aplastic anemia, asthma, brain tumor, hepatocellular carcinoma, immune thrombocytopenia, mixed phenotypic acute leukemia, myelodysplastic syndrome, primary amyloidosis, and systemic lupus erythematosus; the data in this group (with n<5 samples in each disease) is not expressed as median. ^bMild, $2.0\text{--}4.0 \times 10^9/\text{L}$; moderate, $1.0\text{--}2.0 \times 10^9/\text{L}$; severe, $<1.0 \times 10^9/\text{L}$. AML, acute myeloid leukemia; IQR, interquartile range; MPN, myeloproliferative neoplasm; NA, not available; PCM, plasma cell myeloma.

loading STAT slides as well, a slide gripping and positioning system, and a computer system containing the acquisition, pattern recognition, and classification software. The motorized microscope has two objectives (10× and 100×). Each slide can be scanned for WBC images in 4.5 min and for WBC, PLT, and red blood cells (RBCs) images in 5.5 min. The EasyCell assistant monitor consists of a 19" diagonal and/or widescreen LCD display with resolution of $1,280 \times 1,024$ or $1,440 \times 900$, 32 bit color quality, and 96 DPI settings. Up to 10,000 smear images can be stored electronically.

WBC differentials and PLT count

For the evaluation of WBC differentials, 200 cells on each smear were pre-classified and verified independently by the same, two hematology experts. EasyCell assistant pre-classifies WBC classes, including eight leukocyte categories (segmented neutrophils, banded neutrophils, lymphocytes, monocytes, eosinophils, basophils, variant lymphocytes, and others) and two non-leukocyte categories (smudge cells and nucleated RBCs [NRBCs]). Others leukocyte category includes immature, abnormal, and unrecognized cells, and it is subjected to further review. After verification, five leukocyte categories (metamyelocytes, myelocytes, promyelocytes, blasts, and plasma cells) can be further designated.

Concordance was evaluated between EasyCell assistant pre-classification and verification in normal and abnormal samples. The correlation of cell classification was evaluated across EasyCell assistant (pre-classification and verification), MMR, and Pentra DX Nexus. The number of WBC count can be set up as an option in EasyCell

assistant (100 or 200 cells), and this was set by counting 200 cells for the further evaluation of detection performance in the 46 leukopenic samples. These leukopenic samples were further divided into three groups: mild leukopenia ($2.0\text{--}4.0 \times 10^9/\text{L}$, $n=34$), moderate leukopenia ($1.0\text{--}2.0 \times 10^9/\text{L}$, $n=4$), and severe leukopenia (less than $1.0 \times 10^9/\text{L}$, $n=7$).

PLT count is estimated in EasyCell assistant by counting PLTs automatically in the 48 images (12 square field captured images $\times$ four pages) and multiplied by the preset estimation factor that is calculated in the demonstration setting; the estimation factor was set as 2,500 in this study as suggested by the manufacturer. PLT count estimated by EasyCell assistant was compared with PLT count by Pentra DX Nexus in all 225 samples. With the PLT count of $100 \times 10^9/\text{L}$, the total samples were divided into two groups: $<100 \times 10^9/\text{L}$ ($n=31$) and $\geq 100 \times 10^9/\text{L}$ ($n=194$).

Statistical analysis

Data were expressed as median (interquartile range, IQR) or number (percentage, %). MMR and/or Pentra DX Nexus results was considered gold standard for each comparison, appropriately. On the basis of verification, the qualitative performance of preclassification by EasyCell assistant was evaluated by sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and efficiency according to the CLSI EP12-ED3 guidelines [20]. Our sample size fulfilled the minimum requirement recommended by the CLSI guidelines (100 samples for assay comparison) [21].

For comparison of WBC classification, we used seven cell classes (neutrophils, lymphocytes, monocytes, eosinophils, basophils, other cells, and nRBCs); other cells included left-shifted neutrophils, blasts, and plasma cells. They were compared using Passing-Bablok regression analysis and Bland-Altman plot, according to the CLSI EP09C-ED3 guidelines [21]. In Passing-Bablok regression, Pearson's correlation coefficients (r) with 95 % confidence interval (CI) were obtained and interpreted as follows: <0.30 , negligible; $0.30\text{--}0.50$, low; $0.50\text{--}0.70$, moderate; $0.70\text{--}0.90$, high; $0.90\text{--}1.00$, very high [22]. In Bland-Altman plot, mean difference with 95 % CI were compared in seven cell classes. Passing-Bablok regression analysis and Bland-Altman plot were also used to compare PLT count between EasyCell assistant and Pentra DX Nexus, according to the CLSI EP09C-ED3 guidelines [21]. Statistical analyses were performed using MedCalc Statistical Software (version 20.011, MedCalc Software, Ostend, Belgium) and Analyse-It (Analyse-it Software Ltd., Leeds, UK). p -Values less than 0.05 were considered statistically significant, and rounding rules were applied for summary statistics [23].

Results

WBC differentials

With the setting of 200 WBC count, EasyCell assistant counted 220.0 (IQR, 139.8–220.0) WBCs per slide in samples with mild leukopenia; 109.5 (83.0–151.5) WBCs per slide in samples with moderate leukopenia; and 29.0 (14.0–57.5) WBCs per slide in samples with severe leukopenia, respectively (Table 1).

On the basis of user verification, WBC pre-classification by EasyCell assistant showed high values of sensitivity and specificity (range, 85.0–100.0 % and 90.8–100.0 %, respectively) for neutrophils, lymphocytes, other cells, and NRBCs. The NPV and efficiency showed high values in all seven classes (range, 85.7–100.0 % and 90.9–99.7 %, respectively) (Table 2).

WBC differentials between pre-classification by EasyCell assistant and MMR showed moderate correlations for neutrophils ($r=0.58$), lymphocytes ($r=0.69$), and eosinophils ($r=0.51$) in all samples. After user verification, they showed mostly high to very high correlations for neutrophils ($r=0.74$), lymphocytes ($r=0.78$), eosinophils ($r=0.88$), and other cells ($r=0.91$) (Table 3). Similar findings were observed even in abnormal samples; after user-verification, WBC differentials between EasyCell assistant and MMR showed high to very high correlations for neutrophils ($r=0.72$), lymphocytes ($r=0.77$), eosinophils ($r=0.91$), and other cells ($r=0.91$). Such high or very high correlations were also observed in leukopenic samples (Table 4).

In abnormal samples, the sensitivity of pre-classification by EasyCell assistant for detecting other cells and NRBCs were 98.6 and 96.3 %, respectively, while the specificity was 7.4 and 14.1 %, respectively (Table 5). After user verification, the specificity was improved, and the efficiency for detecting other cells and NRBCs were 68.0 and 84.0 %, respectively.

PLT counting

Figure 1 shows comparison of platelet counting between EasyCell assistant and Pentra DX Nexus using Passing-Bablok regression analysis and Bland-Altman plot. PLT count by EasyCell assistant showed a high correlation with that by Pentra DX Nexus ($r=0.80$) without proportional or systematic difference, and the mean difference was $-34.4 \times 10^9/\text{L}$ (Figure 1A and B). In the samples with PLT counts $<100 \times 10^9/\text{L}$ ($n=31$), PLT count by EasyCell assistant also showed a high correlation with that by Pentra DX Nexus ($r=0.82$) without proportional or systematic differences, and the mean difference was $2.5 \times 10^9/\text{L}$ (Figure 1C and D). In the samples with PLT counts $\geq 100 \times 10^9/\text{L}$ ($n=194$), PLT count by EasyCell assistant showed a moderate correlation with that by Pentra DX Nexus ($r=0.69$) with systematic difference ($-37.6 \times 10^9/\text{L}$), and the mean difference was $-40.3 \times 10^9/\text{L}$ (Figure 1E and F).

When we further divided the samples with PLT counts $\geq 100 \times 10^9/\text{L}$, in the samples with PLT counts between 100 and $400 \times 10^9/\text{L}$ ($n=179$), PLT count by EasyCell assistant showed a moderate correlation with that by

Table 2: Performance of WBC pre-classification by EasyCell assistant on the basis of verification (n=225).

Cell class (cell number)	Sensitivity, %			Specificity, %			Positive predictive value, %			Negative predictive value, %			Efficiency, %		
	Overall		Normal	Abnormal		Normal	Overall		Normal	Overall		Normal	Overall		Abnormal
	Normal	Abnormal		Normal	Abnormal		Normal	Abnormal		Normal	Abnormal		Normal	Abnormal	
Neutrophils (n=25,996)	85.0	96.1	73.1	100.0	100.0	100.0	100.0	100.0	100.0	85.7	94.6	80.7	92.1	97.7	87.3
Lymphocytes (n=16,731)	85.2	93.2	79.4	100.0	100.0	100.0	100.0	100.0	100.0	93.0	97.9	89.5	95.0	97.9	92.5
Monocytes (n=3,492)	61.9	68.5	57.8	100.0	100.0	100.0	100.0	100.0	100.0	94.6	98.1	96.4	97.3	98.1	96.6
Eosinophils (n=1,573)	17.8	23.5	13.1	100.0	100.0	100.0	100.0	100.0	100.0	97.4	97.6	97.1	97.4	97.6	97.2
Basophils (n=491)	69.7	84.0	59.3	100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.9	99.6	99.7	99.9	99.6
Other cells (n=479)	100.0	100.0	100.0	95.2	92.9	14.0	0.2	20.3	100.0	100.0	100.0	100.0	94.0	95.2	93.0
NRBCs (n=657)	100.0	NA	100.0	90.8	98.3	84.3	12.8	0.0	13.9	100.0	100.0	100.0	90.9	98.3	84.7

Cell number indicates total number of user-verified cells in EasyCell assistant. NA, not available; NRBC, nucleated red blood cells.

Table 3: Comparison of cell classification between EasyCell assistant and manual counting in all samples (n=225).

Cell class (cell number)	Manual, % (median, IQR)	Pre-classification, % (median, IQR)	Verification, % (median, IQR)	Correlation (r) (95 % CI)				Mean difference, % (95 % CI)			
				Pre-classification vs. verification		Verification vs. manual count		Pre-classification vs. verification		Pre-classification vs. manual count	
				Pre-classification vs. verification	Verification vs. manual count	Pre-classification vs. manual count	Verification vs. manual count	Pre-classification vs. verification	Verification vs. manual count	Pre-classification vs. manual count	Verification vs. manual count
Neutrophils (n=25,996)	52.0 (45.5–59.5)	53.9 (39.9–62.4)	53.9 (42.3–62.2)	0.79 (0.74–0.84)	0.58 (0.49–0.66)	0.74 (0.68–0.80)	–0.7 (–23.5 to 22.1)	–0.5 (–29.4 to 28.4)	0.2 (–24.2 to 24.5)		
Lymphocytes (n=16,731)	34.5 (28.1–41.5)	32.3 (24.5–41.4)	33.3 (25.1–42.4)	0.82 (0.77–0.86)	0.69 (0.61–0.75)	0.78 (0.73–0.83)	–0.8 (–19.7 to 18.2)	0.0 (–22.1 to 22.1)	0.7 (–19.9 to 21.3)		
Monocytes (n=3,492)	7.0 (5.0–9.5)	4.5 (2.3–7.4)	6.2 (4.3–8.9)	0.49 (0.38–0.58)	0.23 (0.10–0.35)	0.57 (0.47–0.65)	–1.9 (–10.9 to 7.1)	–2.2 (–12.0 to 7.7)	–0.3 (–8.6 to 8.0)		
Eosinophils (n=1,573)	2.8 (1.5–4.3)	0.5 (0.0–0.9)	2.3 (0.9–4.0)	0.56 (0.47–0.65)	0.51 (0.40–0.60)	0.88 (0.84–0.90)	–2.5 (–8.8 to 3.8)	–2.8 (–10.1 to 4.5)	–0.3 (–4.2 to 3.5)		
Basophils (n=491)	0.5 (0.0–1.0)	0.5 (0.0–1.4)	0.5 (0.3–1.3)	0.40 (0.29–0.51)	0.12 (–0.01 to 0.25)	0.41 (0.29–0.51)	–0.1 (–2.3 to 2.1)	0.3 (–1.7 to 2.3)	0.4 (–1.7 to 2.6)		
Other cells (n=479)	0.0 (0.0–0.5)	6.4 (3.6–10.5)	0.0 (0.0–0.4)	0.48 (0.37–0.57)	0.47 (0.36–0.57)	0.91 (0.89–0.93)	7.5 (–8.3 to 23.3)	7.1 (–10.0 to 24.2)	–0.4 (–7.1 to 6.3)		
NRBCs (n=657)	0.0 (0.0–0.0)	1.4 (0.5–12.9)	0.0 (0.0–0.2)	0.11 (–0.02 to 0.24)	0.21 (0.08–0.33)	0.60 (0.51–0.68)	17.7 (–96.6 to 132)	18.7 (–94.7 to 132.1)	0.9 (–17.2 to 19.1)		

Cell number indicates total number of user-verified cells in EasyCell assistant. Manual, manual counting; NA, not available; NRBCs, nucleated red blood cells; IQR, interquartile range; CI, confidence interval.

Table 4: Comparison of cell classification between EasyCell assistant and manual counting in abnormal samples (n=125).

Cell class (cell number)	Manual, % (median, IQR)	Pre-classification, % (median, IQR)	Verification, % (median, IQR)	Correlation (r) (95 % CI)		Mean difference, % (95 % CI)			
				Pre-classification vs. verification	Pre-classification vs. manual count	Pre-classification vs. verification	Pre-classification vs. manual count	Verification vs. manual count	Verification vs. manual count
Abnormal samples (n=125)									
Neutrophils (n=12,558)	50.3 (41.1–58.0)	45.9 (31.7–57.5)	49.6 (31.4–59.6)	0.71 (0.61–0.79)	0.52 (0.37–0.63)	0.72 (0.63–0.80)	–1.2 (–31.5 to 29.1)	–5.0 (–40.4 to 30.3)	–3.8 (–33.0 to 25.5)
Lymphocytes (n=9,682)	35.0 (28.0–42.3)	34.5 (26.3–46.4)	37.7 (27.4–53.4)	0.78 (0.69–0.84)	0.65 (0.53–0.74)	0.77 (0.69–0.83)	–0.9 (–26.0 to 24.1)	2.3 (–25.1 to 29.8)	3.3 (–22.0 to 28.6)
Monocytes (n=2,150)	7.5 (3.7–9.6)	5.4 (2.7–9.1)	7.4 (4.4–11.0)	0.41 (0.25–0.54)	0.17 (–0.01 to 0.34)	0.61 (0.48–0.71)	–2.0 (–13.6 to 9.6)	–1.1 (–13.0 to 10.8)	0.9 (–8.6 to 10.5)
Eosinophils (n=875)	2.5 (1.0–4.0)	0.0 (0.0–0.5)	2.3 (0.5–4.0)	0.58 (0.45–0.69)	0.54 (0.40–0.65)	0.91 (0.87–0.93)	–2.7 (–10.2 to 4.9)	–2.8 (–11.8 to 6.1)	–0.2 (–4.3 to 4.0)
Basophils (n=285)	0.0 (0.0–0.5)	0.6 (0.0–1.4)	0.5 (0.0–1.5)	0.32 (0.16–0.47)	0.16 (–0.01 to 0.33)	0.52 (0.38–0.63)	–0.2 (–3.0 to 2.7)	0.5 (–1.6 to 2.6)	0.7 (–1.7 to 3.1)
Other cells (n=477)	0.3 (0.0–1.0)	9.1 (5.5–14.0)	0.0 (0.0–1.2)	0.47 (0.32–0.59)	0.45 (0.30–0.58)	0.91 (0.87–0.94)	9.5 (–10.5 to 29.4)	8.8 (–13.3 to 30.8)	–0.7 (–9.7 to 8.3)
NRBCs (n=657)	0.0 (0.0–1.1)	9.1 (1.4–35.2)	0.0 (0.0–2.6)	0.08 (–0.10 to 0.25)	0.16 (–0.02 to 0.32)	0.59 (0.46–0.69)	29.7 (–117.0 to 176.4)	31.4 (–113.4 to 176.3)	1.7 (–22.6 to 26.0)
Abnormal samples with leukopenia (n=45)									
Neutrophils (n=3,339)	44.5 (35.0–54.8)	46.4 (32.5–58.0)	46.0 (30.7–57.0)	0.78 (0.63–0.87)	0.78 (0.63–0.87)	0.90 (0.83–0.95)	1.5 (–26.4 to 29.5)	1.5 (–24.9 to 27.9)	0.0 (–18.7 to 18.6)
Lymphocytes (n=3,079)	38.5 (28.6–48.3)	40.0 (32.6–57.6)	40.5 (31.0–57.2)	0.86 (0.77–0.92)	0.86 (0.76–0.92)	0.86 (0.77–0.92)	–2.1 (–25.3 to 21.1)	–0.5 (–23.3 to 22.4)	1.7 (–17.3 to 20.6)
Monocytes (n=448)	7.7 (3.4–8.8)	5.6 (2.8–7.8)	5.6 (2.8–7.8)	0.43 (0.15–0.64)	0.22 (–0.08 to 0.48)	0.59 (0.35–0.75)	–1.7 (–10.4 to 7.1)	–2.7 (–13.1 to 7.7)	–1.0 (–9.1 to 7.1)
Eosinophils (n=204)	1.8 (0.2–3.1)	0.0 (0.0–0.5)	1.2 (0.0–4.1)	0.53 (0.24–0.72)	0.40 (0.21–0.62)	0.87 (0.78–0.93)	–2.0 (–7.5 to 3.5)	–2.3 (–8.3 to 3.6)	–0.4 (–3.6 to 2.9)
Basophils (n=73)	0.0 (0.0–0.5)	0.5 (0.0–1.2)	0.5 (0.0–1.1)	0.36 (0.07–0.59)	0.16 (–0.14 to 0.44)	0.66 (0.45–0.80)	–0.2 (–3.5 to 3.1)	0.4 (–1.8 to 2.6)	0.6 (–2.1 to 3.3)
Other cells (n=41)	0.0 (0.0–0.5)	5.9 (3.1–10.2)	0.0 (0.0–0.0)	0.44 (0.17–0.65)	0.18 (–0.12 to 0.45)	0.76 (0.60–0.86)	6.5 (–3.8 to 16.9)	6.4 (–5.1 to 17.9)	–0.1 (–3.0 to 2.8)
NRBCs (n=24)	0.0 (0.0–0.0)	3.5 (0.9–25.0)	0.0 (0.0–0.0)	0.07 (–0.23 to 0.36)	–0.00 (–0.29 to 0.29)	0.65 (0.44–0.79)	38.2 (–180.2 to 256.5)	38.9 (–179.9 to 257.6)	0.7 (–6.5 to 7.9)

Cell number indicates total number of user-verified cells in EasyCell assistant. Manual, manual counting; NRBCs, nucleated red blood cells; IQR, interquartile range; CI, confidence interval.

Table 5: Comparison of detection performances of specific cells (other cells and NRBCs) between EasyCell assistant and manual counting in abnormal samples (n=125).

Cell type	Manual	EasyCell assistant	Compared method	Efficiency, %	Sensitivity, % (95 % CI)	Specificity, % (95 % CI)	True positive	False positive	True negative	False negative
Other cells	71/125	120/125	Pre-classified	59.2	98.6 (92.4–99.9)	7.4 (2.1–17.9)	70	50	4	1
		61/125	Verified	68.0	64.8 (52.5–75.8)	72.2 (58.4–83.5)	46	15	39	25
NRBCs	54/125	113/125	Pre-classified	49.6	96.3 (87.3–99.6)	14.1 (7.0–24.4)	52	61	10	2
		58/125	Verified	84.0	85.2 (72.9–93.4)	83.1 (72.3–91.0)	46	12	59	8

Manual, manual counting; NA, not available; NRBCs, nucleated red blood cells; IQR, interquartile range; CI, confidence interval.

Pentra DX Nexus ($r=0.64$) with systematic difference ($y=1.15x - 66.6$ [95 % CI: -109.0 to -30.8]), and the mean difference was $-29.8 \times 10^9/L$. In the samples with PLT counts $>400 \times 10^9/L$ ($n=15$), PLT count by EasyCell assistant showed a moderate correlation with that by Pentra DX Nexus ($r=0.54$) without proportional or systematic difference ($y=1.10x - 198.3$ [95 % CI: $-7,532.3$ to 115.4]), and the mean difference was $-164.6 \times 10^9/L$.

Discussion

In this study, we evaluated the performance of EasyCell assistant comprehensively, encompassing WBC differentials and PLT counting, in comparison with MMR and Pentra DX Nexus. In our data, the overall performance of EasyCell assistant for WBC classification was satisfactory with a high correlation with MMR after user verification. Such a result was constantly observed in both normal and abnormal samples. Moreover, in normal samples, EasyCell assistant showed a high correlation with MMR for neutrophils and lymphocytes even with pre-classification (Tables 3 and 4).

EasyCell assistant is an assistant device to improve efficiency by eliminating much of the routine work of sorting predominantly normal cells with unattended scanning. It suggests the correct classification of normal cells to allow for the medical personnel to quickly review the suggested classifications and then to devote time to the abnormal cells. One of the distinguished features of EasyCell assistant compared with the well-known DM96 or DI-60 is that WBC is preclassified into 10 classes automatically, including others, and the cells belonging to the others class can be further reviewed and verified by users. Other cells defined by EasyCell assistant include metamyelocytes, myelocytes, promyelocytes, blasts, and plasma cells; this definition corresponds to the CLSI H20-A2 guidelines, where other cells consist of NRBC, blasts, myeloblasts,

lymphoblasts, monoblasts, promyelocytes, myelocytes, and metamyelocytes [3].

In our data, EasyCell assistant showed a high sensitivity for detecting other cells and NRBCs (98.6 and 96.3 %, respectively); this finding also supported the intended use of EasyCell assistant (Table 5). For an appropriate use of EasyCell assistant, each laboratory should verify its own criteria for MMR and optimize them to maximize the workflow efficiency [1, 2, 24].

In routine hematology practice, leukopenia is one of the main criteria for triggering MMR [25, 26]. The international consensus group suggested hematology review criteria for action following automated CBC and WBC differentials; WBC count $<4.0 \times 10^9/L$ or $>30.0 \times 10^9/L$ [26]. Imprecision of WBC count increases when lower numbers of WBC are counted [3, 15]; therefore, if the WBC count is $<4.0 \times 10^9/L$, the CLSI H20-A2 recommended not including this data in the evaluation [3]. In our data, EasyCell assistant demonstrated reliable performance in the mild leukopenic samples, showing almost 200 WBCs being counted. It seems that EasyCell assistant could reduce workload in WBC analysis with satisfying CLSI H20-A2-suggested counting (200 WBCs) in mild leukopenic samples [3]. However, considering the fact that a prolonged MMR may improve the detection of underpopulated cells, further studies on DM analyzers mainly focusing on samples with moderate or severe leukopenia would be of interest and necessity [27].

Based on our data, PLT count estimation by EasyCell assistant was overall satisfactory with high correlation with the data by Pentra DX Nexus, and such a finding was evident especially in thrombocytopenic samples (Figure 1). EasyCell assistant, however, underestimated PLT counts in samples with normal or increased PLT counts. Of note, the mean difference was greater in samples with PLT counts $>400 \times 10^9/L$, than in samples with PLT counts between 100 and $400 \times 10^9/L$ ($-164.6 \times 10^9/L$ vs. $-29.8 \times 10^9/L$).

When estimating PLT counts on blood smear, examining the feathered and lateral edge of the smear is essential to

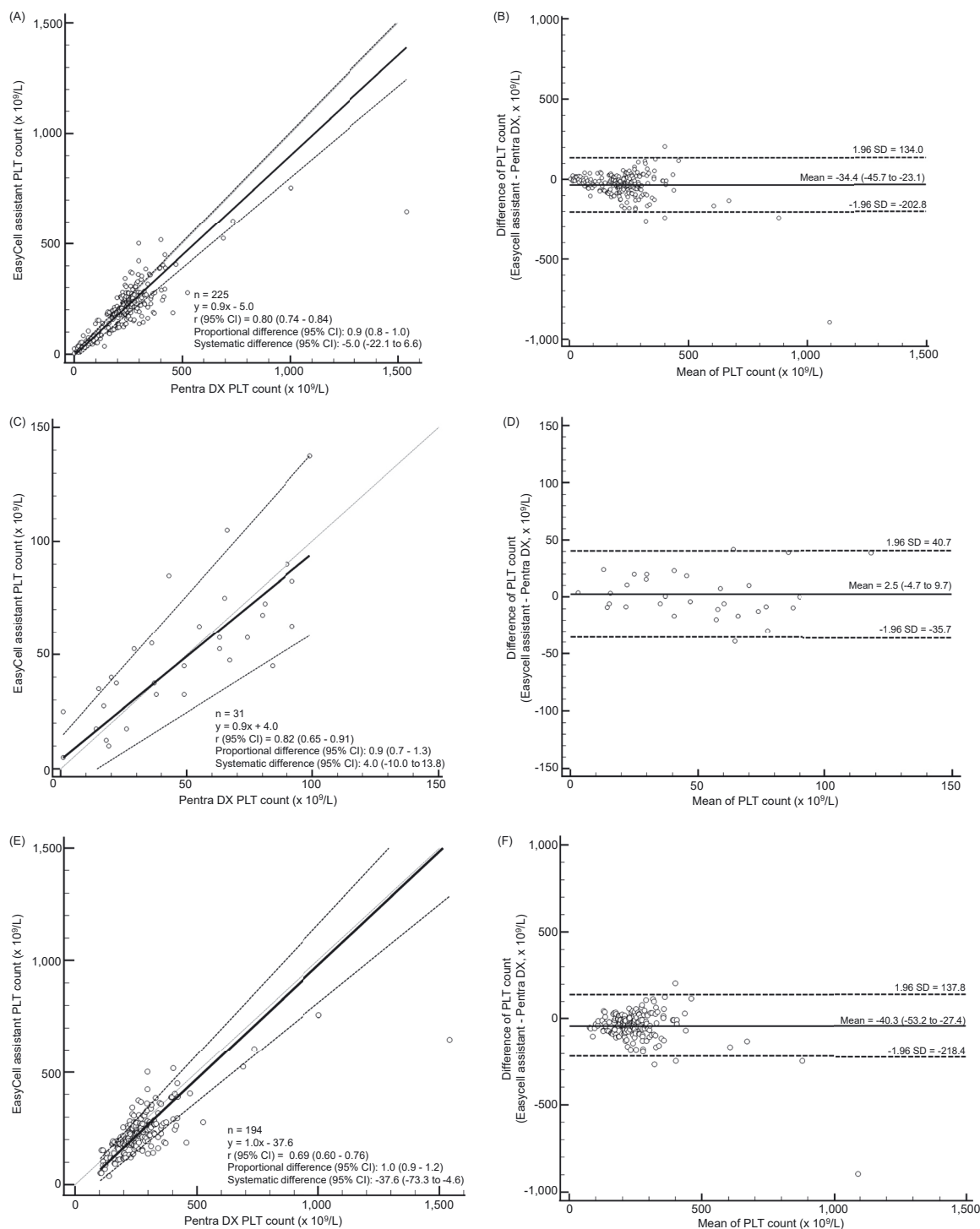


Figure 1: Comparison of platelet counting between EasyCell assistant and Pentra DX Nexus using Passing-Bablok regression analysis and Bland-Altman plot. (A) and (B) are in total samples ($n=225$); (C) and (D) in samples with PLT counts $<100 \times 10^9/\text{L}$ ($n=31$); (E) and (F) in samples with PLT counts $\geq 100 \times 10^9/\text{L}$ ($n=194$). All p values were <0.001 . Solid line, Passing-Bablok regression or mean difference; dashed line, 95 % CI or ± 1.96 SD. CI, confidence interval; SD, standard deviation.

detect PLT clumps [28, 29]. In addition to the EasyCell assistant, the other DM analyzers, including DM96, DI-60, and Mindray MC-80 (MC-80, Mindray Bio-Medical Electronics

Co., Ltd., Shenzhen, China), provide PLT counting by multiplying their own preset estimation factor [6, 13, 30]. The DM 96 showed a high correlation without significant disparity,

but the difference increased as PLT counts increased [30]. The DI-60 estimated PLT counts reliably but underestimated it in samples with marked thrombocytosis [13]. The MC-80 and EasyCell assistant have not yet been evaluated for PLT counting, and this is the first study for PLT counting in EasyCell assistant. Collectively, previous data and our data imply that estimated PLT counts might be falsely less depending on which images are selected, and the gap between estimated and actual PLT counts would be larger as the PLT counts increase. Therefore, it is questionable to multiply a uniform preset estimation factor, regardless of PLT counts; it would be reasonable to adjust preset estimation factor according to the PLT counts. PLT counting issue on various DM analyzers should be more clarified in further studies [12, 31].

This study has several limitations. Although we evaluated EasyCell assistant using a wide range of abnormal samples, there were no samples with Auer rods, Dohle bodies, and malarial parasites; therefore, we could not evaluate the capability of WBC morphology categorization of EasyCell assistant for these abnormal samples [32]. The performance of DM analyzers could vary depending on the smear quality and staining methods [33]; however, we could not explore the performance of EasyCell assistant using different staining methods. We focused on evaluating the qualitative performance of EasyCell assistant, and we did not assess the laboratory efficiency including turnaround time and risk assessment between MMR and EasyCell assistant [16, 17]. Additionally, like other DM analyzers, EasyCell assistant undergo pre-classification only in ideal zone of slides, which can miss pathologic cells located in other sites. The DM analyzers proceeding full field review of slides might solve this problem [34]; accordingly, there is a need to compare the performance of EasyCell assistant with full-field-review analyzers.

Nevertheless, this is the first study that comprehensively investigated an automated DM analyzer, EasyCell assistant. In comparison with MMR and Pentra DX Nexus, the performance of EasyCell assistant for WBC classification seems to be acceptable in both normal and abnormal samples with improvement after user verification. PLT count estimation by EasyCell assistant was overall satisfactory with a high correlation with Pentra DX Nexus. However, our data also shows the inherent limitation of PLT counting on DM analyzers. In conclusion, the EasyCell assistant, with its fair performance on WBC classification and detection of abnormal cells would help optimize the workflow in clinical hematology laboratories with reduced workload of MMR. Further studies are necessary for more comprehensive assessment of various DM analyzers.

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Author contributions: Kim H collected the samples, analyzed the data, and wrote the draft; Lee GH and Yoon S discussed the data and modified the draft; Hur M conceived the study, analyzed the data, and finalized the draft; Kim HN and Park M analyzed the data; Kim SW collected the samples. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

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Informed consent: Written informed consent was obtained from the mother of each neonate for the use of cord blood samples and was waived for the use of other leftover blood samples.

Ethical approval: This study was conducted according to the Declaration of Helsinki, and the study protocol was exempted from the approval of the Institution Review Board of KUMC (KUH1200063).

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