Received: 28 March 2017 | Accepted: 15 July 2017

DOI: 10.1111/ijlh.12728

ORIGINAL ARTICLE

WILEY ISLH International Journal of Laboratory Hematology

Automated cell counts on CSF samples: A multicenter performance evaluation of the GloCyte system

E. A. Hod[1,2] | C. Brugnara[3] | M. Pilichowska[4] | L. M. Sandhaus[5] | H. S. Luu[6] | S. K. Forest[1,2] | J. C. Netterwald[1] | G. M. Reynafarje[2] | A. Kratz[1,2]

[1]Department of Pathology and Cell Biology, Columbia University College of Physicians and Surgeons, New York, NY, USA

[2]NewYork-Presbyterian Hospital, New York, NY, USA

[3]Department of Laboratory Medicine, Boston Children's Hospital, and Harvard Medical School, Boston, MA, USA

[4]Tufts Medical Center, Boston, MA, USA

[5]University Hospitals, Cleveland Medical Center, Cleveland, OH, USA

[6]Children's Medical Center Dallas, Dallas, TX, USA

Correspondence
Alexander Kratz, NewYork-Presbyterian Hospital, Core Laboratory, New York, NY, USA.
Email: ak2651@cumc.columbia.edu

Funding information
This study was funded by Advanced Instruments

Abstract

Objectives: Automated cell counters have replaced manual enumeration of cells in blood and most body fluids. However, due to the unreliability of automated methods at very low cell counts, most laboratories continue to perform labor-intensive manual counts on many or all cerebrospinal fluid (CSF) samples. This multicenter clinical trial investigated if the GloCyte System (Advanced Instruments, Norwood, MA), a recently FDA-approved automated cell counter, which concentrates and enumerates red blood cells (RBCs) and total nucleated cells (TNCs), is sufficiently accurate and precise at very low cell counts to replace all manual CSF counts.

Methods: The GloCyte System concentrates CSF and stains RBCs with fluorochrome-labeled antibodies and TNCs with nucleic acid dyes. RBCs and TNCs are then counted by digital image analysis. Residual adult and pediatric CSF samples obtained for clinical analysis at five different medical centers were used for the study. Cell counts were performed by the manual hemocytometer method and with the GloCyte System following the same protocol at all sites. The limits of the blank, detection, and quantitation, as well as precision and accuracy of the GloCyte, were determined.

Results: The GloCyte detected as few as 1 TNC/μL and 1 RBC/μL, and reliably counted as low as 3 TNCs/μL and 2 RBCs/μL. The total coefficient of variation was less than 20%. Comparison with cell counts obtained with a hemocytometer showed good correlation (>97%) between the GloCyte and the hemocytometer, including at very low cell counts.

Conclusions: The GloCyte instrument is a precise, accurate, and stable system to obtain red cell and nucleated cell counts in CSF samples. It allows for the automated enumeration of even very low cell numbers, which is crucial for CSF analysis. These results suggest that GloCyte is an acceptable alternative to the manual method for all CSF samples, including those with normal cell counts.

KEYWORDS

automated cell counters, cerebrospinal fluid testing, laboratory testing, red cell counts, white cell counts

1 | INTRODUCTION

Until the invention of automated cell counters, hematology laboratories used manual counting chambers to enumerate cells in blood and body fluids.[1] Such manual methods have inherent limitations, especially for low cell counts; for example, at a cell count of 5 cells/μL, the coefficient of variation (CV) of a Neubauer hemocytometer (Hausser Scientific, Horsham, PA) is 45%.[2,3] With the development of the Coulter counter and other automated platforms, most laboratories quickly adopted automated cell counters for the enumeration of blood cells.[1] Cell counts in body fluids remained manual for several more decades, and only in recent years have manufacturers added reliable body fluid modes to their instruments.[4-12]

Today, most automated cell counters can provide reasonably accurate cell counts of almost all body fluids. However, cellular analysis of cerebrospinal fluid (CSF) samples continues to present unique challenges, because reference ranges for total nucleated cells (TNCs) in CSF are 0-5 cells/μL and "normal" CSF samples should not contain any red blood cells (RBCs).[2] To distinguish between a "normal" CSF with less than 5 TNCs/μL and 0 RBCs/μL and an "abnormal" CSF with elevated TNCs and/or RBCs, automated cell counters must be extremely accurate and precise at very low cell counts.

Several instrument platforms on the market offer quantitation of RBCs and TNCs in CSF samples. The ADVIA120i (Siemens Medical Solutions, Malvern, PA) has been shown to allow reliable TNC enumeration in CSF at very low cell counts. However, the presence of high RBC counts can influence the accuracy of the TNC count.[13-15] In the evaluation of the Mindray BC-6800 (Mindray North America, Mahwah, NJ) body fluid mode in cerebrospinal fluid, Buoro et al concluded that the instrument provided an effective alternative to manual cell counts.[16] However, they recommended a microscopic review of all cell counts between 4.0 and 7.0 cells/μL. The Sysmex XT-4000i and XE-5000 cell counters (Sysmex America, Lincolnshire, IL) had limited precision at <20 TNCs/μL.[10,17] The newer Sysmex XN-1000 has a lower limit of quantitation of 5 TNCs/μL, and the minimal reportable number of RBCs is 1000 RBCs/μL.[18] Recently, Fleming and colleagues have described a high-sensitivity analysis (hsA) research mode on the XN-1000 system.[19] This application has a lower limit of quantitation of 10 RBCs/μL and 2 TNCs/μL.[19,20] Buoro and co-workers have investigated the use of the Sysmex UF-1000i Body Fluid Mode and found good correlation with manual TNC enumeration, with a modest overestimation of counts below 30 TNCs/μL.[21] The use of the Iris iQ200 Body Fluid Module for CSF analysis (Beckman-Coulter, Indianapolis, IN) was investigated by Goubard and colleagues as well as Walker and co-workers.[22,23] Both groups found good correlation between the manual method and the results of the iQ200. The CVs were slightly higher on the Iris than on the hemocytometer. Finally, Glasser and colleagues compared the iQ200 and the LH750 (Beckman-Coulter) to manual counts and found unacceptable rates of error at lower cell counts [23] (Table 1).

Because the accuracy and precision at low CSF cell counts is so clinically important, and because most automated cell counters may not have sufficient accuracy and precision at low counts, many laboratories still regard manual chamber counts as the "gold standard" to obtain cell counts on CSF samples, especially on clear (non-bloody) specimens.[20,24] The GloCyte Automated Cell Counter System (Advanced Instruments, Norwood, MA) is a new platform that concentrates and enumerates TNCs and RBCs using fluorescent microscopy and digital image analysis.[25,26] The purpose of this multicenter study was to determine if GloCyte RBC and TNC counts were sufficiently accurate to replace manual CSF cell counts.

TABLE 1 Performance of presently available automated CSF cell counting methods

Instrument	Performance Issues in counting cells in CSF	References
Advia 120i	Presence of high RBC counts can interfere with the accuracy of the WBC count	15,16
Mindray BC-6800	Microscopic review of all cell counts between 4.0 and 7.0 recommended	17
Sysmex XT-4000i and XE-5000	Limited Precision at WBC counts of <20 cells/μL	11,18
Sysmex XN-1000	Minimal reportable number of RBCs is 1000 cells/μL	19
Sysmex XN-1000 high-sensitivity mode	Lower Limit of quantitation of RBCs is 10 cells/μL; not FDA cleared in the US	20,21
Sysmex UF-1000i	Modest overestimation of the WBC counts below 30 cells/μL	7
iQ200	Unacceptable rates of error at counts of less 50 cells/μL	24
LH750	Unacceptable rates of error at counts of less 200 cells/μL	24

2 | MATERIALS AND METHODS

2.1 | The GloCyte system

The GloCyte is an automated cell counter system. It concentrates TNCs and RBCs in CSF samples and enumerates them; 30 μL of sample and 30 μL of reagent containing fluorochrome-labeled antibodies against human RBCs are dispensed into a 0.5-mL tube. Another 30 μL of sample and 30 μL of reagent containing nucleic acid dye with detergent to stain TNCs are dispensed into a second 0.5-mL tube. After mixing, the two samples are transferred into separate cartridges. The TNC stained sample is transferred immediately after mixing, while the RBC stained sample is transferred after a 3-minute incubation in the tube. Vacuum suction is applied to the cartridges, removing all liquid and pulling all cells onto a membrane. A semiconductor laser and a digital image analysis system then capture and enumerate the fluorescent cell images. No manual cell counting is necessary. The instrument does not provide additional qualitative parameters for the cell count. No instrument calibration is necessary.

The samples are contained in a disposable cartridge and do not come in contact with the instrument, eliminating the possibility of carryover and allowing safe handling of potentially infectious specimens.

We did not experience any instrument malfunction during the study that led to sample loss.

2.2 | Manual method

Depending on each clinical site's hemocytometer material preference, manual counts were performed in duplicate on either glass Neubauer hemocytometers (Hausser Scientific, Horsham, PA) or disposable Levy-Neubauer hemocytometers (INCYTO C-Chip, Seoul, Korea) by adding 10 μL to each chamber and counting all 9 squares.[27] The mean of the two manual counts was used in the study.

2.3 | Study protocol

The study was performed at five sites: Boston Children's Hospital, Boston, Massachusetts; Columbia University Medical Center, New York, New York; Tufts Medical Center, Boston, Massachusetts; University Hospitals, Cleveland Medical Center, Cleveland, Ohio; and the University of Texas Southwestern Medical Center, Dallas, Texas. The study was approved by the Institutional Review Board of each site.

Residual CSF samples that had been obtained for clinical purposes and sent for analysis to the clinical laboratory at each participating site were used for the study. Samples were included in the study after all clinically necessary testing had been performed. Only CSF samples that were 4 hours or less after collection and that still had at least 300 μL of sample remaining qualified for study inclusion. The samples were de-identified and coded. The coded samples were then split and analyzed using both the GloCyte test method and the gold standard hemocytometer method. Each sample was analyzed four times: twice using the hemocytometer method followed by two runs on the GloCyte. Every site analyzed samples from its own patient population.

2.4 | Performance parameters

2.4.1 | Limit analysis

We followed CLSI EP 17-A2 for the determination of the LOB, LOD, and LOQ. [28] LoB testing was performed in the Advanced Instruments laboratory with purchased cell-free human CSF. LoD testing was performed at Tufts Medical Center due to availability of CSF samples with low cell counts. LoQ was calculated from LoB and LoD.

- *Limit of the Blank (LOB):* The LOB is the highest number of cells expected to be measured in a blank sample.[28-30] The LOB was defined as the 95th percentile of measurements made on blank samples (samples with no cells detected with the hemocytometer); 240 counts were performed for each cell type, with five blank samples, using two GloCyte devices. Counts were made over 3 days for each instrument, with two reagent lots for each blank sample and four replicates per reagent lot per day.
- *Limit of Detection (LOD):* The LOD is the smallest number of cells that can reliably be detected as different from zero; it was defined as the value where at least 95% of the measured counts fell above the LOB. LOD testing was performed using CSF specimens with low cell counts (1-2 cells/μL). 240 counts were performed for each cell type using two GloCyte devices. Six specimens were tested for TNC and 6 were tested for RBC. Each specimen was tested 10 times with one reagent lot and one cartridge lot and 10 times with a second reagent lot and a second cartridge lot.
- *Limit of Quantitation (LOQ):* The LOQ is the lowest cell count in a sample that can both be reliably detected and also meet set guidelines for precision and bias; it was defined as the lowest count at which the GloCyte provides quantitative measurements with a total error of 20% or less. This cutoff was used because surveys by the College of American Pathologists (CAP) indicate CVs of 20%-40% for manual chamber counts.[4,31] The LOQ was determined utilizing the same samples used to determine the LOD, six samples with low cell counts (LOD samples) for each cell type. The total error (TE) method was used in accordance with CLSI EP 17-A2.[28] For each sample by instrument and reagent lot, the replicated measurements were used to estimate the total error (TE) and the percent total error (%TE). The "truth" was assumed to be the manual reference count for each sample. The root mean square (RMS) approach to calculating TE was employed utilizing the %TE of the LOD counts:

$$\mathrm{TE} = \sqrt{\mathrm{bias}^2 + \mathrm{stddev}^2} \text{ and } \%\mathrm{TE} = \frac{\mathrm{TE}}{\text{manual ref count}} \times 100\%$$

Bias was defined as the difference between the automated count and the gold standard hemocytometer method. Based on the data from the assay runs detailed for the LOD experiments, a scatter plot was generated with %TE on the vertical axis and the manual reference count on the horizontal axis. A linear regression line was fit to the %TE data. The LOQ was then determined as the manual count for which the accuracy met the prespecified level of 20%TE.[32]

2.4.2 | Receiver-operating curve analysis

Receiver-operating curve analysis (ROC) analysis was conducted using a manual count of 5 cells/μL as the cutoff between normal/abnormal. Any samples with a manual count of five or less were assigned zero; all others were assigned one. At all GloCyte TNC levels, sensitivity and 1-specificity were calculated and plotted, resulting in the ROC curve presented.

2.4.3 | Precision

- *Intrarun Precision:* Intrarun precision studies were performed at three sites, using 14 samples representing the clinically important range for total nucleated cells (TNCs) and 16 samples representing the clinically relevant range for red blood cells (RBCs), as well as 6 TNC and 6 RBC GloCyte controls. Each sample and control were run 10 times by a single operator for RBC and/or TNC counts.
- *Inter-run Precision:* Reproducibility was evaluated at three clinical sites over 20 days. The sites were selected based on the availability of resources at the sites to perform testing. Testing

was performed twice daily, using the same sets of controls, for 20 days. Each control set was tested in duplicate, independently by two operators, at each site; 160 controls were tested for each level of the two controls at each site. The total number of controls tested at each site was 640. Standard deviations and %CV were calculated for each factor of interest (residual, run, day, site, and operator). A prespecified acceptance criterion for this assay was set at 20%.[33]

2.4.4 | Method comparison (Accuracy)

Due to the low prevalence of abnormal samples, a sampling plan was developed to ensure collection of adequate numbers of both normal and abnormal samples. Each clinical trial site initially tested both TNC and RBC for each specimen. Once a specified number of samples were tested for a given result range, either TNC or RBC counts were tested, as needed, so that a valid statistical analysis could be performed. For example, by the end of the study, samples with high TNC and low RBC counts were tested for TNC counts only, as a sufficient number of low RBC counts had already been collected. There were therefore differences in the number of specimens tested for TNC and RBC counts.

Accuracy studies were performed with 321 samples for TNC counts (203 adult specimens and 118 pediatric specimens) and 422 samples for RBC counts (243 adult specimens and 179 pediatric specimens). Each sample was counted twice manually and twice using the GloCyte System. For method comparison, the mean of the two manual counts, both performed by the same operator, was used as the gold standard. Only the first GloCyte count was used for data analysis. In addition to the clinical samples, manipulated CSF samples (31 samples for TNC and 41 samples for RBC) were created by diluting human TNC and RBC into pooled blank human CSF. These manipulated samples were necessary to provide data at the upper end of the reportable range. Separate pediatric samples (pediatric venous blood and pediatric CSF) and adult samples (adult venous blood and adult CSF) were created for separate collection of pediatric and adult data. Two manual counts were performed with the Neubauer hemocytometer, and two counts were performed using the GloCyte.

CUSUM statistics were used to test the assumption of linearity for the samples. A *P*-value of less than .05 would have indicated that there was a significant deviation from linearity between the hemocytometer and the GloCyte method; as all *P*-values in this analysis were greater than .05, there was no evidence against the assumed linear relationship and Passing-Bablok analysis was appropriate to determine bias between results from the hemocytometer and the GloCyte System.

2.4.5 | Linearity/Reportable range

Linearity was determined using manipulated CSF samples with concentrations spanning the linear range of the instrument. A total of 14 concentration levels ranging from 0 to 8000 TNCs/μL and 15 concentration levels ranging from 0 to 800 000 RBCs/μL were tested on three GloCyte instruments by three operators. Samples were run in triplicate at each level, resulting in 126 TNC counts and 135 RBC counts.

2.5 | Statistical analysis

R version 3.3.0 (2016-05-03) was used for Limit of Blank Histograms, and SPSS (IBM, Armonk, NY) was used for mixed-model analysis for precision studies. Mixed-model analysis was confirmed with SAS® statistical software version 9.4 (SAS Institute, Cary, NC) and Minitab (Minitab Inc., State College, PA). SAS software was used for all other analysis.

3 | RESULTS

3.1 | Limits of blank, detection, quantitation

The LOB for the GloCyte instrument was 0.73 cells/μL for RBCs and 0.47 cells/μL for TNCs. Thus, when performing cell counts on samples without cells, the GloCyte System provides a count of less than 1 cell/μL. The LOD for the GloCyte System was 0.8 RBCs/μL and 1.2 TNCs/μL, indicating that the instrument is able to distinguish samples with approximately 1 cell/μL from samples with less than 1 cell/μL. Finally, the LOQ for the new device was 2.0 RBCs/μL and 2.6 TNCs/μL (Figure 1), indicating that it is able to reliably and precisely enumerate cells in samples with at least 2 RBCs/μL and/or 3 TNCs/μL (Table 2).

3.2 | Precision

For the inter-run precision, the total CV for each control level (N = 480), including within-run, between-run, between-day, between-site, and between-operator variation, was <12%. The largest contributor to the total CV was the within-run CV of 10.1% (Table 3). For the intrarun precision, the total CV of 14 TNC and 16 RBC patient samples was <18% (Table 4). All samples had CVs below 20%, which is the standard acceptance criterion for this type of assay.[4,31] Samples with low cell counts also showed low CVs, indicating good repeatability at low cell counts.

3.3 | Accuracy

For adult TNC counts, differences under 4.0% were observed in 95% of counts up to 10 000 cells/μL (Figure 2). For pediatric TNC counts, differences up to 6.8% were observed in 95% of counts up to 8000 cells/μL. The differences between hemocytometer counts and GloCyte enumerations are much smaller at lower cell counts, with both adult and pediatric samples showing differences grouped closely around zero when manual counts are <100 cells/μL. These very low counts include the decision points used by clinicians when interpreting CSF results and are therefore key for a correct diagnosis and

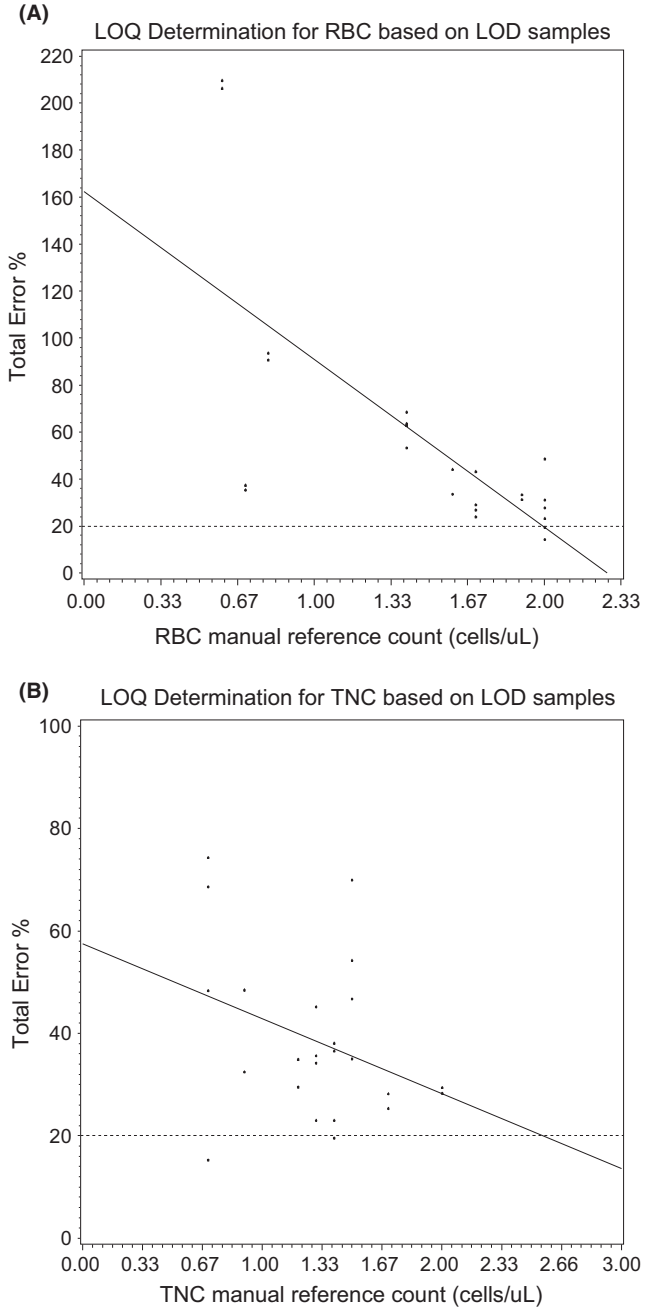

FIGURE 1 Scatterplots of Limit of Detection (LOD) Experiments. Limit of Quantitation (LOQ) was determined as the manual count for which accuracy met the level of 20% total error

treatment plan. For RBCs, differences between the GloCyte System and chamber counts were larger than for TNCs. However, the percent difference was still small (5.6%), as these measurements range into the 900 000s. Like for TNCs, differences between manual and GloCyte counts were close to zero at low counts.

Passing-Bablok analysis showed no significant bias of GloCyte TNC counts vs the manual method (Figure 2 and Table 5). The data showed equivalence between the two methods up to 9900 TNCs/μL for samples from adults and 7672 TNCs/μL for pediatric samples (Figure 3). Manual adult RBC counts were interchangeable with GloCyte counts up to 901 250 RBCs/μL. There was a small proportional bias in pediatric RBC counts, undercounting RBCs by approximately 9% up to 817 500 RBCs/μL. Separate analysis of pediatric samples from general hospitals (which analyzed both adult and pediatric samples) showed no bias, indicating that the GloCyte method could be used interchangeably with the manual method in these samples. Samples collected at dedicated pediatric hospitals showed a 13% bias on the GloCyte System vs the hemocytometer. This indicated that the difference may be due to different procedures for handling samples in pediatric hospitals as opposed to general hospitals. The bias was not present at manual counts below 100 RBCs/μL, which includes the clinical decision thresholds used by clinicians. As the presence of any RBCs in CSF is considered abnormal, bias in higher counts should not be an issue, and for lower counts, GloCyte counts were equivalent to manual counting. Bland-Altman plots also showed high accuracy and no significant bias (Figure 3).

3.4 | Clinical applications

A cutoff or 5 cells was used for ROC analysis and for sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) determination (Table 6). At all GloCyte TNC levels, sensitivity and 1-specificity were calculated and plotted, resulting in the ROC curve presented in Figure 4. The area under the curve (AUC) was 0.985, indicating strong agreement with the manual diagnosis. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were all greater than 0.92, which also indicates strong agreement (Table 6). Table 4 shows the intrarun precision at the clinically relevant TNC levels of 5 to 20 cells/μL, and the lowest levels of RBCs tested.

TABLE 2 Limit of the blank, limit of detection, and limit of quantitation of the GloCyte system

Cell type	Reagent lot	LOB: Count/μL	LOD: Manual Count/μL[a]	LOQ: based on total error <20%, assuming manual count (gold standard) as truth
RBC	1	0.33 cells/μL	0.6 cells/μL	2.0 cells/μL
	2	0.73 cells/μL	0.8 cells/μL	
	Combined	0.53 cells/μL	0.8 cells/μL	
TNC	1	0.33 cells/μL	0.7 cells/μL	2.6 cells/μL
	2	0.47 cells/μL	1.2 cells/μL	
	Combined	0.40 cells/μL	1.2 cells/μL	

LOB, Limit of the blank; LOD, limit of detection; LOQ, limit of quantitation.
[a]100% of the GloCyte counts were greater than the LOB.

TABLE 3 Overall interassay precision summary statistics

Cell type	Control	N	Mean (cells/μL)	Within run		Between run		Between day		Between site		Between operator		Total	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
TNC	Low	480	10.56	1.07	10.11	0.00	0.00	0.17	1.58	0.41	3.86	0.25	2.40	1.18	11.20
	High	480	122.89	7.30	5.94	0.00	0.00	0.00	0.00	3.81	3.10	1.80	1.46	8.43	6.86
RBC	Low	480	11.25	1.03	9.19	0.00	0.00	0.21	1.86	0.43	3.78	0.30	2.68	1.18	10.46
	High	480	130.00	6.83	5.25	0.00	0.00	0.87	0.67	2.24	1.72	1.33	1.02	7.36	5.66

TABLE 4 Intrarun precision at the clinically relevant thresholds of 5, 10, and 20 TNCs/μL and at 5 and 10 RBCs/μL

Cell type	Sample	N	Mean	Median	Standard Deviation	%CV
TNC	128	10	7.80	8.00	0.92	11.78
	129	10	5.50	5.50	0.85	15.45
	199	10	7.90	8.00	0.99	12.59
	300	10	4.10	4.00	0.74	18.00
	303	10	17.90	18.00	1.10	6.15
	306	10	4.90	5.00	0.57	11.58
	310	10	11.70	12.00	0.95	8.11
	L1103	10	9.80	10.00	0.92	9.38
	L1103	10	10.60	11.00	0.84	7.96
	L1105	10	10.30	10.50	0.95	9.21
RBC	126	10	5.00	5.00	0.82	16.33
	195	10	7.10	7.00	0.99	14.01
	307	10	6.30	6.00	0.67	10.71
	L1103	10	10.00	10.00	1.05	10.54
	L1105	10	10.30	10.50	0.95	9.21

4 | DISCUSSION

Accurate CSF cell counts are vital for the diagnosis of several diseases, including subarachnoid hemorrhage, meningeal infection, demyelinating disease, and central nervous system malignancies. These samples represent a significant percentage of body fluid cell counts performed in hematology laboratories. Until recently, many manufacturers of automated cell counters were unable to achieve sufficient precision and accuracy at low cell counts to validate their instruments for clinical use for clear (nonbloody) CSF samples. Fleming and co-workers described a high-sensitive analysis (hsA) research mode on the Sysmex XN-1000 (Sysmex, Kobe, Japan) specifically for counting cells in fluids that contain low cell counts.[19] The lower limit of quantitation of this method is 10 RBCs/μL and 2 TNCs/μL; thus, this method cannot quantitate RBCs around the "normal" CSF range of zero RBCs. Until the FDA 510K clearance of the GloCyte System, no automated cell counter was validated and FDA 510K cleared for very low cell counts; thus, laboratories had to use manual chamber counts for many CSF samples. Sandhaus and colleagues performed CSF cell counts with the GloCyte and the hemocytometer on samples from patients with acute leukemia, lymphoma, malignant neoplasms involving the brain, meningitis, and encephalitis; results correlated very well between the manual and the automated method.[25]

The strengths of this study are the participation of multiple medical centers, making it possible to obtain large numbers of samples and spanning the entirety of the clinically significant measurement ranges, including extremely low cell counts, which are crucial for CSF samples. The standardized study protocol ensured consistent data collection at all sites. This multicenter study at five major medical centers shows that the GloCyte Automated Cell Counter System

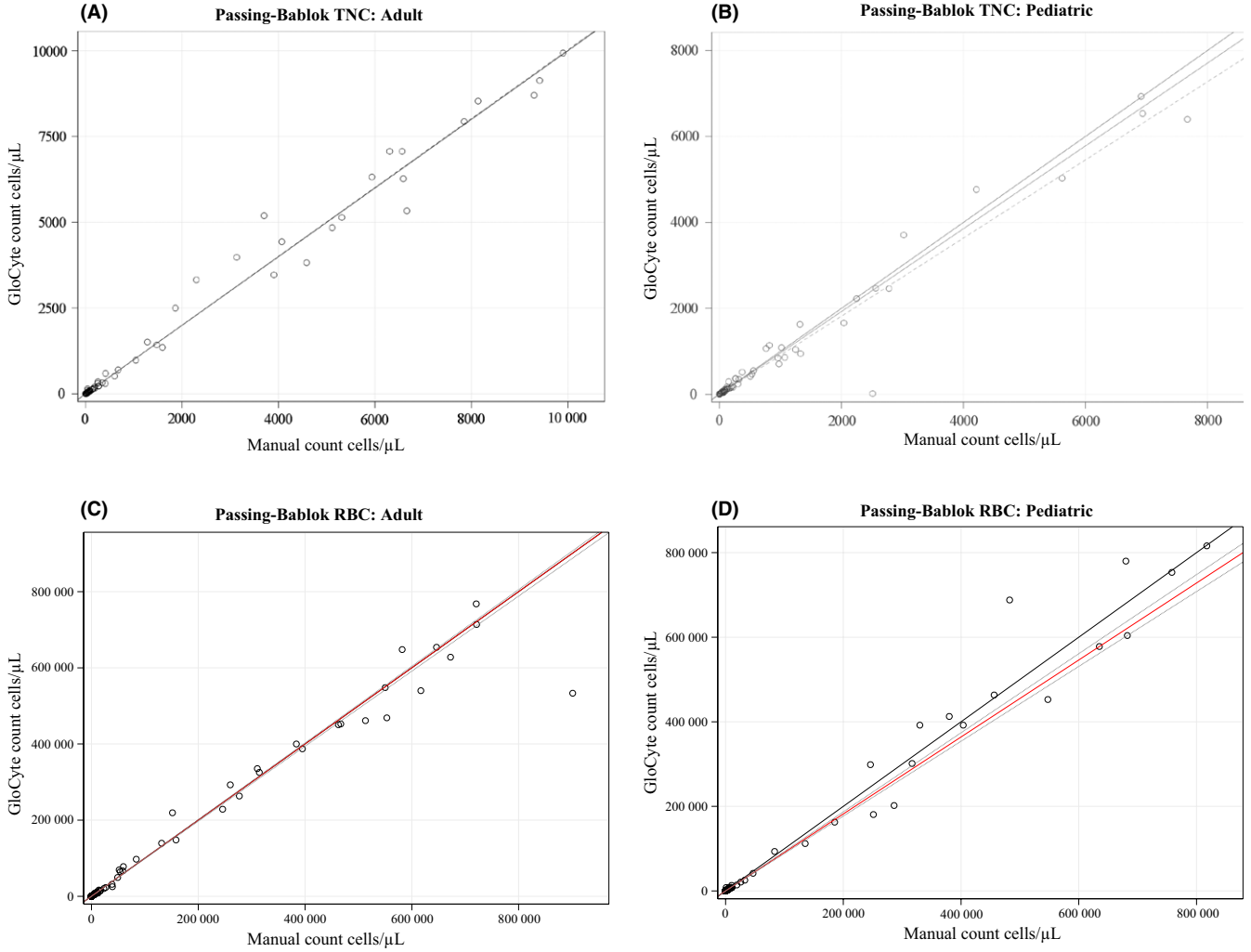

FIGURE 2 Passing-Bablok (PB) Correlation plots from method comparison for all (A) adult TNC, (B) pediatric TNC, (C) adult RBC, and (D) pediatric RBC counts

TABLE 5 Results of Passing-Bablok analysis

			Passing-Bablok analysis		
Cell type	Age Group	CUSUM test for linearity (*P*-value)	Parameter	Estimate	95% Confidence Interval
TNC	Adult	.524	Slope	1.000	(1.00, 1.00)
			Intercept	0.000	(0.00, 0.00)
	Pediatric	.415	Slope	0.963	(0.91, 1.00)
			Intercept	0.037	(0.00, 0.18)
RBC	Adult	.107	Slope	1.000	(0.99, 1.01)
			Intercept	0.000	(0.00, 0.01)
	Pediatric	.788	Slope	0.910	(0.89, 0.93)
			Intercept	0.000	(-0.05, 0.06)

provides results interchangeable with the counts obtained by trained technologists with a hemocytometer. With a CV below 20%, the GloCyte's precision met the standard acceptance criterion for this type of assay.[20] The correlation between GloCyte and hemocytometer results was excellent. These findings indicate that the GloCyte System can provide results with the same clinical value as manual methods even for extremely low cell counts that are within or near the reference ranges. These results can be obtained within approximately 5 minutes, without the need for staff who have the pattern recognition skills necessary for cell identification. This should allow

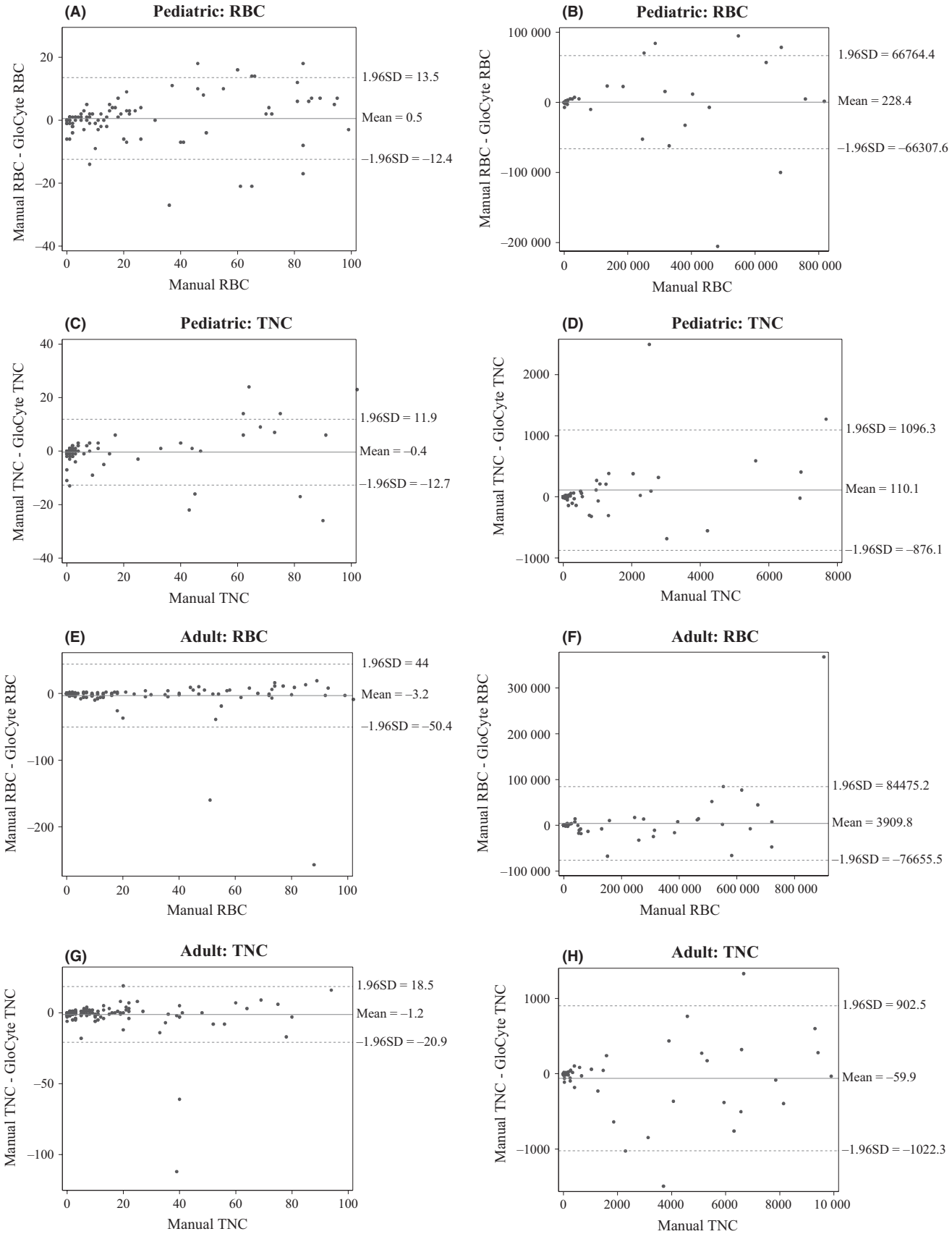

FIGURE 3 Bland-Altman Plots for Pediatric and Adult Samples. To demonstrate accuracy and lack of significant bias at low cell counts, separate graphs are provided for counts between zero and 100 cells/microliter (A,C,E,G) and for all cell counts (B, D, F, H). Results are also shown for pediatric (A, B, C, D) and adult (E, F, G, H) specimens separately, as labeled

TABLE 6 Sensitivity, specificity, positive predictive value, and negative predictive value of the GloCyte System

	Frequency of GloCyte counts									
Cell type	≤5	>5	TP	TN	FN	FP	Sensitivity	Specificity	PPV	NPV
TNC	176	176	163	164	12	13	0.931	0.927	0.926	0.932

A manual count of 5 was used as the cutoff between normal and abnormal.
NPV, Negative Predictive Value; PPV, Positive Predictive Value.

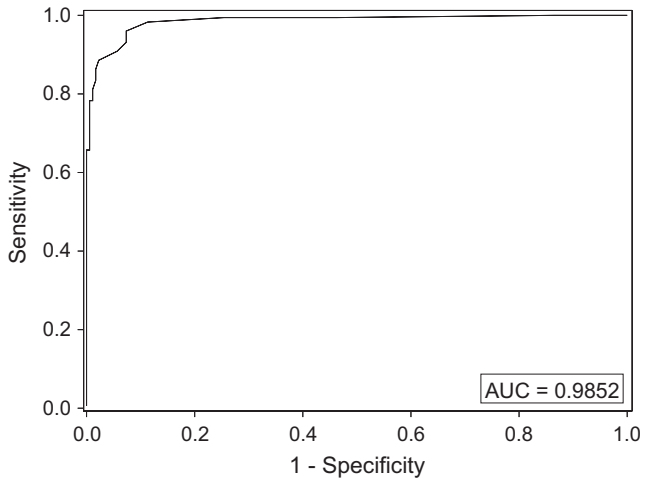

FIGURE 4 Receiver-operating characteristic (ROC) curve for total nucleated cells (TNC)

for the ability to obtain CSF cell counts on all shifts, including nights and holidays.

It has recently been suggested that hemocytometers may soon be obsolete in the clinical laboratory.[20] The availability of the GloCyte System, with the ability to provide accurate and precise counts at extremely low cell numbers, may make that prediction a reality in the near future. This study raises the hope that the GloCyte System, in combination with the body fluid modes of other automated cell counters and digital image analysis, will allow laboratories to discontinue all manual cell counting methods. However, until automated cell counters can reliably classify all cells that are present in CSF samples, including neoplastic cells, there will still be a need for microscopic examination, either by manual microscopy or digital image analysis.

ACKNOWLEDGEMENTS

The authors thank Dr. George Hong and Julie MacKenzie (Advanced Instruments, Norwood, MA) for their input and comments on the paper, and Warren Hinkle (BioStat Solutions, Inc, Frederick MD) for providing the statistical analysis.

REFERENCES

1. Green R, Wachsmann-Hogiu S. Development, history, and future of automated cell counters. In: Kratz A, Brugnara C, eds. *Clinics in Laboratory Medicine 35*. Philadelphia, PA: Elsevier; 2015:1-10.
2. Karcher DS, McPherson RA. Cerebrospinal, synovial, serous body fluids, and alternative specimens. In: McPherson RA, Pincus MR, eds. *Henry's Clinical Diagnosis and Management by Laboratory Methods*, 22nd edn. Philadelphia, PA: Elsevier; 2011:480-506.
3. Danise P, Maconi M, Rovetti A, et al. Cell counting of body fluids: comparison between three automated haematology analysers and the manual microscope method. *Int J Lab Hematol*. 2013;35:608-613.
4. Sandhaus LM. Body fluid cell counts by automated methods. In: Brugnara C, Kratz A eds. *Automated Hematology Analyzers: State of the Art*. Clinics in Laboratory Medicine. Philadelphia, PA: Elsevier; 2015:93-103.
5. Boer K, Deufel T, Reinhoefer M. Evaluation of the XE-5000 for the automated analysis of blood cells in cerebrospinal fluid. *Clin Biochem*. 2009;42:684-691.
6. Buoro S, Apassiti Esposito S, Alessio M, Crippa A, Ottomano C, Lippi G. Automated cerebrospinal fluid cell counts using the new body fluid mode of sysmex uf-1000i. *J Clin Lab Anal*. 2015;30:381-391.
7. Paris A, Nhan T, Cornet E, Perol JP, Malet M, Troussard X. Performance evaluation of the body fluid mode on the platform Sysmex XE-5000 series automated hematology analyzer. *Int J Lab Hematol*. 2010;32:539-547.
8. Yang D, Zhou Y, Chen B. Performance evaluation and result comparison of the automated hematology analyzers Abbott CD 3700, Sysmex XE 2100 and Coulter LH 750 for cell counts in serous fluids. *Clin Chim Acta*. 2013;419:113-118.
9. Aulesa C, Mainar I, Prieto M, Cobos N, Galimany R. Use of the Advia 120 hematology analyzer in the differential cytologic analysis of biological fluids (cerebrospinal, peritoneal, pleural, pericardial, synovial, and others). *Lab Hematol*. 2003;9:214-224.
10. Li A, Gronlund E, Brattsand G. Automated white blood cell counts in cerebrospinal fluid using the body fluid mode on the platform Sysmex XE-5000. *Scand J Clin Lab Invest*. 2014;74:673-680.
11. Buoro S, Appassiti Esposito S, Vavassori M, et al. Reflex testing rules for cell count and differentiation of nucleated elements in pleural and ascitic fluids on Sysmex XE-5000. *J Lab Autom*. 2016;21:297-304.
12. Buoro S, Mecca T, Azzara G, et al. Cell Population Data and reflex testing rules of cell analysis in pleural and ascitic fluids using body fluid mode on Sysmex XN-9000. *Clin Chim Acta*. 2016;452:92-98.
13. Bremell D, Mattsson N, Wallin F, et al. Automated cerebrospinal fluid cell count–new reference ranges and evaluation of its clinical use in central nervous system infections. *Clin Biochem*. 2014;47:25-30.
14. Tanada H, Ikemoto T, Masutani R, Tanaka H, Takubo T. Evaluation of the automated hematology analyzer ADVIA(R) 120 for cerebrospinal fluid analysis and usage of unique hemolysis reagent. *Int J Lab Hematol*. 2014;36:83-91.
15. Mahieu S, Vertessen F, Van der Planken M.. Evaluation of ADVIA 120 CSF assay (Bayer) vs. chamber counting of cerebrospinal fluid specimens. *Clin Lab Haematol*. 2004;26:195-199.
16. Buoro S, Seghezzi M, Mecca T, Vavassori M, Crippa A, La Gioia A. Evaluation of Mindray BC-6800 body fluid mode for automated cerebrospinal fluid cell counting. *Clin Chem Lab Med*. 2016;54:1799-1810.
17. Kleine TO, Nebe CT, Lower C, Geilenkeuser WJ, Dorn-Beineke A. Cell analysis in cerebrospinal fluid (CSF) using Sysmex(R) hematology

analyzers XT-4000i and XE-5000: evaluation with CSF controls of the Joint German Society for Clinical Chemistry and Laboratory Medicine (DGKL). *Cytometry A*. 2012;81:255-264.
18. Fleming C, Brouwer R, Lindemans J, de Jonge R. Validation of the body fluid module on the new Sysmex XN-1000 for counting blood cells in cerebrospinal fluid and other body fluids. *Clin Chem Lab Med*. 2012;50:1791-1798.
19. Fleming C, Russcher H, Brouwer R, Lindemans J, de Jonge R. Evaluation of Sysmex XN-1000 High-Sensitive Analysis (hsA) Research Mode for Counting and Differentiating Cells in Cerebrospinal Fluid. *Am J Clin Pathol*. 2016;145:299-307.
20. Sandhaus LM. Is the Hemocytometer Obsolete for Body Fluid Cell Counting? *Am J Clin Pathol*. 2016;145:294-295.
21. Buoro S, Apassiti Esposito S, Alessio M, Crippa A, Ottomano C, Lippi G. Automated Cerebrospinal Fluid Cell Counts Using the New Body Fluid Mode of Sysmex UF-1000i. *J Clin Lab Anal*. 2016;30:381-391.
22. Walker TJ, Nelson LD, Dunphy BW, Anderson DM, Kickler TS. Comparative evaluation of the Iris iQ200 body fluid module with manual hemacytometer count. *Am J Clin Pathol*. 2009;131:333-338.
23. Glasser L, Murphy CA, Machan JT. The clinical reliability of automated cerebrospinal fluid cell counts on the Beckman-Coulter LH750 and Iris iQ200. *Am J Clin Pathol*. 2009;131:58-63.
24. DeMott WR, Skikne BS. Cerebrospinal Fluid Analysis. In: Jakobs DS, DeMott WR, Oxley DK, eds. *Jacobs & DeMott Laboratory Test Handbook*. Hudson, Ohio: Lexi-Comp; 2001:416-418.
25. Sandhaus LM, Dillman CA, Hinkle WP, MacKenzie JM, Hong G. A new automated technology for cerebrospinal fluid cell counts: comparison of accuracy and clinical impact of glocyte, sysmex xn, and manual methods. *Am J Clin Pathol*. 2017;147:507-514.
26. *CSF Cell Counter: Advanced Instruments*, Norwood, MA; 2017 [Available from: http://www.aicompanies.com/product/glocyte/ - features.
27. (CLSI) CaLSI. *Body Fluid Analysis for Cellular Composition; Approved Guideline; CLSI Document H56-A*. Wayne, PA; 2006.
28. CLSI. *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures: Approved Guideline, 2nd edition; EP 17-A2*. Wayne, PA: CLSI; 2012.
29. Bourner G, De la Salle B, George T, et al. ICSH guidelines for the verification and performance of automated cell counters for body fluids. *Int J Lab Hematol*. 2014;36:598-612.
30. *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures: Approved Guideline, 2nd edition. CLSI document EP17-A2*, (2012).
31. College of American Pathologists. *Hemocytometer Fluid Count, Participant Summary*. Northfield, IL: College of American Pathologists; 2015.
32. Macdonald R. Quality assessment of quantitative analytical results in laboratory medicine by root mean square of measurement deviation. *J Lab Med*. 2006;30:111-117.
33. *Evaluation of Precision of Quantitative Measurement Procedures (EP05-A3)*, (2014).

How to cite this article: Hod EA, Brugnara C, Pilichowska M, et al. Automated cell counts on CSF samples: A multicenter performance evaluation of the GloCyte system. *Int J Lab Hem*. 2017;00:1–10. https://doi.org/10.1111/ijlh.12728