# A New Automated Technology for Cerebrospinal Fluid Cell Counts

## Comparison of Accuracy and Clinical Impact of GloCyte, Sysmex XN, and Manual Methods

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**Key Words:** Cerebrospinal fluid cell counts; Automated CSF cell counts; GloCyte; Sysmex XN; Body fluid cell counts; Total nucleated cells

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## ABSTRACT

***Objectives:*** *The purpose of the study was to compare the performance of GloCyte (Advanced Instruments, Norwood, MA), a new semiautomated instrument for cerebrospinal fluid cell counting, with the manual hemocytometer method and the automated Sysmex XN (Sysmex, Kobe, Japan) body fluid mode. The clinical impact of replacing the manual method with either automated method was determined.*

***Methods:*** *Fifty-seven samples from 38 patients were analyzed by all three methods. Pearson correlation and Passing-Bablok regression were used to compare methods. Cytospin smears were reviewed on all samples, and clinical histories were obtained.*

***Results:*** *There was a strong linear relationship between the manual and automated methods for WBC counts (*$R = 0.988$ *for GloCyte;* $R = 0.980$ *for Sysmex XN). Positive bias was absent or negligible for WBC counts less than 30/μL. GloCyte and manual RBC counts were equivalent. There were no samples for which replacement of manual WBC counts by automated counts would have changed the diagnosis. Both automated methods showed improved precision for WBC counts compared with the manual method.*

***Conclusions:*** *Replacing manual WBC counts by GloCyte or Sysmex XN WBC counts would improve consistency of results without compromising diagnostic accuracy.*

Cerebrospinal fluid (CSF) cell counts have traditionally been performed by hemocytometer chamber counts. As with other manual microscopic techniques, this method is subject to high interobserver variability and poor reproducibility.[1,2] Body fluid (BF) cell counts are now widely performed on various hematology analyzers in automated laboratories.[3-26] However, positive bias at very low cell counts has limited implementation of automated CSF cell counting to the same extent as for other body fluid cell counts.[27]

GloCyte (Advanced Instruments, Norwood, MA) is a semiautomated method that was designed to achieve accurate cell counts in the range of values that are typically present in normal and abnormal CSF samples. Unlike flow-through hematology analyzers, GloCyte uses digital image capture of fluorescence-labeled cells immobilized on a membrane to determine the concentration of total nucleated cells (TNCs) and RBCs in CSF samples. The purpose of this study was (1) to compare the GloCyte method with manual hemocytometer and Sysmex XN methods for CSF cell counts and (2) to determine the potential clinical impact of replacing the manual method by either of the automated methods.

## Materials and Methods

The study was approved by the University Hospitals Cleveland Medical Center Institutional Review Board. CSF samples were eligible for inclusion in the study if there was

sufficient volume for analysis by all three methods. For all samples, manual cell counts were performed first, followed by Sysmex XN and then GloCyte. All analyses were completed within 4 hours of sample receipt in the laboratory.

Manual counts were performed in duplicate on disposable Levy-Neubauer hemocytometers (INCYTO C-Chip, Seoul, Korea) by adding 10 µL to each chamber and counting all nine squares. Duplicate counts agreed within 20% of each other.

The Sysmex XN aspirates 88 µL CSF for analysis in the BF mode. It uses sheath flow impedance technology to count RBCs (RBC-BF) and fluorescence flow cytometry to enumerate TNCs (TC-BF) and WBCs (WBC-BF). TC-BF include highly fluorescent cells that fall outside the normal WBC distribution; therefore, TC-BF will always be greater than or equal to WBC-BF. For this study, WBC-BF values were used for method comparison for all samples. To further evaluate the Sysmex XN capabilities and compare Sysmex WBC-BF and TC-BF, the TC-BF values for 50 of the study samples were added to the data set at a later date; TC-BF values for seven of the samples were not available. RBC-BF are enumerated by impedance technology using a rounding algorithm: values less than 500/µL are rounded down to zero and counts of 500/µL or more are rounded up to 1,000/µL and so on until the RBC count exceeds 10,000/µL.

For GloCyte, 30 µL CSF sample is dispensed into each of two microcentrifuge tubes (one for TNCs and the other for RBCs), to which 30 µL RBC fluorescent staining reagent and 30 µL TNC fluorescent staining reagent are added, respectively. Then, 30 µL of the stained TNC sample is dispensed into a test cartridge and subjected to a vacuum pump that accelerates the capture of the cells onto a membrane. The cartridge is then inserted into the GloCyte instrument, where the emitted fluorescence from labeled cells is imaged via the charge-coupled device camera and converted by the software counting algorithm to a result expressed as cells/µL. The same process is repeated for the RBC count. If the cell count for either assay exceeds 53 cells/µL, the instrument prompts the operator to add a smaller volume of sample-reagent mixture to a new cartridge or make dilutions and repeat the analysis.

Cytospin smears were prepared from all CSF samples using a Shandon Cytospin 4 (Thermo Electron, Waltham, MA) and stained with a Wright stain. Manual differential counts were done by a technologist and all smears were reviewed by a hematopathologist (L.M.S.). Clinical histories and diagnoses were obtained by review of the electronic medical record.

To evaluate precision, four patient samples demonstrating a range of RBC and WBC counts were independently analyzed by five medical technologists by all three methods. Analyses were completed within 4 hours of the first technologist's analysis for samples 2, 3, and 4 and within 8 hours for sample 1. The technologists were blinded to each other's results.

### Statistical Analysis

SAS statistical software (SAS Institute, Cary, NC) versions 9.2 and 9.4 were used for descriptive statistics and analyses. Pearson correlation coefficients were used to assess the linear relationship between methods. Passing-Bablok regression was used for method comparisons, using the average of duplicate manual hemocytometer cell counts as the reference method. Precision (reproducibility) was evaluated by calculating the coefficient of variation (CV = SD/mean * 100) for repeated analyses. Sensitivity and specificity were calculated for CSF WBCs or TNCs using more than 5 cells/µL as the definition of a true positive for all samples.

## Results

From January to June 2015, 57 CSF samples from 38 patients met the criteria for study inclusion. The patients ranged in age from 2 days to 75 years. There were 43 samples from 25 adults and 14 samples from 13 patients younger than 18 years, including five neonates. Twenty-three patients had one sample, 12 patients had two samples, two patients had three samples, and one patient with a ventricular drain had four samples. There were eight samples from seven patients with acute leukemia, three samples from two patients with lymphoma, and six samples from five patients with other malignant neoplasms involving the brain. A total of 18 samples were from nine patients with clinical diagnoses of meningitis or encephalitis. Two of the patients with meningitis also had malignancies involving the brain (ependymoma in a child and metastatic breast cancer in an adult). The remaining samples were negative, hemorrhagic, or consistent with peripheral blood contamination.

Pearson correlation coefficients ($R$) and Passing-Bablok (PB) regression results with confidence intervals for WBC/TNC method comparisons are shown in **Table 1** and **Figure 1**. All of the automated methods demonstrate a strong linear relationship with the manual reference method with $R$ values of 0.98 or more over the entire range of data. The $R$ values are somewhat lower for samples with manual cell counts of 30/µL or less but still demonstrate a strong linear relationship.

The PB intercept provides an estimate of constant bias. A slight constant bias of 1.1 cells/µL was estimated for Sysmex TNC counts less than 30/µL but not for Sysmex WBC or GloCyte TNC counts less than 30/µL. The PB slope provides an estimate of proportional bias, for which


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**Table 1**
**Pearson Correlation (*R*) and Passing-Bablok Regression Estimates for GloCyte and Sysmex XN Cell Counts Compared With Manual Method**

| Method | Range, Cells/μL | Pearson *R* | Passing-Bablok | | | |
|---|---|---|---|---|---|---|
| | | | Intercept (95% CI) | Constant Bias | Slope (95% CI) | Proportional Bias, % (95% CI) |
| Manual TNC (0-2,833 cells/μL, n = 57) | | | | | | |
| GloCyte TNC | 0-4,087 | 0.988 | 0.000 (−0.172 to 0.990) | No | 1.049 (1.005 to 1.172) | 5 (0.5 to 17) |
| Sysmex WBC | 0-5,728 | 0.980 | 0.367 (−0.470 to 0.897) | No | 1.204 (1.103 to 1.470) | 20 (10 to 47) |
| Sysmex TNC[a] | 0-5,728 | 0.985 | 0.633 (−0.412 to 1.468) | No | 1.367 (1.161 to 1.516) | 37 (16 to 52) |
| Manual TNC (0-29 cells/μL, n = 37) | | | | | | |
| GloCyte TNC | 0-29 | 0.894 | 0.000 (−0.250 to 0.000) | No | 1.000 (1.000 to 1.250) | No |
| Sysmex WBC | 0-30 | 0.903 | 0.948 (0.000 to 1.313) | No | 1.052 (0.938 to 1.444) | No |
| Sysmex TNC[b] | 0-30 | 0.909 | 1.065 (0.290 to 2.056) | 1.1 cells/μL (0.3 to 2.1) | 1.087 (0.963 to 1.421) | No |
| Manual RBC (0-62,222 cells/μL, n = 57) | | | | | | |
| GloCyte RBC | 0-78,000 | 0.995 | 0.000 (−0.946 to 0.005) | No | 1.065 (0.996 to 1.135) | No |
| Manual RBC (0-432 cells/μL, n = 50) | | | | | | |
| GloCyte RBC | 0-1,084 | 0.743 | 0.000 (−0.712 to 0.012) | No | 1.056 (0.977 to 1.135) | No |

CI, confidence interval; TNC, total nucleated cell.
[a]Sysmex TNC was available for 50 samples.
[b]Sysmex TNC was available for 34 samples.

the difference between counting methods is expected to be greater in samples with higher cell counts. GloCyte TNC had the least proportional bias (5%) in comparison to the Sysmex WBC (20%) and Sysmex TNC (37%) over the complete range of values. Proportional bias was not detected by any method when manual cell counts were 30/μL or less.

For GloCyte RBC counts, Pearson *R* and PB regression (**Table 1** and **Figure 1G**) indicated a strong correlation with the manual reference method and absence of bias. The Sysmex XN rounding algorithm limits the value of correlation and regression models for RBC counts less than 10,000/μL. There were 50 samples with manual RBC counts less than 500/μL. For Sysmex XN, 43 (86%) of these had RBC values of zero, and seven (14%) had RBC values ranging from 1,000 to 3,000/μL. For GloCyte, 49 (98%) of these 50 samples had RBC counts less than 500/μL, and one sample had a higher RBC value of 1,084/μL. The Sysmex XN RBC result for this sample was 1,000/μL, demonstrating good correlation between the two automated methods. For the remaining seven of 57 samples, RBC counts ranged from 1,000 to 62,222/μL by the manual method, 1,620 to 78,000/μL by GloCyte, and 2,000 to 95,000/μL by Sysmex XN.

Results of the precision study for manual TNC, GloCyte TNC, and Sysmex WBC counts are shown in **Table 2**. Both automated methods demonstrated improved reproducibility in comparison to the manual method. The CVs for GloCyte and Sysmex XN were comparable with the exception of sample 4, for which a CV of 39% was obtained for GloCyte. The relatively high CV for this sample was due to the cell counts from the last two technologists, whose results (49/μL and 27/μL) were considerably lower than the first three technologists' results (83/μL, 81/μL, and 81/μL). The GloCyte counts were performed as the last method, and it is likely that cellular degradation over time is the explanation of the lower cell counts.

The patient results were compared for discordant results between methods and potential impact on clinical diagnoses. **Table 3** lists the results for all pediatric samples (n = 14). Overall, there is excellent agreement for WBC/TNC by all methods. There was one child with meningitis (sample 31) with a manual TNC count of 56 cells/μL compared with GloCyte TNC and Sysmex XN WBC counts of 108 cells/μL. Although the manual and automated counts appear discordant, they are all concordant with the diagnosis of meningitis. There was also excellent overall agreement of GloCyte and manual RBC counts for pediatric samples. There were 12 samples that had manual and GloCyte RBC counts less than 500/μL, for which the Sysmex XN yielded RBC counts of zero, as expected, based on the rounding algorithm. For the two samples with high RBC counts (samples 28 and 55), the Sysmex XN RBC counts were substantially higher than the other methods, but this discrepancy had no clinical impact. Cytospin smears confirmed that none of the samples from children with acute leukemia or ependymoma had malignant cells.

Cell counts from patients with clinical diagnoses of meningitis, ventriculitis, or encephalitis are shown in **Table 4**. Direct comparison of cell counts confirms that the same diagnostic interpretation would have been reached on each sample by any of the three methods. Although patient 4 (samples 34, 44, and 46) had a clinical diagnosis of encephalitis, an infectious etiology was never confirmed. The



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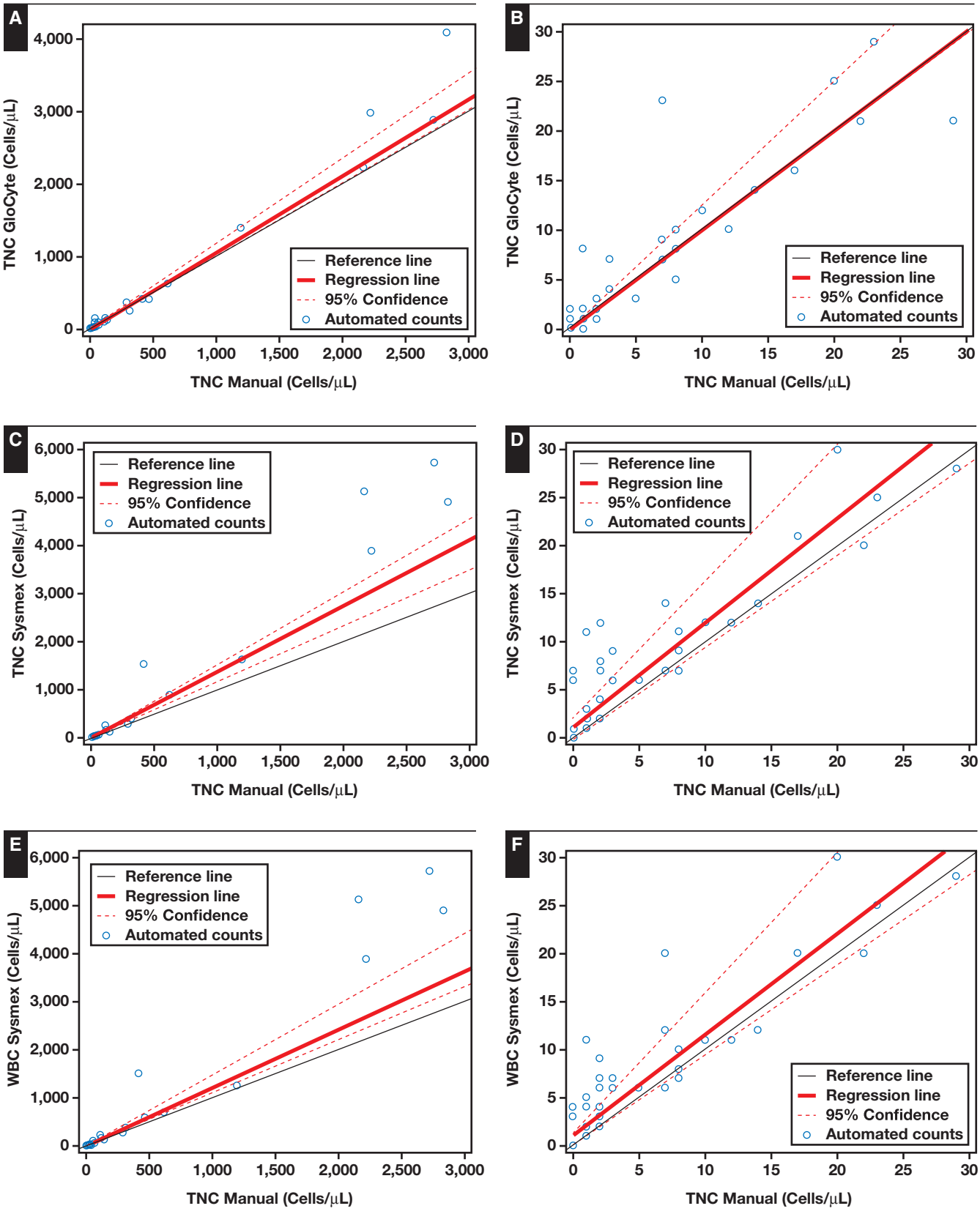


**Figure 1** Scatterplots for automated vs manual counts. **A**, **C**, **E**, Manual 0 to 2,833 cells/µL, n = 57. **B**, **D**, **F**, Manual 0 to 29 cells/µL, n = 37. TNC, total nucleated cell.


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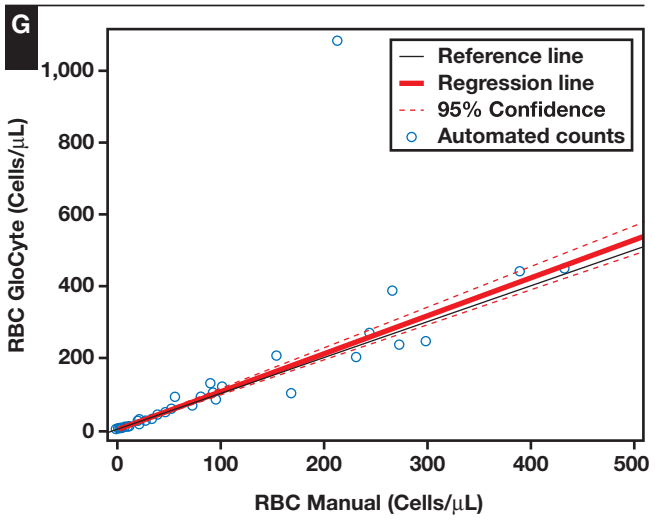


**Figure 1** (cont) **G**, Manual 0 to 432 cells/μL, n = 50.

**Table 2**
**Precision Study Results for Cerebrospinal Fluid WBC/TNC Methods**

| Sample | Manual TNC | GloCyte TNC | Sysmex WBC |
|---|---|---|---|
| Sample 1 | | | |
| Mean (SD), cells/μL | 35.0 (9.0) | 48.2 (3.8) | 57.4 (2.4) |
| CV, % | 26 | 8 | 4 |
| Sample 2 | | | |
| Mean (SD), cells/μL | 0.6 (0.5) | 1.2 (0.4) | 1.2 (0.4) |
| CV, % | 91 | 37 | 37 |
| Sample 3 | | | |
| Mean (SD), cells/μL | 21.8 (3.5) | 26.6 (1.8) | 41.2 (2.2) |
| CV, % | 16 | 7 | 5 |
| Sample 4 | | | |
| Mean (SD), cells/μL | 63.2 (28.6) | 64.2 (25.2) | 96.0 (5.8) |
| CV, % | 45 | 39 | 6 |

CV, coefficient of variation; TNC, total nucleated cell.

mildly elevated WBC counts and higher RBC counts in tube 1 than in tube 4 (data not shown) were more consistent with peripheral blood contamination than an inflammatory process. Sequential WBC counts from ventricular drainage fluid from patient 8, who had brain abscesses, are shown in **Figure 2**. Sysmex XN WBC and TNC values are nearly identical and are slightly higher than the other two methods. Cytospin smears from these samples showed many neutrophils, degenerated neutrophils, and macrophages.

Sensitivity was comparable for the automated methods when the hemocytometer count was used as the reference method (97% for GloCyte vs 100% for Sysmex WBC and Sysmex TNC). GloCyte was the most specific (91%), followed by Sysmex WBC (70%) and Sysmex TNC (57%). Two samples were classified as false positive (FP) by GloCyte, seven classified as FP by Sysmex XN WBC, and nine classified as FP by Sysmex XN TNC. One of the two GloCyte FP samples was grossly bloody with a TNC count of 8 cells/μL compared with 1 cell/μL by the manual method. Sysmex XN results for this sample were 11 cells/μL for both WBCs and TNCs. The second GloCyte FP sample was from a child with ependymoma; TNC count by GloCyte was 7 cells/μL, Sysmex XN WBC and TNC yielded 6 cells/μL, and the manual result was 3 cells/μL. With the exception of the bloody sample mentioned above, the FP Sysmex XN WBC values ranged from 6 to 9 cells/μL for corresponding manual counts of 2 to 5 cells/μL. The FP Sysmex XN TNC values ranged between 6 and 12 cells/μL for corresponding manual counts of 0 to 5 cells/μL. None of the automated results classified as FP by this analysis would be expected to have a clinical impact.

## Discussion

The most common body fluids submitted for cellular analysis in clinical laboratories are CSF, pleural fluid, and peritoneal fluid. An important difference between CSF and the serous cavity body fluids is that mesothelial cells are present in normal serous cavity fluids and contribute to TNC count, whereas CSF lining cells are rarely present in CSF samples and do not contribute significantly to TNC count even when they are present. Therefore, for most CSF samples, the WBC and TNC values are equivalent and may be used interchangeably. Exceptions might be samples with large numbers of malignant cells that fall outside of the normal WBC cluster on automated hematology analyzers. For these samples, as for any samples for which malignancy is a diagnostic consideration, microscopic examination of cytospin smears by an experienced observer is indicated.

Samples from patients with bacterial meningitis or central nervous system (CNS) leukemia with a manual TNC count of more than 300/μL (n = 9) had Sysmex XN WBC and TNC results that were consistently higher than the manual or GloCyte methods. This discrepancy became more pronounced at higher cell counts, as expressed by the estimate of proportional bias by PB regression (Table 1). Cytospin smears showed many macrophages and degenerated neutrophils in some samples, as well as many apoptotic leukemic blasts in the two samples from an adult patient with CNS leukemia. Whether dead cells and macrophages account for the higher Sysmex XN WBC and TNC values in these samples is an interesting question and deserves further study.

The most common indication for CSF cellular analysis is the diagnosis or exclusion of meningitis. The results of this study show that the GloCyte and manual methods are substantially equivalent for WBC counts that are at or near the decision threshold for all age groups. The PB regression



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**Table 3**
**WBC/TNC Counts on 14 CSF Samples From 13 Pediatric Patients**

| | | | Methods, Cells/μL | | | | | |
|---|---|---|---|---|---|---|---|---|
| Sample No. | Age | Diagnosis | Manual TNC | Sysmex WBC | GloCyte TNC | Manual RBC | Sysmex RBC | GloCyte RBC |
| 50 | 2 d | Newborn | 8 | 7 | 10 | 7 | 0 | 7 |
| 9 | 3 d | Newborn | 8 | 8 | 5 | 81 | 0 | 91 |
| 33 | 10 d | Newborn | 8 | 10 | 8 | 2 | 0 | 1 |
| 55 | 15 d | Newborn | 20 | 30 | 25 | 432 | 3,000 | 449 |
| 28 | 22 d | Intracranial hemorrhage | 289 | 285 | 365 | 4,972 | 6,000 | 3,587 |
| 25[a] | 2 y | Seizure | 2 | 2 | 2 | 72 | 0 | 67 |
| 26[a] | 2 y | Seizure | 2 | 2 | 1 | 95 | 0 | 83 |
| 31 | 2 y | Ependymoma/meningitis | 56 | 108 | 108 | 168 | 0 | 100 |
| 2 | 5 y | ALL | 0 | 3 | 1 | 8 | 0 | 6 |
| 23 | 5 y | ALL | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | 9 y | ALL | 1 | 1 | 0 | 0 | 0 | 0 |
| 24 | 9 y | ALL | 1 | 1 | 1 | 273 | 0 | 237 |
| 17 | 10 y | Ependymoma | 2 | 7 | 3 | 2 | 0 | 4 |
| 29 | 17 y | ALL | 1 | 4 | 1 | 47 | 0 | 47 |

ALL, acute lymphoblastic leukemia; TNC, total nucleated cell.
[a]Tube 4 (sample 25) and tube 1 (sample 26) are from the same procedure.

**Table 4**
**WBC/TNC Counts on Patients With Clinical Diagnoses of Meningitis, Ventriculitis, or Encephalitis**

| | | Methods, Cells/μL | | |
|---|---|---|---|---|
| Patient No. | Sample | Manual TNC | Sysmex WBC | GloCyte TNC |
| 1 | 10[a] | 41 | 45 | 43 |
| | 11[a] | 41 | 46 | 43 |
| 2 | 31 | 56 | 108 | 108 |
| 3 | 32 | 12 | 11 | 10 |
| 4 | 34 | 23 | 25 | 29 |
| | 44[a] | 29 | 28 | 21 |
| | 46[a] | 5 | 6 | 3 |
| 5 | 41[a] | 2,833 | 4,907 | 4,087 |
| | 42[a] | 2,222 | 3,896 | 2,980 |
| | 43 | 412 | 1,504 | 416 |
| 6 | 47[a] | 2,167 | 5,126 | 2,233 |
| | 48[a] | 2,722 | 5,728 | 2,887 |
| 7 | 49 | 10 | 11 | 12 |
| | 51 | 142 | 139 | 135 |
| 8 | 52 | 117 | 175 | 149 |
| | 53 | 312 | 380 | 257 |
| | 54 | 111 | 239 | 93 |
| | 56 | 67 | 77 | 51 |
| 9 | 57 | 467 | 596 | 410 |

TNC, total nucleated cell.
[a]Pairs of samples that represent tubes 1 and 4 from the same procedure.

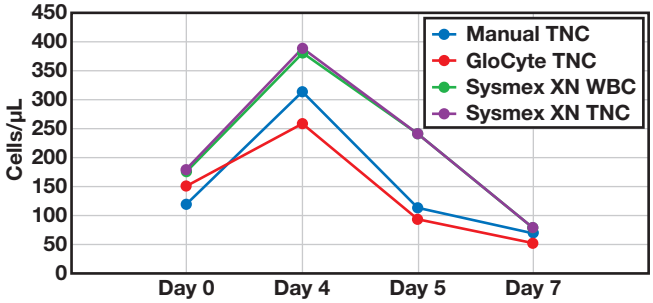


**Figure 2** Sequential cerebrospinal fluid WBC/total nucleated cell (TNC) counts on ventricular drainage fluid from patient 8.

statistics for Sysmex XN suggest that there may be a slight positive bias for cell counts less than 30/μL, which was reflected in the higher FP rate for Sysmex XN. However, CSF WBCs that are only minimally elevated above the reference range are unlikely to be interpreted as evidence of meningitis without other supportive clinical or laboratory evidence. For CSF WBCs more than 30/μL, a proportional bias was most evident in the samples with extremely high WBC counts, as discussed above, for which the diagnosis of meningitis or CNS leukemia was clear-cut.

The CSF RBC count is primarily useful as an indication of intracranial hemorrhage or traumatic tap, whereby RBCs are introduced into the CSF sample by the procedure. Comparison of the ratio of RBCs/WBCs in tubes 1 and 4 is often used to assess the likelihood of traumatic tap.[28-30] The ratio of RBCs/WBCs can be useful in determining whether the source of WBCs in CSF is CNS inflammation or peripheral blood, either from intracranial hemorrhage or traumatic tap. For patients with acute leukemia who have leukemic blasts circulating in peripheral blood, the exclusion of traumatic tap is especially important in the assessment of CNS disease. The results of this study confirm that GloCyte RBC and manual RBC counts may be used interchangeably. However, the Sysmex XN RBC algorithm for reporting RBC counts less than 1,000/μL limits its usefulness for this purpose.

GloCyte and Sysmex XN offer two alternatives for the automation of CSF cell counting. The main benefit of the automated methods is their objective methodology for cell quantitation that improves consistency of results.


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Depending on laboratory workflow and the number of CSF samples received, turnaround times for CSF cell counts might also improve. GloCyte involves more sample preparation than Sysmex. Sysmex, on the other hand, requires switching the analyzer from the CBC mode to the BF mode, which may require several minutes for the analyzer to perform background cell counts. An advantage of GloCyte is that it produces an accurate RBC count, even for low RBC counts. Sysmex has recently introduced a high-sensitivity BF mode on XN analyzers that is capable of reporting RBC counts as low as 10/μL.[31,32] This software enhancement is not approved by the Food and Drug Administration but can be implemented as a laboratory-developed test in the United States. Sysmex XN also offers a limited WBC differential count and flagging algorithm.[31,32] However, neither automated method eliminates the need for microscopic evaluation for abnormal cells and microorganisms. The relative advantages and disadvantages of each method must be evaluated in the context of each laboratory's test volume, case mix, level of automation, and technical staff.

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