Use of the Cell-Dyn Sapphire Hematology Analyzer for Automated Counting of Blood Cells in Body Fluids

Dieter De Smet, MD,1 Guy Van Moer,1 Geert A. Martens, MD, PhD,2 Nikolaos Nanos, MD,1 Lutgarde Smet,1 Kristin Jochmans, MD, PhD,1 and Marc De Waele, MD, PhD1

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Abstract

The enumeration and identification of blood cells in body fluids offers important information for the diagnosis and treatment of various medical conditions. Manual microscopic methods (hemacytometer total cell count and cytocentrifuged differential count) have inherent analytic and economic disadvantages but are still considered the “gold standard” methods. We evaluated the analytic and clinical performance of the Cell-Dyn Sapphire hematology analyzer (Abbott Diagnostics Division, Santa Clara, CA) for automated blood cell counting and leukocyte differential counting in cerebrospinal fluid, serous fluid (peritoneal and pleural fluid), and continuous ambulatory peritoneal dialysis fluid, and we compared the performance with the respective manual methods. In the present article, we describe its applicability for the distinct body fluids, and we highlight limitations and caveats.

The enumeration and identification of blood cells in body fluids offers important information for the diagnosis and treatment of various medical conditions. Elevated WBC counts in cerebrospinal fluid (CSF) are seen in meningitis, encephalitis, and other neurologic or neoplastic disorders. RBC counts are important for the diagnosis of intracerebral hemorrhage and for the exclusion of a traumatic tap as the cause of an elevated WBC count.1 Serous fluid cell counts are helpful in the diagnosis of peritonitis and allow classification of the fluid as a transudate or an exudate.2,3 An elevated WBC count in continuous ambulatory peritoneal dialysis (CAPD) fluids is an argument for peritonitis.4 For peripheral blood, the automated cell count and leukocyte differential count are well established and precise, whereas for body fluids, manual hemacytometer counting of blood cells and leukocyte differentiation in a cytocentrifuged preparation are still considered the “gold standard” methods.5,6 Manual cell counting is extremely labor-intensive and time-consuming, and skilled personnel have to be available 24 hours a day, and 7 days a week. Apart from the organizational and inherent economic disadvantages, the manual method also has poor precision and large interobserver variability.7 Automation seems to be the answer to overcome these difficulties.

The Cell-Dyn Sapphire (Abbott Diagnostics Division, Santa Clara, CA) is an automated hematology analyzer based on cell counting with an optical and an impedance channel.8 As a hematology analyzer, it is, in contrast with urine analyzers, designed for the enumeration of high cell counts and not of low cell concentrations, as in body fluids. Yet, several attempts to use automated hematology analyzers for this purpose have been described over the years.9-17 Published data are, however, not conclusive enough to indicate that one of the...
currently available automated cell counters has adequate precision for reliable low WBC counts in body fluid specimens. However, manual hemacytometer counts could even be more imprecise at low cell concentrations.12,18

We evaluated the analytic and clinical performance of the Cell-Dyn Sapphire for blood cell counting and WBC differential count in body fluids. RBC and WBC counts were compared with the conventional manual hemacytometer counts, whereas leukocyte differential counts were compared with microscopic counts of cytocentrifuged preparations.

Materials and Methods

Patient Samples

For method comparison, we used 167 CSF, 101 serous fluid (61 pleural and 40 peritoneal), and 127 CAPD fluid samples that were collected for routine diagnostic purposes. All samples were collected into sterile tubes without anticoagulants. First, all requested tests were performed. When the results were completely determined following standard operating procedures, the RBC count, WBC count, and WBC differential count were performed on the leftover samples, provided there was a sufficient amount of residual sample. The analyses were performed as soon as possible and for CSF, always within 1 hour from the time of sample collection. Our study was performed with full respect for individuals’ rights to confidentiality and in accordance with procedures supervised by local authorities responsible for ethical research.

Manual Microscopic Examination

Manual microscopic RBC and nucleated cell (NC) counts, after staining with brilliant cresyl blue, were performed in Bürker counting chambers using ×250 magnification with a standard microscope. Differential NC counts were performed after cytocentrifugation (7 minutes at 1,250 rpm) of the samples, followed by May-Grünwald staining. Experienced observers examined at least 100 nucleated cells.

Cell-Dyn Sapphire Hematology Analyzer

The Cell-Dyn Sapphire is an automated hematology analyzer designed for counting peripheral blood cells in whole blood samples. The counting and differentiation principles are based on an optical and an impedance channel. RBC counts can be performed with both methods, namely the RBC impedance count (RIC) and the RBC optical count (ROC), whereas the WBCs are counted and differentiated optically by flow cytometry with forward, side, polarized, and depolarized scatter. The required specimen volume for the Cell-Dyn Sapphire is 120 μL. Total counts of WBCs and RBCs are reported (with the lowest detectable RBC count of 1,000 cells/μL), as is the differential count of lymphocytes, monocytes, neutrophils, eosinophils, and basophils.

In view of the different WBC/RBC proportions that can be expected in body fluids other than blood, we investigated the Cell-Dyn Sapphire RBC count for WBC interference. For this purpose, we analyzed the correlations with RIC and ROC with and without subtraction of the WBC count. For assessing the differential count, the percentages of lymphocytes and monocytes determined by the Cell-Dyn Sapphire were added, and this sum was compared with the mononuclear cell (MNC) count found on the cytocentrifuged preparations, which included lymphocytes, monocytes, and, in some samples, epithelial cells and macrophages. The remaining WBCs (neutrophils, eosinophils, and, occasionally, basophils) were regarded as polymorphonuclear cells (PMNCs). When no good correlation was obtained, we investigated whether the Cell-Dyn Sapphire “correctly” classified macrophages and epithelial cells within the MNC count, by adding the macrophages and epithelial cells found in the cytocentrifuged preparation to the PMNC count instead of to the MNC count. If correlation improved, this would imply that the Cell-Dyn Sapphire classified the epithelial cells and macrophages within the PMNC count rather than in the MNC count.

Performance Characteristics

Background Concentration Limits

Background concentrations represent apparent sample-related constituents that actually originate from blood-free reagents and/or electronic “noise” and are used to confirm the system’s baseline performance, where no actual sample is aspirated. The manufacturer’s acceptable background concentration limits that must be met before using the instrument are 100/μL or less for the WBC count and 20,000/μL or less for the RBC count. We investigated whether these limits can be modified to make them applicable for body fluids with much lower cell concentrations compared with blood. Therefore, we determined the WBC and RBC background count 20 consecutive times. The mean WBC and RBC values + 2 SD were used as background concentration limits.

Carryover

Because the Cell-Dyn Sapphire is normally running blood samples with higher cell concentrations than in body fluids, carryover is an important issue. After a normal blood sample was analyzed, we determined the RBC and WBC count in a series of 6 tubes containing sodium chloride buffer only.

Linearity

Linearity was determined on serial sample dilutions prepared at relevant linearity ranges. Therefore, CSF samples with respectively high WBC and RBC counts were diluted
with sodium chloride buffer (0.14 mol/L sodium chloride, 0.01 mol/L phosphate, and 50 g/L human albumin). All samples were measured twice, and the means were compared with the expected cell counts.

**Imprecision and Functional Sensitivity**

For within-run imprecision, a sample of CSF with a very high RBC or WBC count was diluted by cell-free CSF to obtain samples with known cell concentrations. Different cell concentrations were prepared, and 10 aliquots of each cell concentration were counted. Within-run imprecision of the microscopic NC count was also investigated at low cell counts. For this purpose, 4 CSF samples were each counted 10 times in 10 different Bürker counting chambers by the same laboratory professional. Functional sensitivity was defined as the lowest cell concentration that can be measured with 95% confidence of a coefficient of variation (CV) of 20% or less. It was determined from the precision study, which generated a mean and an SD for each cell concentration. The concentration in which the CV equals 20% was determined mathematically from the power regression equation. To obtain 95% confidence, 2 SD were added to the found concentration to obtain functional sensitivity [Figure 1].

**Statistical Analysis**

Statistical analyses were performed with Analyse-it software, version 1.73 (Analyse-it Software, Leeds, England) and Microsoft Excel 2003 (Microsoft, Redmond, WA). Linearity of the automated method was assessed by using linear regression analysis. Agreement between the automated cell counts and the microscopic data was examined by Passing-Bablok regression analysis. Wilcoxon tests for paired samples were used because normal distribution could not be demonstrated. A P value of less than .05 was considered statistically significant. Clinical performance studies were performed using Bayesian statistics.

**Results**

**Background Concentration Limits**

The following background concentrations (mean ± 2 SD) were obtained: WBC, 0.5/μL ± 1.8/μL; RIC, 100/μL ± 616/μL; and ROC, 0/μL. In view of these results, we decided to lower the background concentration limits proposed by the manufacturer for WBC counts from 100/μL or less to 3/μL or 0/μL. 

[Figure 1] Within-run imprecision (coefficient of variation [CV], %) of the Cell-Dyn Sapphire at different cell concentrations. Several dilutions of cerebrospinal fluid (CSF) samples with a very high WBC count (A) or RBC count (B and C) were made with a pool of cell-free CSF. Each dilution was examined 10 times. Functional sensitivities were defined as the upper 95% confidence limit (+2 SD) of the concentration corresponding to a CV of 20%. It was determined mathematically from the corresponding power regression equation by solving the equation toward x for y = 20%. The solid lines represent the power regression lines, and the equations with corresponding correlation coefficients are the following: A, WBC count: y = 112.6x−0.499 (r² = 0.98); B, RBC impedance count (RIC): y = 1922.0x−0.556 (r² = 0.98); C, RBC optical count (ROC): y = 995.2x−0.515 (r² = 0.90).
less and for RBC counts from 20,000/μL or less to 1,000/μL or less, which makes them applicable for body fluid analysis.

**Carryover**

Evaluation of carryover from normal (high cell count) blood samples to low cell count body fluid samples indicated that after analysis of a blood sample, at least 1 blank sample should be run before the WBC count and the RIC and ROC drop to the background level (data not shown).

**Linearity**

Linearity data are shown in **Table 1**. Excellent linearity was found, as shown by linear regression analysis for the WBC count for the range from 5/μL to 90/μL and for the RIC and ROC, respectively, for ranges from 3,000/μL to 83,000/μL and from 3,000/μL to 90,000/μL.

**Imprecision and Functional Sensitivity**

Within-run imprecision of the WBC and RBC counts in CSF was determined at relevant ranges encompassing normal and pathologic levels. Figure 1 shows the imprecision (CV) as a function of the cell count. Overall, the imprecision (CV) increased with decreasing RBC and WBC counts. Imprecision ranged from 83.7% to 5.7% (CV) for WBC counts between 2/μL and 706/μL, from 31.6% to 1.1% (CV) for an RIC between 2 × 10^3/μL and 629 × 10^3/μL, and from 27.0% to 1.6% (CV) for an ROC between 2 × 10^3/μL and 642 × 10^3/μL. Imprecision was further examined to determine functional sensitivity (Figure 1). The functional sensitivity, defined as the lowest RBC and WBC concentrations that can be measured with 95% confidence of a CV of 20% or less, was 50/μL for the RIC, and 3,000/μL for the ROC. These calculated cutoff values were confirmed by analyzing body fluids with the respective cell counts for 10 consecutive times (data not shown).

The within-run imprecision of the microscopic NC count at low cell counts was comparable to that of the automated method and ranged from 49.7% to 13.9% (CV) for NC counts between 6/μL and 90/μL.

**Comparison With Counting Chamber Results**

For all types of body fluids with microscopic cell counts below the functional sensitivities of the Cell-Dyn Sapphire (microscopic NC count, <50/μL [137 CSF, 11 serous fluid, and 102 CAPD fluid samples]; microscopic RBC count, <3,000/μL [130 CSF, 69 serous fluid, and 127 CAPD fluid samples]), we found a significant difference (P < .0001) between the manual and automated methods. Passing-Bablok regression analysis indeed showed lack of agreement between both methods in these samples.

However, for all types of body fluids with microscopic NC counts of 50/μL or more (30 CSF, 90 serous fluid, and 25 CAPD fluid samples) and RBC counts of 3,000/μL or more (37 CSF, 32 serous fluid, and no CAPD fluid samples), we found no statistically significant difference between the microscopic count and the Cell-Dyn Sapphire **Figure 2**. However, the good agreement for the RBC count could only be obtained with the ROC and with subtraction of the WBC count, which indicates that WBCs, if present, interfere with the RBC count in body fluids (data not shown). Correlation data for all body fluids with an NC count of 50/μL or more and an RBC count of 3,000/μL or more are summarized in **Table 2**.

**Differential Count: Comparison With Cytocentrifuged Preparations**

When all samples were taken into account, the differential counts obtained on the cytocentrifuged preparations and by the Cell-Dyn Sapphire were significantly different (P < .0001) for all types of body fluids (data not shown).

We then evaluated the samples with a microscopic NC count of 50/μL or more. Data are summarized in **Table 3**.

For CSF and CAPD fluids, no statistically significant difference was found, and good agreement was shown between both methods for the PMNC and MNC counts.

For serous fluids, there was a statistically significant difference (P < .0001) between both methods for the PMNC and MNC counts. When macrophages and epithelial cells, if present, were added to the PMNC count for the cytocentrifuged preparation, Wilcoxon testing still yielded a statistically significant difference.

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**Table 1**

**Linearity of Blood Cell Counting With the Cell-Dyn Sapphire**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>No. of Samples</th>
<th>Slope (95% CI)</th>
<th>Intercept (95% CI)</th>
<th>r^2</th>
<th>Range (/μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>7</td>
<td>0.97 (0.92 to 1.02)</td>
<td>3.4 (1.0 to 5.9)</td>
<td>1.00</td>
<td>10-900</td>
</tr>
<tr>
<td>RIC</td>
<td>7</td>
<td>0.97 (0.90 to 1.04)</td>
<td>1,450.1 (~1,102.9 to 4,003.1)</td>
<td>1.00</td>
<td>3,000-83,000</td>
</tr>
<tr>
<td>ROC</td>
<td>7</td>
<td>1.00 (0.98 to 1.01)</td>
<td>483.0 (~304.0 to 1,269.9)</td>
<td>1.00</td>
<td>3,000-90,000</td>
</tr>
</tbody>
</table>

CI, confidence interval; RIC, RBC impedance count; ROC, RIC optical count.

* Linearity was inferred from separate sample dilutions at relevant ranges. Linear regression analysis was performed for assessment of linear agreement between the measured cell count and the expected cell count.

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Figure 2: Passing-Bablok regression analysis comparing WBC counts (≥50/µL) and RBC counts (≥3,000/µL), respectively, in cerebrospinal fluid (A and B) and in serous fluids (C and D) performed on the Cell-Dyn Sapphire compared with the conventional hemacytometer count. Solid lines are the regression lines and dotted lines, the identity lines. The parameters of the regression equation are summarized in Table 2. NC, nucleated cells; ROC, RBC optical count.

Table 2: Agreement Between the Cell-Dyn Sapphire and Hemacytometer for WBC Counts (≥50/µL) and RBC Counts (≥3,000/µL) in Body Fluids*

<table>
<thead>
<tr>
<th></th>
<th>No. of Samples</th>
<th>Slope (95% CI)</th>
<th>Intercept (95% CI)</th>
<th>r²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF WBC</td>
<td>30</td>
<td>1.04 (0.76 to 1.28)</td>
<td>−9.4 (−62.6 to 13.7)</td>
<td>1.00</td>
<td>NS</td>
</tr>
<tr>
<td>ROC-WBC</td>
<td>37</td>
<td>1.06 (1.00 to 1.16)</td>
<td>−77.6 (−693.7 to 634.7)</td>
<td>1.00</td>
<td>NS</td>
</tr>
<tr>
<td>Serous fluid WBC</td>
<td>90</td>
<td>0.94 (0.88 to 1.01)</td>
<td>5.6 (−15.6 to 25.0)</td>
<td>1.00</td>
<td>NS</td>
</tr>
<tr>
<td>ROC-WBC</td>
<td>32</td>
<td>0.96 (0.91 to 1.11)</td>
<td>743.7 (−670.8 to 1,262.8)</td>
<td>1.00</td>
<td>NS</td>
</tr>
<tr>
<td>CAPD fluid WBC</td>
<td>25</td>
<td>1.03 (0.89 to 1.15)</td>
<td>4.7 (−32.4 to 25.0)</td>
<td>1.00</td>
<td>NS</td>
</tr>
<tr>
<td>ROC-WBC</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

CI, confidence interval; CAPD, continuous ambulatory peritoneal dialysis; CSF, cerebrospinal fluid; NS, not significant; ROC, RBC optical count.

* Passing-Bablok regression analysis of WBC counts (≥50/µL) and RBC counts (≥3,000/µL) in body fluids measured by both methods. Differences between measurements were investigated with Wilcoxon tests for paired samples, and P < .05 was considered statistically significant.
Agreement Between the Cell-Dyn Sapphire and Hemacytometer for Differential Counts of Nucleated Cells (≥50/μL) in Body Fluids

<table>
<thead>
<tr>
<th>No. of Samples</th>
<th>Slope (95% CI)</th>
<th>Intercept (95% CI)</th>
<th>r²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymorphonuclear cells</td>
<td>30</td>
<td>0.87 (0.58 to 1.15)</td>
<td>10.2 (~9.6 to 33.4)</td>
<td>1.00</td>
</tr>
<tr>
<td>Mononuclear cells (including epithelial cells and macrophages)</td>
<td>30</td>
<td>0.89 (0.59 to 1.16)</td>
<td>2.1 (~5.4 to 7.8)</td>
<td>1.00</td>
</tr>
<tr>
<td>Serous fluid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymorphonuclear cells</td>
<td>90</td>
<td>1.55 (1.21 to 2.19)</td>
<td>9.6 (5.9 to 13.1)</td>
<td>1.00</td>
</tr>
<tr>
<td>Mononuclear cells (including epithelial cells and macrophages)</td>
<td>90</td>
<td>1.55 (1.21 to 2.20)</td>
<td>-64.6 (~123.5 to ~34.0)</td>
<td>1.00</td>
</tr>
<tr>
<td>Polymorphonuclear cells (including epithelial cells and macrophages)</td>
<td>90</td>
<td>0.88 (0.75 to 1.03)</td>
<td>-3.7 (~6.8 to 2.9)</td>
<td>1.00</td>
</tr>
<tr>
<td>Mononuclear cells</td>
<td>90</td>
<td>0.87 (0.75 to 1.03)</td>
<td>16.3 (5.7 to 22.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>CAPD fluid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymorphonuclear cells</td>
<td>25</td>
<td>0.88 (0.74 to 1.05)</td>
<td>8.5 (1.7 to 14.9)</td>
<td>1.00</td>
</tr>
<tr>
<td>Mononuclear cells (including epithelial cells and macrophages)</td>
<td>25</td>
<td>0.88 (0.74 to 1.03)</td>
<td>3.4 (~4.9 to 11.7)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Table 3: Agreement Between the Cell-Dyn Sapphire and Hemacytometer for Differential Counts of Nucleated Cells (≥50/μL) in Body Fluids

CAPD, continuous peritoneal dialysis; CI, confidence interval; CSF, cerebrospinal fluid; NS, not significant.

* Passing-Bablok regression analysis of differential counts of nucleated cells (≥50/μL) in body fluids measured by both methods. Differences between measurements were investigated with Wilcoxon tests for paired samples, and P < .05 was considered statistically significant.

significant difference (P < .0001) between both methods for the PMNC and MNC counts, but Passing-Bablok regression analysis showed better agreement and the confidence interval of the slopes even included unity [Figure 3]. This indicates that the Cell-Dyn Sapphire presumably counts macrophages and epithelial cells within the PMNC count rather than within the MNC count.

Clinical Performance of the Cell-Dyn Sapphire

To assess the ability of the Cell-Dyn Sapphire to distinguish normal from abnormal body fluid samples, Bayesian statistics were used with the manual hemacytometer count as the comparison method. Table 4 summarizes the clinical performance characteristics of the NC count for the different body fluids with their respective cutoff values for normality.

CSF samples with a microscopic NC count of more than 5/μL were considered abnormal. In 2 cases with borderline positive microscopic counts of 6/μL and 7/μL, the Cell-Dyn Sapphire yielded, respectively, 3/μL and 5/μL, thus resulting in false-negative samples in 1.2% of the cases (2/167). As a consequence, the Cell-Dyn Sapphire had a very good sensitivity (97.4%) and a very good negative likelihood ratio (0.06) for detection of abnormal NC counts in CSF. In contrast, in 49 cases with normal microscopic counts of 5/μL or less, the Cell-Dyn Sapphire assessed elevated cell counts between 6/μL and 28/μL, resulting in false-positive results in 29.3% of the cases (49/167). Hence, the specificity (45.6%) and the positive likelihood ratio (1.79) were poor.

Serous fluid samples with a microscopic NC count of more than 500/μL were considered abnormal. Sensitivity (90.3%) and specificity (94.3%) of the Cell-Dyn Sapphire were very good. Similarly, an excellent positive likelihood ratio (15.81) and a negative likelihood ratio (0.10) could be demonstrated. In 10 samples of serous fluids, metastatic cells were detected with microscopic NC differentiation of the cytocentrifuged preparation. The Cell-Dyn Sapphire is able to detect aberrant cell types by generating the flag “out of bound greater than preset limit,” meaning that a population of large aberrant cells is present that are, for that reason, not counted within the total WBC cell count. We evaluated the out of bound greater than preset limit flagging efficiency for the presence of metastases. The Cell-Dyn Sapphire flagged 7 samples, but in only 2 of those samples were metastatic cells present according to the differentiation of the cytocentrifuged preparation. On the other hand, the Cell-Dyn Sapphire failed to flag the remaining 8 samples with metastatic cells. This yields a very poor sensitivity (20.0%) and a specificity of 94.5% for the flagging efficiency in detecting metastatic cells. The positive and negative likelihood were respectively 3.64 and 0.85, and their 95% confidence intervals even included unity, thereby not leading to conclusive shifts from pretest to posttest probability for the presence of metastases.

CAPD fluid samples with a microscopic NC count of more than 100/μL were considered abnormal. The Cell-Dyn Sapphire showed good sensitivity (94.4%) and an excellent specificity (100.0%). Likewise, a very good negative likelihood ratio (0.06) and a more than excellent positive likelihood ratio (approaching infinity) could be demonstrated.

Discussion

Because hematology analyzers are normally running blood samples with inherently much higher cell concentrations, the accepted background cell concentration and the elimination of carryover are important issues. We have shown that it is possible to lower the manufacturer’s background limits toward 1,000/μL or less for the RBC count and 3/μL or...
**Figure 3** Passing-Bablok regression analysis comparing differential counts of serous fluid samples with microscopic WBC counts ≥50/μL on the Cell-Dyn Sapphire with those obtained with the conventional hemacytometer count. **A**, Polymorphonuclear cells. **B**, Mononuclear cells (with the cytocentrifuged preparation possibly including epithelial cells and macrophages). **C**, Polymorphonuclear cells (with the cytocentrifuged preparation possibly including epithelial cells and macrophages). **D**, Mononuclear cells. Solid lines are the regression lines and dotted lines, the identity lines. The parameters of the regression equation are summarized in Table 3.

**Table 4**
Clinical Performance Characteristics of the Cell-Dyn Sapphire for Nucleated Cell Count for Different Body Fluids Based on Their Respective Cutoff Values

<table>
<thead>
<tr>
<th></th>
<th>CSF (cells/μL)</th>
<th>Serous Fluid</th>
<th>CAPD Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>ULN (cells/μL)</td>
<td>5</td>
<td>500</td>
<td>100</td>
</tr>
<tr>
<td>No. of samples</td>
<td>167</td>
<td>101</td>
<td>127</td>
</tr>
<tr>
<td>True positive</td>
<td>75</td>
<td>28</td>
<td>17</td>
</tr>
<tr>
<td>False negative</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>True negative</td>
<td>41</td>
<td>66</td>
<td>109</td>
</tr>
<tr>
<td>False positive</td>
<td>49</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Sensitivity, % (95% CI)</td>
<td>97.4 (90.9-99.6)</td>
<td>90.3 (74.2-97.9)</td>
<td>94.4 (72.6-99.1)</td>
</tr>
<tr>
<td>Specificity, % (95% CI)</td>
<td>45.6 (35.0-56.4)</td>
<td>94.3 (86.0-98.4)</td>
<td>100.0 (86.6-100.0)</td>
</tr>
<tr>
<td>PPV, % (95% CI)</td>
<td>60.5 (51.3-69.1)</td>
<td>87.5 (71.0-96.4)</td>
<td>100.0 (80.3-100.0)</td>
</tr>
<tr>
<td>NPV, % (95% CI)</td>
<td>95.3 (84.2-99.3)</td>
<td>95.7 (87.8-99.0)</td>
<td>99.1 (95.0-99.9)</td>
</tr>
<tr>
<td>LR+, % (95% CI)</td>
<td>1.79 (1.48-2.17)</td>
<td>15.81 (6.06-41.22)</td>
<td>+ ∞</td>
</tr>
<tr>
<td>LR−, % (95% CI)</td>
<td>0.06 (0.01-0.23)</td>
<td>0.10 (0.03-0.30)</td>
<td>0.06 (0.01-0.37)</td>
</tr>
</tbody>
</table>

CAPD, continuous ambulatory peritoneal dialysis; CI, confidence interval; CSF, cerebrospinal fluid; LR−, negative likelihood ratio; LR+, positive likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; ULN, upper limit of normal.

* Results were considered normal or abnormal based on manual hemacytometer counts.
less for the WBC count, which makes them suitable for body fluid analysis. We were also able to show that it suffices to run 1 blank sample after a normal blood sample to minimize carryover for the WBC and RBC counts. The Cell-Dyn Sapphire further showed excellent linearity for WBC and RBC counts within the relevant ranges.

Our results are in agreement with previous studies showing limited precision of automated cell counters at low cell concentrations. For example, 2 automated hematology analyzers, Sysmex XE-2100 (TOA Medical Electronics, Kobe, Japan) and Bayer Advia 120 (Siemens Healthcare Diagnostics, Deerfield, IL), have been shown to have a lower limit of detection of 50/μL for the WBC count. Likewise, the body fluids module of the Iris iQ200 automated urine microscopy analyzer (Iris Diagnostics, Chatsworth, CA) was found to have a lower detection limit of 35/μL for NCs based on a within-run CV of 20% or less.9,17 However, we were able to show that the “poor” precision at low cell counts also holds true for the manual gold standard method and was comparable to that of the automated counting system.

The functional sensitivity of the Cell-Dyn Sapphire for WBC counts of 50/μL is troublesome for CSF specimens, which often fall below this cutoff and are clinically considered abnormal at WBC counts of more than 5/μL and >30/μL for adults and for children, respectively.1 The observed Cell-Dyn Sapphire’s functional sensitivity is, on the other hand, acceptable for many serous and CAPD fluids, eg, the clinical decision cutoff for peritonitis is a WBC count of 500/μL, and the cutoff is 1,000/μL for the distinction between transudates and exudates.2,3 For CAPD fluids, a WBC count of more than 100/μL is a well-accepted argument for CAPD-associated peritonitis.4

For the RBC count, we calculated a functional sensitivity of the Cell-Dyn Sapphire of 3,000/μL (provided that the RBCs are measured within the optical channel, ROC). Again, this limits the reliability of reporting low RBC counts in CSF because increased RBC counts resulting from cerebral bleeding will remain undetected as long as they are less than 3,000/μL. On the other hand, the Cell-Dyn Sapphire is clinically useful for distinguishing WBC admixture from peripheral blood caused by traumatic lumbar puncture. For example, 1,000 RBCs/μL of normal blood is accompanied by approximately 1 or 2 WBC/μL, which implies that as long as the RBC count remains below the limit of 3,000/μL, any increased WBC count indicates true pleocytosis.15,19 However, a functional sensitivity of 3,000 RBCs/μL remains high compared with urine analyzers like, for example, the Iris iQ200 with a functional sensitivity of 30 RBCs/μL, and many samples will still require manual analysis when an exact enumeration of the RBCs is desirable.

Our results are in contrast with those of Heller et al,20 who investigated the performance of the Cell-Dyn Sapphire for CSF blood enumeration and who reported CSF RBC values of less than 100/μL. According to the manufacturer, it is technically impossible to generate such low RBC counts because the lowest detectable value for the RIC and the ROC is 1,000/μL.

The poor reproducibility at low cell concentrations that we encountered is most likely to be held responsible for the absence of agreement with the manual microscopic count. On the other hand, we were able to show that for all body fluids, very good agreement existed with the manual method for samples with microscopic WBC and RBC counts greater than the respective functional sensitivities of 50/μL and 3,000/μL, provided that the RBC count was measured with the ROC and that a correction was made for WBC interference. Consistent with our findings, other investigators have also demonstrated good correlation when comparing body fluid cell counts by automated hematology analyzers such as the Sysmex XE-2100 (r² = 0.90–0.98), Bayer Advia 120 (r² = 0.92 for WBCs), and the Abbott Cell-Dyn 4000 (Abbott Diagnostics Division) (r² = 0.98 for WBCs) with manual hemacytometer counts.9,11,15

Whereas for CSF and CAPD fluids the WBC differential count showed acceptable analytic agreement with the manual method, it was clearly a major problem in serous fluids. In serous fluids, our results led to the impression that the Cell-Dyn Sapphire counts epithelial cells and macrophages, if present, within the PMNC count, resulting in a serious overestimation of the PMNC count. Because an increase in the PMNC count of more than 250/μL is also considered an argument for peritonitis,3 this could lead to many false-positives if a substantial amount of macrophages or epithelial cells is present in the serous fluid. For CAPD fluids, however, we did not encounter the same problem, and the frequently present macrophages and epithelial cells were “correctly” classified within the MNC count. We further showed that metastatic cells are not well flagged by the Cell-Dyn Sapphire so that microscopic evaluation of a cytocentrifuged preparation remains necessary.

To evaluate the clinical performance of the Cell-Dyn Sapphire, we looked at the decision cutoff values for the different body fluids and investigated whether the Cell-Dyn Sapphire correctly distinguishes normal from abnormal WBC counts. The results were good for serous fluids and CAPD fluids, with their respective cutoff values of 500/μL and 100/μL. For CSF specimens, many cases were falsely classified as abnormal, leading to a very poor specificity of 45.6%. But more interestingly, a screening opportunity exists within the very good sensitivity of 97.4% and the negative likelihood ratio of 0.06. Provided that there is enough sample volume (120 μL is required for automated analysis with the Cell-Dyn Sapphire), one should consider the possibility of presenting all CSF samples to the Cell-Dyn Sapphire because a value of less
than 5 cells/μL reported by the Cell-Dyn Sapphire is also very likely to be a normal cell count with the manual method. For a substantial part of samples, in our sample set approximately 25% (43/167), this could eliminate the need for the (labor-intensive) manual count.

We were able to show that the Cell-Dyn Sapphire can be very useful as a screening tool for total WBC and RBC counts in all body fluid samples. We recommend reporting only total WBC and RBC counts more than 50/μL and 3,000/μL, respectively, owing to the high imprecision at low cell counts. Exact enumeration of WBCs with a manual microscopic chamber count remains necessary for CSF when the Cell-Dyn Sapphire WBC count is less than 50/μL, unless values of less than 5/μL are obtained, because we found an excellent sensitivity for the Cell-Dyn Sapphire with regard to distinguishing normal from abnormal WBC counts. Furthermore, we do not recommend automated differentiation of NCs by the Cell-Dyn Sapphire because of its variable analytic performance in the presence of epithelial cells and macrophages for the distinct body fluids. Moreover, the inability to detect malignant cells or other pathologic cell types (eg, cell clusters and extracellular and intracellular bacteria) strongly justifies the need for sustained visual morphologic examination of cytocentrifuged preparations.

From the Departments of 1Hematology and 2Clinical Chemistry and Radioimmunology, Universitair Ziekenhuis Brussel, Brussels, Belgium.

Address reprint requests to Dr De Smet: Dept of Hematology, Universitair Ziekenhuis Brussel, Laarbeeklaan 101, Brussels, Belgium.

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