Evaluation of the HemoCue WBC analyzer to count leucocytes in body fluids

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Hemocytometric analysis of body fluids aids in the management and diagnosis of several diseases. Counting of white blood cells (WBC) in synovial fluid discriminates between inflammatory and non-inflammatory forms of joint swelling (1). Differential counting of WBC and erythrocytes (RBC) in cerebrospinal fluid (CSF) forms important and rapid available information in the diagnosis of meningitis, encephalitis and neuroinflammatory diseases like multiple sclerosis. Bacterial peritonitis is suspected when a large number of polymorph nucleated cells (PMN >250 x 10^6/L) (2) or WBC (WBC >100 x 10^6/L with ≥50% PMN) (3) are present in ascites and in continuous ambulatory peritoneal dialysis (CAPD) fluid, respectively. Microscopic analysis has been the gold standard for determination of the (differential) WBC and RBC counts in fluids but suffers from high imprecision (4), long turnaround times, and requirement of skilled personnel and mostly is not available 24 hours a day. Automated hemocytometric analysis may be the answer to these problems. Dedicated body-fluid modules have been developed by some manufacturers and are available on two commercial hemocytometers (5, 6). However, these machines are relatively costly and are mostly only available in central laboratory facilities. Moreover, the aspirated volume is relatively large (ca. 130 µL) and the matrix of some fluids such as drain fluids, synovial fluids, and bronchoalveolar lavage (BAL) fluids mostly is complex and not always suitable for automated hemocytometric analysis. Furthermore, no POC-instrument is on the market today to count WBC in body fluids. HemoCue recently launched a very small POC-instrument to count WBC in blood. We investigated whether this POC-analyzer also can be used to count WBC in body fluids.

Methods
The following body fluids were prospectively studied: CSF, pleural fluid, ascites, CAPD fluid, and synovial fluid. CSF was delivered in plain tubes, synovial fluid in heparin-anticoagulated tubes and the other fluids in EDTA-anticoagulated tubes. Because only material was used that was leftover from routine analysis, informed patient consent was not required. Routine body fluids were mixed and first counted using the Sysmex XE-5000 Body-Fluid (BF) Module (Sysmex, Etten-Leur, The Netherlands) in the open-manual mode. Directly after, fluids were measured on the HemoCue WBC analyzer (HemoCue Diagnostics B.V., Waalre, The Netherlands) in the open-manual mode. After mixing, fluid was pipetted on parafilm and ca. 10 µL of fluid was drawn into a single-use microcuvette by capillary action. In the microcuvette, red blood cells were lysed (saponin) and WBC stained (methylen blue). An image of the stained WBC was referenced.

References

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taken automatically after introduction of the micro-
cuvette in the analyzer and WBC were counted within
3 minutes by the software image analysis. Agreement
between methods was analyzed by creating Bland &
Altman difference plots and Passing & Bablok regres-
sion analyses in Analyse-it for Excel.

Results

Combining all fluids, good agreement was observed
between both analyzers: HemoCue = 0.93 × XE-5000
BF-mode + 86 (n=56; WBC in 10⁶/L; figure 1). The
HemoCue WBC analyzer counted significantly less
WBC in the higher ranges and more WBC in the lower
ranges because the 95% confidence interval (95% CI)
of the slope (0.90 - 0.99) did not include 1 and the 95%
CI of the intercept (43 - 137) did not include 0. The
mean bias was - 297 × 10⁶/L and non-significant (95%
CI: - 716 - 122 × 10⁶/L; figure 2). The slightly nega-
tive bias was mostly caused by some extreme outliers
in the higher WBC counts, because in the lower cell
counts the HemoCue in general counted more cells
than the XE-5000. Exclusion of 5 extreme high WBC
counts (≥10000 × 10⁶/L) resulted in a non-significant
positive bias of 47 × 10⁶/L (95% CI: - 52 - 146 × 10⁶/L);
similarly, exclusion of 5 outliers did not dramatically
change the regression results (HemoCue = 0.95 × XE-
5000 BF-mode + 82; n=51; 95% CI slope: 0.90 - 1.04
× 10⁶/L, 95% CI intercept: 18 - 134 × 10⁶/L). Good
agreement was also observed between both analyzers
for the individual fluids ascites (HemoCue = 0.99 ×
XE-5000 BF-mode + 145; n=24; WBC in 10⁶/L) and
pleural fluid (HemoCue = 0.89 × XE-5000 BF-mode
+ 102; n=15; WBC in 10⁶/L). For the other individual
fluids, too few samples were measured to perform
Passing-Bablok regression analyses.

Conclusion

In this study, we show that the HemoCue WBC ana-
lyzer agrees well with the Sysmex XE-5000 BF-mode
and allows to counting WBC in body fluids. The
HemoCue WBC analyzer could be of use in POC set-
tings such as at the emergency department, in situa-
tions where skilled personnel or financial resources
are scarce (e.g., peripheral locations, underdeveloped
countries), or when the sample matrix or volume does
not permit automated counting in the clinical chemistry
laboratory. The use of the HemoCue WBC analyzer is
limited because of several reasons. First, like all other
blood modes on automated cell counters, false-positive
counts are generated when large cells (macrophages,
mesothelial cells, tumor cells) are present. Second,
the measuring range (300 - 30000 × 10⁶/L) does not al-
low cell counting in clinically relevant ranges in CSF
(most counts will be below 300 ×10⁶/L) and CAPD
fluid (decision cut-off at WBC >100 ×10⁶/L). Third,
no differential is performed, which is important informa-
tion in the diagnosis of bacterial peritonitis in ascites
(PMN >250 ×10⁶/L) or CAPD fluid (WBC>100x10⁶/L
with ≥50% PMN) and to distinguish bacterial menin-
gitis from other causes of meningitis in CSF fluid.

References

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Figure 1. HemoCue vs. XE-5000 BF-mode. ◇ Ascites/CAPD;
○ CSF; △ Pleural fluid; □ Synovial fluid; — identity line.

Figure 2. Bland & Altman bias plot for the HemoCue WBC
analyzer versus Sysmex XE-5000 BF-mode. ——— Bias (-297);
------- 95% CI; ———- 95% Limits of agreement (-3362.1 to
2768.9).