Reliability of automated synovial fluid cell counting with Mindray BC-6800 body fluid mode

S. BUORO*, M. SEGHEZZI*, B. MANENTI*, T. MECCA*, E. CANDIAGO†, M. VIDALI‡, C. OTTOMANO§, C.C. CASTELLI¶, R. FERRARI¶, G. ZAPPALÀ¶, G. LIPPI**

*Clinical Chemistry Laboratory, Hospital Papa Giovanni XXIII, Bergamo, Italy
†Pathology, Hospital Papa Giovanni XXIII, Bergamo, Italy
‡Immunohaematology and Transfusion Medicine Unit, SS Trinità Hospital, Borgomanero, Italy
§Synlab, Monza, Italy
¶Orthopedics and Traumatology Unit, Hospital Papa Giovanni XXIII, Bergamo, Italy
**Section of Clinical Biochemistry, University of Verona, Verona, Italy

Correspondence:
Dr Sabrina Buoro, Clinical Chemistry Laboratory, Hospital Papa Giovanni XXIII –piazza OMS, 1, 24128 Bergamo, Italy.
Tel.: +39 352674550;
Fax: +39 352674939;
E-mail: sbuoro@asst-pg23.it

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ABSTRACT

Introduction: The enumeration and differentiation of nuclear elements in synovial fluid is a cornerstone for diagnosis and follow-up of many orthopedic and rheumatologic diseases. In this study, we evaluated the analytical performance of Mindray BC-6800 BF mode (BC-6800-BF) for synovial fluid analysis.
Methods: Overall, 78 synovial fluids were collected and analyzed with both BC-6800-BF and light microscopy. The study also entailed the assessment of limit of blank (LoB), limit of detection (LoD), limit of quantification (LoQ), carryover and linearity.
Results: The LoB for the parameters total cells and white blood cells was 6×10^6 cells/L, and the LoD and LoQ were instead 15 and 16×10^6 cells/L, respectively. Linearity was excellent and carryover was negligible. The agreement between BC-6800-BF and light microscopy was satisfactory for all samples pretreated with hyaluronidase, displaying a bias between −5.9% and 8.2%.
Conclusions: The use of BC-6800-BF for synovial fluid analysis enables rapid and accurate assessment, especially for total cell and polymorphonuclear counts. The use of BC-6800-BF may therefore allow the replacement of optical analysis, especially in samples pretreated with hyaluronidase, thus allowing its routine use for the screening of synovial specimens.

INTRODUCTION

The synovial fluid, which is specifically limited to the joint cavity, is produced by synovial cells and by exudation of fluids through the capillaries of the synovial membrane. A variety of orthopedic and rheumatologic diseases may cause joint pathologies, thus making synovial fluid analysis a crucial aspect in the differential diagnosis and therapeutic monitoring of these conditions. In particular, the accurate enumeration and differentiation of leukocytes in synovial fluid are both useful for distinguishing between inflammatory or infectious joint effusions, especially in patients bearing orthopedic implants [1, 2].

The light microscopy (LM) analysis using counting chambers (e.g., Burker, Nageotte) is still considered the reference technique for enumeration of total nuclear elements (TC) in synovial fluid, whereas the differential leukocyte count is typically achieved by means of LM analysis of slides stained with May–Grünwald–Giemsa stain [3]. As it is now clearly established that the reference LM analysis is plagued by notorious drawbacks such as high imprecision, need for skilled personnel for accurate identification of cellular elements, long turnaround time (TAT), and high cost [1–6], the possibility of performing automated analysis of many synovial fluids in a short time may be regarded as a valuable opportunity for overcoming many of these limitations [7–12]. Notably, the automated analysis of synovial fluid also carries some problems, the most important of which is attributable to the high viscosity of the specimen due to the presence of hyaluronic acid, a molecule that is absent from other biological fluids such as ascites and pleural effusions [1, 4]. The Mindray BC-6800 (Mindray, Shenzhen, China) is an automated hematological analyzer equipped with a specific module for body fluid analysis. Some previous studies have evaluated the performance of this instrumentation for the analysis of ascites or pleural or cerebrospinal fluids [13–15], but information on its reliability for analyzing synovial fluids is lacking to the best of our knowledge. Therefore, this study was planned to verify the analytical performance of Mindray BC-6800 (Mindray, Shenzhen, China) body fluid (BF) mode (BC-6800-BF) in synovial fluids according to the CLSI document H56-A [3] and the recommendations of the International Council for Standardization in Hematology (ICSH) [16].

MATERIALS AND METHODS

Synovial fluid samples

A total of 78 consecutive synovial fluid samples (68 from knee and 10 from hip synovia) were received by the local clinical chemistry and hematology laboratory for routine analysis, between July 2015 and April 2016. The samples were collected from 43 women and 35 men (median age 59.5 years) hospitalized in different medical and surgical wards of the Papa Giovanni XXIII General Hospital. All samples were collected in blood tubes containing K_3EDTA (Becton Dickinson, Franklin Lakes, NJ) and were simultaneously assessed with LM and BC-6800-BF within 2 hours from sampling. Both collection and analysis of all samples were performed according to the CLSI document H56-A [3]. The study was approved by the ethics committee of the Papa Giovanni XXIII Hospital and was carried out in accordance with the Declaration of Helsinki, under the terms of all relevant local legislations.

Pretreatment with hyaluronidase

Before analysis, the synovial fluid samples were divided into two paired aliquots, one of which was pretreated with 0.5 mg/mL of hyaluronidase (HY) (bovine hyaluronidase type IV-S: 750–3000 U/mg solid; Sigma, Saint Louis, Missouri, USA). Briefly, a HY solution was initially prepared by dissolving 5 mg of HY in 10 mL of 20 mM sodium phosphate buffer (pH 7.0). A volume of 20 μL of this solution was then mixed with 1 mL of synovial fluid and finally incubated at room temperature for 5 min before analysis, as suggested by the manufacturer's instructions and in agreement with previous data published by Seghezzi *et al.* [17].

Light microscopy

Manual LM cell count was performed using Burker's counting chamber [17]. The samples pretreated with HY were preliminarily diluted 1:20 or 1:200 with Stromatol's reagent (Mascia Brunelli, Milano, Italy), and the nuclear elements were then counted in four squares with a light microscope at ×400 magnification by two skilled operators, and by a third

operator when disagreement between the first two results was up to 5% [17]. For differential cell counts, synovial fluid samples were centrifuged at 100 *g* for 3 min (Cytospin2; Thermo Scientific, Massachusetts, USA) and stained with May–Grünwald–Giemsa reagent (Carlo Erba Reagents, Italy). The slide review was carried out at ×400 magnification with x40 oil-immersion objective (Objective Plan-Apochromat 40 × /1.3 Oil DIC M27, $D = 0.17$ mm; Carl Zeiss S.p.A., Italy) on 200 cells by two skilled operators, and by a third operator when disagreement between the first two results was up to 5% [17–19]. When necessary, a more accurate cytomorphological evaluation by means of a second microscopic analysis was performed at ×1000 magnification with ×100 oil-immersion objective (Objective Plan-Apochromat).

BC-6800-BF mode analysis

The principle of cell enumeration in the BC-6800 body fluid mode (BC-6800-BF) entails cell quantification through fluorescent flow cytometry with hydrodynamic focusing after selective lysis and fluorescent staining of nucleated elements. Stained cells are then classified using laser side scatter (SS), forward scatter (FS), and fluorescence (FL) into a 3D scattergram according to their internal complexity (SS axis), size (FS axis), and nucleic acid content (FL axis). The red blood cells (RBC) are identified and counted in the impedance channel of the BC-6800-BF. The default parameters provided by the BC-6800-BF include total cell (TC-BF), white blood cell (WBC-BF), polymorphonuclear cell (PMN), and mononuclear cell (MN) counts. Additional research parameters include differentiation and enumeration of neutrophils (NE-BF), eosinophils (Eos-BF) and high fluorescence cells (HF-BF).

The BC-6800-BF automatically performs a rinse cycle, followed by a background check after each analysis to prevent carryover and cross-contamination with peripheral blood. All BC-6800-BF measurements were carried out according to the manufacturer's instructions. A proprietary material was used for calibration materials, and the quality of results was validated using three levels of internal quality controls (R&D Body Fluid Hematology controls; R&D Systems, Inc. USA).

Evaluation of carryover

The carryover of the BC-6800-BF was assessed using two synovial fluid samples with high cellularity (7186–35 782 × 10^6 cells/L). Both samples were analyzed in triplicate (A1, A2, A3), followed by the triplicate analysis of saline solution (B1, B2, B3). The carryover was expressed in percent (%) and finally calculated according to the equation [(B1–B3)/(A3–B3)]×100 [3, 16].

Limit of blank (LoB) and limit of detection (LoD)

The limit of blank (LoB) and the limit of detection (LoD) of the BC-6800-BF were calculated in accord with CLSI document EP17-A2, 2012 [20]. Accordingly, LoB was calculated using nonparametric analysis, as the 95th percentile value of 60 replicates of synovial fluid samples with undetectable cells at LM. The LoD was instead calculated using six HY-pretreated synovial fluid samples diluted with saline solution to obtain a low number of TCs. Ten replicate analyses of each sample were performed, for a total of 60 measurements. The mean TC values of samples were between 7 and 36 × 10^6 cells/L. The LoD was finally expressed as the lowest TC and WBC values that could be detected (with 95% probability) over their respective LoBs, using the following formula: LoD = LoB + 1.645 × SDs (where SD is the pooled standard deviation of results on samples with low cellularity).

Functional sensitivity (limit of quantification [LoQ])

The functional sensitivity of the BC-6800-BF was assessed on 10 replicates of 14 HY-pretreated synovial fluid samples with different cell values, as follows: 148–16 036 × 10^6 cells/L for TC-BF and WBC-BF, 119–15 291 × 10^6 cells/L for PMN, and 29–2510 × 10^6 cells/L for MN. The mean TC-BF, WBC-BF, PMN, and MN counts of each sample were plotted against the coefficient of variation (CV), and the LoQ was then calculated from the relative regression, as the concentration with 20% imprecision [20].

Imprecision

In accordance with CLSI document EP05-A3 [21], the within-run imprecision of BC-6800-BF was evaluated

by measuring 10 replicates of six synovial fluids pretreated with HY and displaying TC values between 216 and 10 611 $\times$ 10^6 cells/L.

Linearity

The linearity of BC-6800-BF was assessed using serial HY-pretreated synovial fluid dilutions within the clinically meaningful linearity ranges. Briefly, a sample with high cellularity (i.e., TC = 29 234 $\times$ 10^6 cells/L) was serially diluted 1:2 with phosphate-buffered saline (PBS) to obtain nine aliquots displaying scalar values. Each serial dilution was then measured in five replicates, and the mean was calculated. A graphic representation of data was generated between theoretical and measured values, and the correlation coefficient (r) was finally calculated according to the CLSI document EP06-A, 2003 [22].

Comparison between BC-6800-BF and light microscopy

A total of 78 synovial fluid samples were used for comparing BC-6800-BF total cell counts with the reference LM analysis. According to the morphological differentiation using LM, cells were clustered in the following classes: neutrophils (NE), lymphocytes (LY), monocytes (MO), eosinophils (EO), macrophages (MA), synoviocytes (SY), and other cells (OTH), as for CLSI document H56-A, 2006 [3].

Due to different classification and cell designation between BC-6800-BF mode and LM, cells were clustered in a reasonable number of homogeneous cell categories to enable a direct comparison between the two methods, as follows:

- TC-BF vs TC-LM (=all nucleated cells present in SF samples)
- WBC-BF vs WBC1-LM (=all nucleated cells present in SF samples without only other cells)
- WBC-BF vs WBC2-LM (=all nucleated cells present in SF samples without only other cells and synoviocytes)
- MN vs MN1-LM (=lymphocytes plus monocytes)
- MN vs MN2-LM (=lymphocytes plus monocytes and macrophages);
- MN vs MN3-LM (=lymphocytes plus monocytes, macrophages, and synoviocytes)
- PMN vs PMN-LM (=neutrophils plus eosinophil and basophils)
- NE-BF vs NE-LM (=only neutrophils)

The difference between the mean (or median) cell count in synovial fluids with or without HY pretreatment was evaluated with Wilcoxon paired test after distribution analysis carried out with Shapiro–Wilk test. The degree of statistical significance was set at $P < 0.05$. The agreement between BC-6800-BF and LM analyses was evaluated by Passing–Bablok regression and Bland–Altman plot analysis. Slope and intercept of the Passing–Bablok regression were calculated along with their 95% confidence interval (95% CI). In Bland–Altman plots, the relative difference between methods was plotted against the result of LM analysis and a significant bias was defined as 95% CI of the mean not containing the value zero. For those data not characterized by normal distribution, the bias was calculated as the median of the differences between the two methods (BC-6800-BF an LM), with a 95% interval between the 2.5th and 97.5th percentiles.

Statistical analysis

The statistical analysis was carried out using Analyse-it™ software, version 3.90.5 (Analyse-it Software Ltd, Leeds, UK).

RESULTS

Analytical performance of BC-6800-BF

The carryover of BC-6800-BF was found to be excellent (i.e., <0.3%) for all the different parameters. The LoB was found to be 6 $\times$ 10^6 cells/L for both TC-BF and WBC, whereas the LoD and LoQ were 15 $\times$ 10^6 cells/L for TC-BF and 16 $\times$ 10^6 cells/L for WBC-BF, respectively. The LoQ was 23 $\times$ 10^6 cells/L for MN and 16 $\times$ 10^6 cells/L for PMN, respectively (Table 1). As shown in Table 1, the linearity was also excellent (i.e., correlation coefficient r between 0.92 and 0.98) for all the parameters (Table 1). The results of the within-run imprecision of BC-6800-BF are also shown in Table 1. The CV was between 1.6% and 7.5%, being slightly higher for MN (1.9–7.5%) and PMN (1.7–6.3%) than for TC-BF (1.6–4.2%) and WBC-BF (1.6–4.1%).

Comparison between BC-6800-BF and light microscopy

The original sample size, including 78 synovial fluid samples, ought to be reduced to 76 because two

Table 1. Limit of blank (LoB), limit of detection (LoD), limit of quantification (LoQ), linearity, and imprecision within run of the BC-6800-BF parameters (total cell [TC], leukocytes [WBC], polymorphonuclear cells [PMN], and mononuclear cells [MN]): mean value, standard deviation (SD), and coefficient of variation (CV)

BC-6800-BF analytical performance					Imprecision within run				
	LoB (10^6 cells/L)	LoD (10^6 cells/L)	LoQ (10^6 cells/L)	Linearity range (10^6 cells/L) Regression coefficient	Mean ± SD (10^6 cells/L) CV	Mean ± SD (10^6 cells/L) CV	Mean ± SD (10^6 cells/L) CV	Mean ± SD (10^6 cells/L) CV	Mean ± SD (10^6 cells/L) CV
TC-BF	6	15	15	42–29 234 r = 0.97	586 ± 15.1 2.6%	1315 ± 40.4 3.0%	2432 ± 41.8 1.7%	4324 ± 182.6 4.2%	10611 ± 170.1 1.6%
WBC-BF	6	16	16	42–29 221 r = 0.96	585 ± 15.5 2.7%	1314 ± 40.6 3.0%	2429 ± 41.1 1.7%	4216.0 ± 172.4 4.1%	10607 ± 170.2 1.6%
PMN#	\\	\\	16	40–25 421 r = 0.98	369 ± 7.7 2.1%	1001 ± 41.7 4.2%	1860.5 ± 33.0 1.8%	2718.±171.2 6.3%	8097 ± 136.5 1.7%
MN#	\\	\\	23	2–3975 r = 0.92	216 ± 16.2 7.5%	312 ± 20.0 6.4%	568.7 ± 23.7 4.2%	1497.6 ± 50.5 3.4%	2509.9 ± 47.2 1.9%

MN, mononuclear cells; PMN, polymorphonuclear cells; TC-BF, total cell count; WBC-BF, leukocytes.

samples were excluded due to high cellularity (i.e., TC >70 000 × 10^6 cells/L). Interestingly, a significant difference was observed between LM counts in synovial fluid samples with or without pretreatment with HY. More specifically, the TC carried out by means of LM in specimens without HY pretreatment was −13.3% lower than in those with HY pretreatment ($P < 0.001$) (Table 2). Notably, the differential count with LM could not be performed as the quality of cytospin in synovial fluid was poor in samples without pretreatment with HY, thus not allowing an accurate morphological differentiation of the different nucleated elements. A similar trend was observed using BC-6800-BF, in that the number of TCs, as well as that of TC-BF, WBC-BF, Eos-BF, MN, and HF-BF, was up to 13.3% higher in synovial fluid samples pretreated with HY than in those untreated (Table 2). Notably, both the PMN and NE-BF parameters show a relative bias of 0.0%. Due to the better quality, the comparison between LM and BC-6800-BF was only performed in HY-pretreated specimens. Two additional samples ought to be excluded from this analysis as accurate differentiation by LM was unfeasible. In the former case, this was due to high cell degeneration. A paradigmatic example is shown in Figure 1, which shows the samples with cell degeneration (Figure 1a). In the latter case, the comparison between LM and BC-6800-BF could not be carried out due to the presence of intra- and extracellular amorphous material, which ultimately compromised the accurate cell differentiation (Figure 1b). Therefore, 74 hyaluronidase-pretreated specimens were finally included in the comparison study between LM and BC-6800-BF. As shown in Table 3 and Figure 2, the slopes of the Passing–Bablok regression were between 0.9 and 1.47, whereas the values of the intercepts ranged between −16.28 and 49.17 The relative bias was between −5.9% and 31.3%.

DISCUSSION

Synovial fluid analysis remains a challenge for modern routine laboratories. Manual assessment by means of LM raises considerable analytical and organizational problems, whereas automated analysis is still regarded as a valuable perspective, needing supplementary validation. Taken together, the results of our study confirm the good performance of BC-6800-BF for synovial fluid analysis in the clinically significant

Table 2. Passing–Bablok regression and relative bias between light microscopy (LM) and BC-6800-BF parameters in paired synovial fluid samples before and after pretreatment with hyaluronidase (HY).

	Median value on samples without HY pretreatment (95% CI)	Median value on samples with HY pretreatment (95% CI)	Passing–Bablok regression Slope (Sl) and Intercept (In) (95% CI)	Relative bias (%) (95% CI)
Light microscopy				
TC-LM ($\times 10^6$/L)	1500* (600–3950)	2250 (850–5800)	Sl: 0.93 (0.83 to 1.01) In: −30.73 (−108 to 11)	−13.3% (−29.9 to -5.1)
BC-6800-BF evaluation				
TC-BF ($\times 10^6$/L)	2051† (784–5533)	2223 (674–5638)	Sl: 1.00 (0.99 to 1.02) In: 12.09 (−0.17 to 41.48)	1.5% (0.6 to 10.4)
WBC-BF ($\times 10^6$/L)	1977† (771–5525)	2069 (640–5631)	Sl: 1.00 (0.99 to 1.02) In: 10.42 (−0.69 to 39.94)	1.7% (−0.15 to 5.0)
PMN ($\times 10^6$/L)	368† (122–1850)	390 (117–2352)	Sl: 1.00 (0.99 to 1.01) In: −3.56 (−11.36 to 4.53)	0.0% (−6.4 to 2.1)
PMN (%)	39.5 (22.6–50.4)	43.1 (27.0–52.9)	Sl: 1.01 (0.99 to 1.04) In: −0.88 (−3.12 to 0.014)	−0.3% (−3.4 to 0.19)
MN ($\times 10^6$/L)	1069 (448–1553)	1175 (468–1468)	Sl: 1.01 (0.98 to 1.04) In: 8.54 (1.09 to 22.6)	4.2% (0.0 to 6.3)
NE-BF ($\times 10^6$/L)	339† (89–2162)	366 (101–2160)	Sl: 1.00 (0.98 to 1.01) In: −0.50 (−7.99 to 3.08)	0.0% (−4.7 to 2.9)
NE-BF (%)	25.5* (13.8–49.4)	31.3 (19.5–50.0)	Sl: 1.00 (0.98 to 1.02) In: −0.31 (−1.86 to 0.25)	−0.7% (−4.8 to 0.1)
Eos-BF ($\times 10^6$/L)	22 (16–38)	20 (11–33)	Sl: 1.25 (1.00 to 1.74) In: −1.75 (−5.71 to 0.75)	9.2% (−1.3 to 45.7)
HF-BF ($\times 10^6$/L)	8 (5–12)	6 (5–9)	Sl: 1.17 (1.00 to 1.34) In: −0.33 (−1.50 to 0.93)	13.3% (0.0 to 22.2)

*Difference between median value of sample with and without HY pretreatment with $P < 0.001$.
†Difference between median value of sample with and without HY pretreatment with $P < 0.01$.

ranges of cellularity [1–4, 23, 24]. We also provided evidence that routine pretreatment of synovial fluids with HY may be advisable for a more accurate enumeration and differentiation of cells, especially using LM. Interestingly, the impact of pretreatment with HY was lower using BC-6800-BF, wherein the bias between native and HY-pretreated samples was always <7% with exception some of research parameters (i.e., Eos-BF and HF-BF), which is lower than the quality specifications for both leukocyte (i.e., ±6%) and neutrophil (i.e., ±9%) count in peripheral blood [25, 26]. Interestingly, BC-6800-BF appeared also scarcely vulnerable to technical issues emerging from the high viscosity of synovial fluids, wherein no functional problems (i.e., aspiration errors) were recorded throughout the evaluation as described for other similar analyzers [17].

The data obtained by comparing LM and BC-6800-BF cell counts in paired synovial fluid specimens suggests that synoviocytes may be included in the leukocyte count using BC-6800-BF, probably within the cluster of mononuclear cells. In fact, the WBC-BF parameter correlated better with WBC1-LM (corresponding to TC-LM minus other cells), whereas MN correlated better with MN3-LM (corresponding to the cluster of lymphocytes, monocytes, macrophages, and synoviocytes).

The DIFF scattergram of BC-6800-BF also provides qualitative information on cells potentially present in the synovial fluids, thus widening its potential use previously established for the analysis of ascites and pleural and cerebrospinal fluids [11,13,15]. A paradigmatic example is reported in Figure 1, which shows two DIFF scattergrams of the specimens excluded from the comparative analysis between LM and BC-6800-BF.

In both DIFF scattergrams, the PMN cluster was not accurately distinguished from the so-called ghost

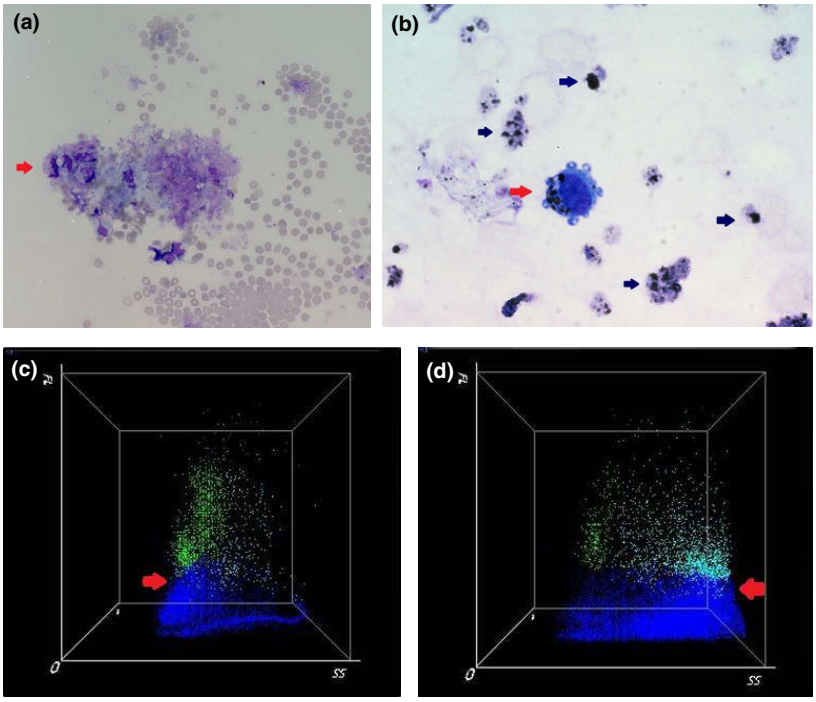


Figure 1. Synovial fluid sample DIFF scattergrams and cell morphology. (a) Synovial fluid sample obtained from a patient with a suspected knee infection: morphological features of cells by light microscopy (LM) (400 × magnification) on cytospin stained with May–Grünwald–Giemsa (MGG) stain. The red arrow indicates a cluster of degenerated cells. The abnormal DIFF scattergram of this sample is shown in c. (b) Synovial fluid sample obtained from a patient with a hip prosthesis and suspected infection. Morphological features of cells by light microscopy (LM) (1000 × magnification) on cytospin stained with MGG stain. The red arrow indicates synoviocyte with inorganic amorphous inclusion, and the blue arrows indicate the inorganic amorphous material, probably of metal/inorganic nature. The abnormal DIFF scattergram of this sample is shown in d. (c) BC-6800-BF DIFF scattergram of synovial fluid sample obtained from a patient with a suspected knee infection, displaying an abnormal 3D DIFF scattergram. The morphology cell of this sample is shown in Figure 2b. The MN area is close to the background area, with inaccurate discrimination (between MN and background area, highlighted with an arrow) (TC-BF: 588×10^6 cells/L; WBC-BF: 570×10^6 cells/L; PMN: 20.5%; MN: 79.5%; HF-BF: 18×10^6 cells/L by BC-6800-BF). (d) BC-6800-BF DIFF scattergram of synovial fluid sample obtained from a patient with a hip prosthesis and suspected infection, displaying an abnormal 3D DIFF scattergram. The PMN area is close to the background area, with inaccurate discrimination (between PMN and background area, highlighted with an arrow) (TC-BF: 903×10^6 cells/L; WBC-BF: 878×10^6 cells/L PMN: 88.2%; MN 11.8%; HF-BF: 25×10^6 cells/L by BC-6800-BF). [Colour figure can be viewed at wileyonlinelibrary.com]

area compared to the standard scattergram (Figure 1c and d) [13]. In the first case, the BC-6800-BF data were as follows: 588×10^6 cells/L TC-BF, 570×10^6 cells/L WBC-BF, and 117×10^6 cells/L PMN (i.e., 20.5%), and the microscopic review showed only degenerated cells as shown in Figure 1a. The second specimen was collected from a patient with a hip prosthesis, and the analysis was requested for suspected infection. The BC-6800-BF data were as follows: 903×10^6 cells/L TC-BF, 892×10^6 cells/L WBC-BF, and 787×10^6cells/L PMN (i.e., 88%). Although the recommended cutoffs of leukocytes for diagnosing infections in synovial fluids vary widely (i.e., between 1700 and 30 000 $\times 10^6$ cells/L) [23, 24], this would have been always classified as a false-negative sample. Nevertheless, the presence of amorphous refractive material and rare synoviocytes was identified by LM (Figure 1b). The quality of the

Table 3. Passing–Bablok regression and relative bias for different cell clusters recognized by means of light microscopy and then compared to BC-6800-BF in samples pretreated with hyaluronidase.

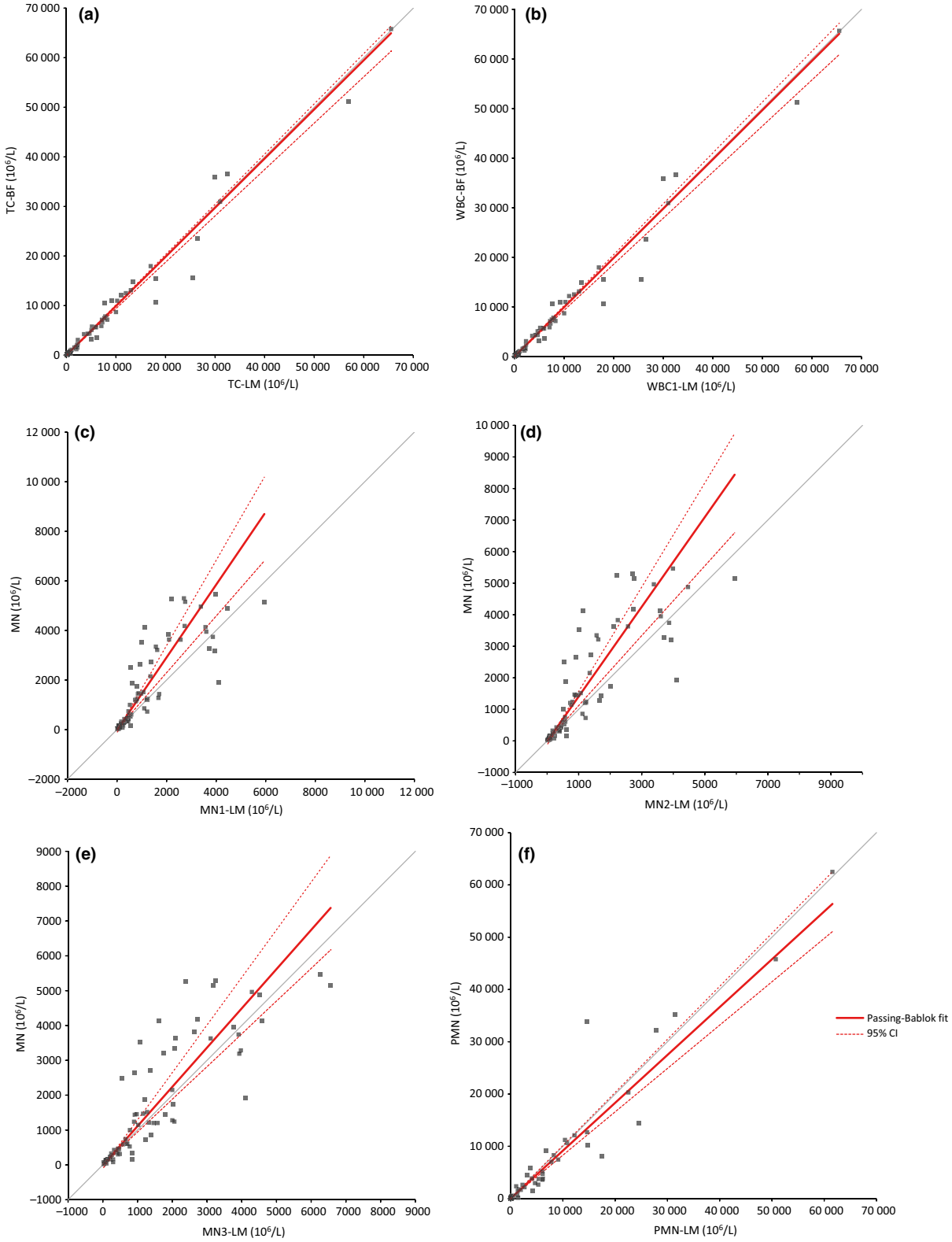
LM parameters	Median value (95% CI) Range (R)	Passing–Bablok regression Slope (Sl) and intercept (In) (95% CI)	Relative bias (%) (95% CI)
TC-LM ($\times 10^6$/L)	2300 (867 to 5800) *R* = 33 to 65 500	Sl = 0.98 (0.94 to 1.01) In = 20.74 (−7.04 to 45.06)	0.0% (−3.8 to 7.7)
WBC1-LM ($\times 10^6$/L)	2300 (866 to 5771) *R* = 33 to 65 500	Sl = 0.98 (0.94 to 1.01) In = 22.5 (−3.9 to 42.9)	0.4% (−4.2 to 4.9)
WBC2-LM ($\times 10^6$/L)	2231 (720 to 4938) *R* = 24 to 65 500	Sl = 1.00 (0.98 to 1.05) In = 49.17 (18.59 to 77.32)	7.7% (0.4 to 12.1)
PMN-LM ($\times 10^6$/L)	666 (175 to 2650) *R* = 0 to 61 570	Sl = 0.91 (0.86 to 1.00) In = 17.84 (8.87 to 23.30)	8.2% (−0.3 to 20.5)
PMN-LM (%)	32 (22 to 57) *R* = 0 to 97	Sl = 0.90 (0.80 to 0.97) In = 5.92 (1.50 to 9.21)	1.1% (−3.4 to 15.6)
MN1-LM ($\times 10^6$/L)	782 (520 to 1100) *R* = 19 to 5462	Sl = 1.47 (1.19 to 1.68) In = −17.97 (−109.4 to 36.48)	31.3% (13.3 to 42.9)
MN2-LM ($\times 10^6$/L)	782 (540 to 1130) *R* = 19 to 5945	Sl = 1.42 (1.15 to 1.61) In = −16.28 (−112.8 to 34.57)	24.5% (9.2 to 39.5)
MN3-LM ($\times 10^6$/L)	947 (690 to 1377) *R* = 24 to 6560	Sl = 1.13 (0.96 to 1.30) In = −14.5 (−117.6 to 22.1)	−5.9% (−9.1 to 14.9)
NE-LM ($\times 10^6$/L)	499 (144 to 2650) *R* = 0 to 61 570	Sl = 0.89 (0.84 to 0.96) In = 5.87 (2.54 to 13.98)	−5.0% (−16.9 to 7.3)
NE-LM (%)	35 (23 to 64) *R* = 1 to 97	Sl = 0.94 (0.86 to 1.02) In = 0.42 (−2.97 to 4.05)	−2.5% (−13.1 to 0.5)

sample was so poor that an accurate cell differentiation was unfeasible. However, a specific comment highlighting the presence of amorphous material was included in the laboratory report, thus enabling an accurate differential diagnosis between infection and swelling due exploitation of the prosthesis, which was finally replaced.

The use of BC-6800-BF may hence allow to replace the routine optical analysis, except for samples with an abnormal scattergram and those with a very high number of synovial cells.

In conclusion, the results of this study suggest that BC-6800-BF displays satisfactory analytical performance for enumeration and differentiation of nuclear elements in synovial fluids, especially in samples pretreated with hyaluronidase, thus supporting its routine use for the preliminary screening of synovial specimens.

Figure 2. Comparison of the BC-6800-BF parameters with those of light microscopy analysis in synovial fluid samples pretreated with hyaluronidase (HY). (a) Passing–Bablok regression analysis for total cell count (TC) by BC-6800-BF with respect to TC-LM in synovial fluid samples. Passing–Bablok regression: $y = 0.98x + 20.74$, (b) Passing–Bablok regression analysis for leukocytes (WBC) by BC-6800-BF with respect to WBC1-LM in synovial fluid samples. Passing–Bablok regression: $y = 1.00x + 49.17$. (c) Passing–Bablok regression analysis for mononucleated cells (MN) by BC-6800-BF with respect to MN1-LM in synovial fluid samples. Passing–Bablok regression: $y = 1.47x - 17.97$. (d) Passing–Bablok regression analysis for mononucleated cells (MN) by BC-6800-BF with respect to MN2-LM in synovial fluid samples. Passing–Bablok regression: $y = 1.42x + 16.28$ (e) Passing–Bablok regression analysis for mononucleated cells (MN) by BC-6800-BF with respect to MN3-LM in synovial fluid samples. Passing–Bablok regression: $y = 1.13x - 14.5$ (f) Passing–Bablok regression analysis for polymorphonuclear cell cells (PMN) by BC-6800-BF with respect to PMN-LM in synovial fluid samples. Passing–Bablok regression: $y = 0.91x + 17.84$. [Colour figure can be viewed at wileyonlinelibrary.com]


(a)
TC-BF (10⁶/L)
TC-LM (10⁶/L)
(b)
WBC-BF (10⁶/L)
WBC1-LM (10⁶/L)
(c)
MN (10⁶/L)
MN1-LM (10⁶/L)
(d)
MN (10⁶/L)
MN2-LM (10⁶/L)
(e)
MN (10⁶/L)
MN3-LM (10⁶/L)
(f)
PMN (10⁶/L)
PMN-LM (10⁶/L)
Passing-Bablok fit
95% CI

CONFLICT OF INTEREST

The authors declare no potential conflict of interests with respect to the research, authorship, and/or publication of this article.

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