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# AUTOMATED BODY FLUID CELL COUNT BY MINDRAY BC-6800 ON SYNOVIAL FLUIDS WITH OR WITHOUT THE HYALURONIDASE PRE-TREATMENT

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## INTRODUCTION

The study of body fluids (BF), is of great clinical importance. The cellular analysis is often required in as urgency procedure. The modern automated hematology analyzers offer a platform which has the advantages for example the rapidity and standardization of BF analysis. The synovial fluid (SF) is a viscous fluid that is present in shoulder, knee and hip joints. The cellular analysis is difficult because it is viscous. For this reason, usually, SF are analyzed after a pre-treatment with Hyaluronidase. The new automated hematology analyzer BC-6800 (Mindray, Shenzhen-China), has shown good counting performances on ascitic and pleural fluids. Interestingly, no data is currently available about the performances on SF. The aim of this study was to evaluate the analytical performances of Mindray BC-6800 BF mode (BC-6800-BF) in the cytometric analysis of SF compared to current gold standard method (Optical Microscopy) according to CLSI standard H56-A guidelines [1].

## METHODS

A total of 65 consecutive SF samples, ranging from 33 to 161333x$10^6$cell/L, collected in K3EDTA tubes (Becton Dickinson, Franklin Lakes, NJ) were analyzed with and without Hyaluronidase (Sigma, Saint Louis, Missouri, USA) pre-treatment using BC-6800-BF and the Optical Microscopy count

The total cell count (TC) performed by Mindray BC-6800-BF (TC-BC) were compared to those obtained by Optical Microscopy (TC-OM)

Manual Optical microscopic cell count was performed in a Burker's counting chamber to count Total nucleated Cells (TC). SFs samples were diluted (1:20 or 1:200) with Stromatol's reagent (Mascia Brunelli, Italy). TCs count was performed by two skilled operators with a light microscope at x400 magnification and by a third person if the first two results disagreed.

The agreement between BC-6800-BF and OM was assessed with Pearson's correlation coefficient (r), Passing Bablok regression, Bland-Altman plot analysis. Slope and intercept of Passing Bablok regression were calculated with their 95% confidence interval (95% CI) to check statistical significant proportional or systematic difference between methods. In Bland-Altman plot, absolute differences were plotted against the results of the OM. A significant bias is appreciated when the 95% CI of mean of differences did not contain the value.We evaluated also carryover according to the CLSI standard H56-A[1] and ICSH [2] guidelines. The statistical analysis was carried out with Analyse-it 3.90.5 (Analyse-it Software Ldt, Leeds, UK).

## RESULTS

The comparison between BC-6800-BF to OM of TCs (TC-BF vs TC-OM) in the samples without pre-treatment showed a Pearson's correlation of r=0.80; a Passing and Bablok regression y=1.03x+47.38 (95%IC Slope: 0.86 to1.27; Intercept: -14.69 to 139.3) (Fig 1) and a Bland Altman Bias of2392.3x$10^6$cell/L (95%IC -2504.9 to 728.6) (Fig 2). The same comparison of TC-BF parameter and OM-TC in the samples with the Hyaluronidase treatment showed a Pearson's correlation of r=0.99; a Passing and Bablok regression y=0.99x+27.42 (95%IC Slope: 0.90 to 1.02; Intercept: -6.35 to 5.92) (Fig 3) and a Bland Altman Bias of -262.7x$10^6$ cell/L (95%IC -1260.0 to 734.6) (Fig 4).

In all case the carryover was negligible.

**Table 1:** Pearson's correlation, Passing and Bablok regression and Bland Altman Bias for TC with and without the Hyaluronidase pre-treatment

| | **Pearson's correlation (r) p value** | **Passing and Bablok (95%CI)** | **Bland Altman Bias (95%CI)** |
|---|---|---|---|
| **Pre Hyaluronidase treatment** | 0.80 p<0.0001 | y=1.03x+47.38 (Intercept: -14.69 to 139.3 Slope: 0.86 to 1.27) | 2392.3x$10^6$cell/L (-2504.9 to 7289.6) |
| **Post Hyaluronidase treatment** | 0.99 P<0.0001 | y=0.99x+27.42 (Intercept: -6.35 to 5.92 Slope: 0.90 to 1.02) | -262.7x$10^6$cell/L (-1260.0 to 734.6) |

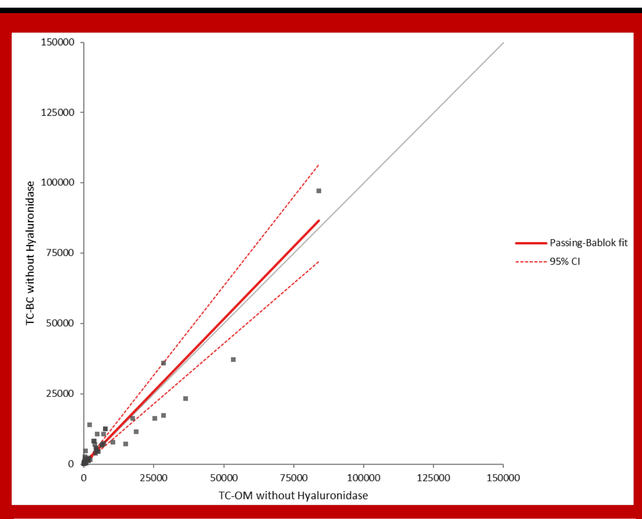


**Figure 1:** Passing and Bablok regression for total leukocyte count without Hyaluronidase pre-treatment

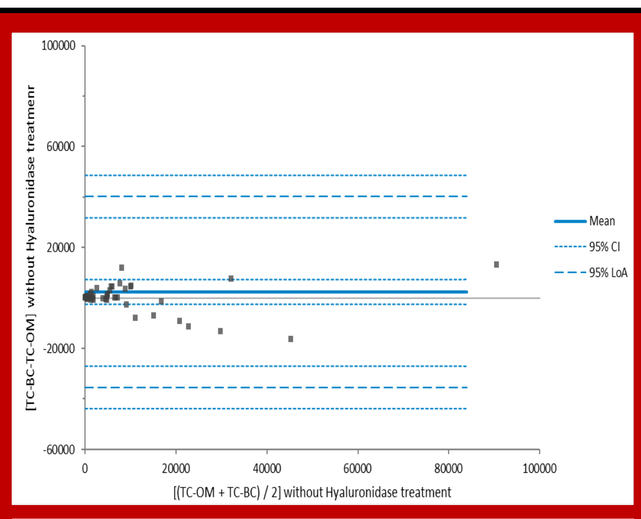


**Figure 2**: Bland Altman Bias for total leukocyte count without Hyaluronidase pre-treatment

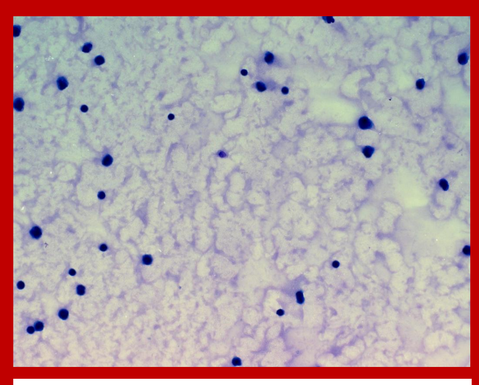

**Figure 5**: Microscopic analysis of a of synovial fluid's sample before the Hyaluronidase pre-treatment

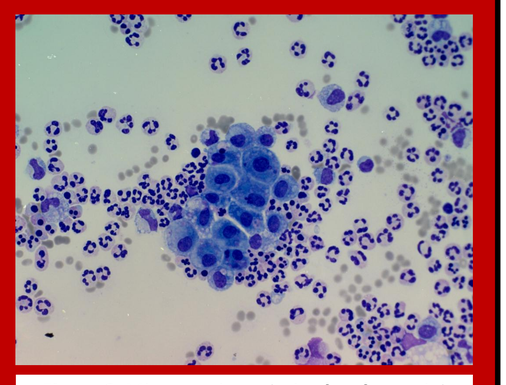

**Figure 6**: Microscopic analysis of a of synovial fluid's sample after the Hyaluronidase pre-treatment

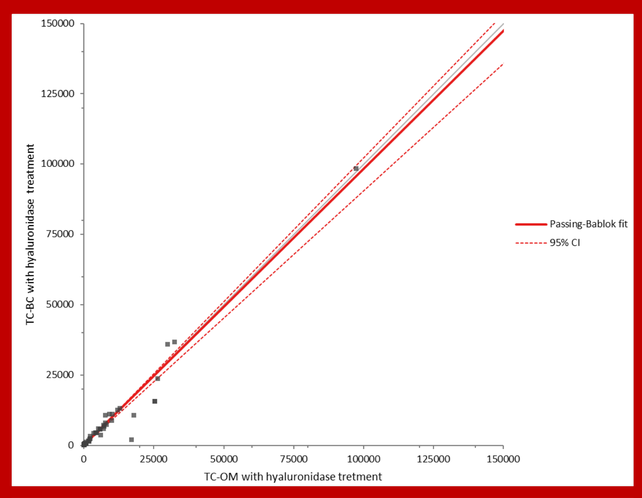


**Figure 3**: Passing and Bablok regression for total leukocyte count with Hyaluronidase pre-treatment

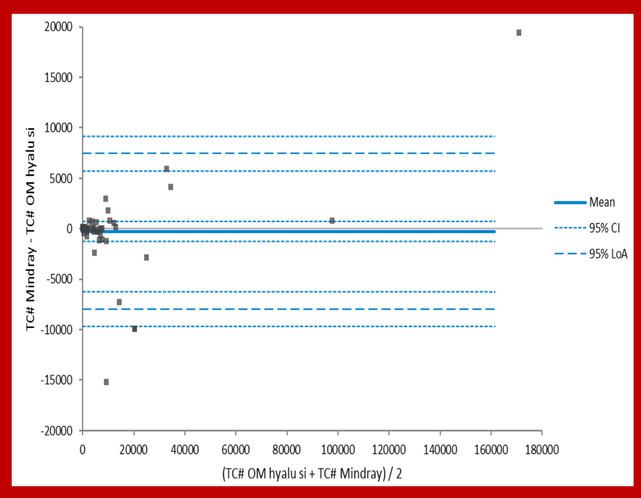


**Figure 4:** Bland Altman Bias for total leukocyte count with Hyaluronidase pre-treatment

## CONCLUSIONS

The BC-6800-BF offers rapid and accurate TC counts in clinically relevant concentration ranges in SF, replacing the counting chamber for most samples. Moreover our study showed the importance of the use of the Hyaluronidase, as pretreatment, in the analysis of this kind of body fluid, not only for the TC count but also for the optical microscope review as shown in figure 5 and 6.

## REFERENCES

[1] Clinical Laboratory Standards Institute. Body Fluid Analysis for cellular composition; approved guideline. CLSI document H56-A. Clinical and Laboratory Standards Institute: Wayne, PA; 2006.

[2] Bourner G, De la Salle B, George T, Tabe Y, Baum H, Culp N, et al. ICSH guidelines for the verification and performance of automated cell counters for body fluids. Int J Lab Hematol. 2014;36:598-612

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