

## Determining White Blood Cell Count In Sterile Body Fluids: Cell Counting Chamber vs Sysmex UF-1000i

Steril Vücut Sıvılarında Beyaz Kan Hücre Sayısının Belirlenmesi: Hücre Sayım Kamarasına Karşı Sysmex UF-1000i

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### ABSTRACT

**Background:** Counting of cells in sterile body fluids other than blood and urine provides important diagnostic information. The aim of the study is to evaluate the performance of the body fluid module of Sysmex UF-1000i system in comparison with cell counting chamber results.

**Methods:** 71 routinely collected sterile body fluid samples were evaluated. The samples were cerebrospinal fluid, peritoneal fluid, pleural fluid, synovial fluid, drain fluid and bile. Cell counts were simultaneously determined with both bright lined Neubauer Cell Counting Chamber® (Marienfeld, Germany) and Sysmex UF-1000i BF (Sysmex, Japan) according to the manufacturers' recommendations. The results were evaluated statistically with interclass correlation coefficient (ICC) and related p-values were calculated.

**Results:** There were seven purulent samples. Outliers were seen between microscopic examination and Sysmex UF-1000i results with purulent samples. The difference between the cell counts of the two methods was 1979 cells (cells/ml) with outliers, and 69 cells (cells/ml) without outliers. The ICC with and without outliers were 0.128 and 0.963, respectively. With outliers, correlation between the two methods was not statistically significant (p=0.139). Without outliers, correlation between two methods was statistically significant (p<0.001) and good agreement was observed.

**Conclusions:** our results showed that especially for macroscopically purulent samples, counting chambers would still be the best choice for WBC counting, however for macroscopically non-purulent samples Sysmex UF1000i is a good alternative instrument for rapid workflow especially in routine microbiology laboratory in sterile body fluids other than blood and urine.

**Key Words:** cell counting; cell counting chamber; Sysmex UF 1000i; sterile body fluids

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### ÖZET

**Amaç:** Kan ve idrar dışındaki steril vücut sıvılarında hücrelerin sayılması, önemli tanısal bilgi sağlar. Bu çalışmanın amacı, Sysmex UF-1000i sisteminin vücut sıvısı modülünün, hücre sayım kamarası sonuçlarına göre performansını değerlendirmektir.

**Yöntem:** Rutin olarak laboratuvara gelen 71 steril vücut sıvı örneği değerlendirildi. Örnekler beyin omurilik sıvısı, periton sıvısı, pleural sıvı, sinovyal sıvı, drenaj sıvısı ve safra idi. Hücre sayımları, üreticilerin tavsiyelerine göre hem parlak çizgili Neubauer Hücre Sayım Kamarası® (Marienfeld, Almanya) hem de Sysmex UF-1000i BF (Sysmex, Japonya) ile aynı anda belirlendi. Sonuçlar, sınıflar arası korelasyon katsayısı (ICC) ile istatistiksel olarak değerlendirildi ve ilgili p değerleri hesaplandı.

**Bulgular:** Yedi tane pürülan örnek vardı. Pürülan örneklerin mikroskopik incelemesi ve Sysmex UF-1000i sonuçları arasında sapan değerler görülmüştür. İki yöntemin hücre sayımları arasındaki fark, sapan değerler dahil edildiği zaman 1979 hücre (hücre / ml) ve sapan değerler dahil edilmediği zaman 69 hücre (hücre / ml) idi. Sapan değerler dahil edildiğinde ve edilmediğinde ICC, sırasıyla 0.128 ve 0.963 idi. Sapan değerlerle, iki yöntem arasındaki korelasyon istatistiksel olarak anlamlı değildi (p = 0.139). Sapan değerler olmadan iki yöntem arasındaki korelasyon istatistiksel olarak anlamlıydı (p < 0.001) ve iyi bir uyum gözlemlendi.

**Sonuç:** Elde ettiğimiz sonuçlar, özellikle makroskopik olarak pürülan örnekler için, hücre sayım kamarasının WBC sayımı için hala en iyi seçenek olduğunu göstermiştir, ancak makroskopik olarak pürülan olmayan örnekler için Sysmex UF1000i, özellikle kan ve idrar dışındaki steril vücut sıvıları için, rutin mikrobiyoloji laboratuvarında hızlı iş akışı için iyi bir alternatif araçtır.

**Anahtar Sözcükler:** Hücre sayımı; hücre sayım kamarası; Sysmex UF 1000i; steril vücut sıvıları

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## INTRODUCTION

Counting of cells in sterile body fluids other than blood and urine, such as cerebrospinal fluid (CsF), peritoneal fluid (PeF), pleural fluid (PIF), synovial fluid (SyF), pericardial fluid (PrF),...etc provides important diagnostic information (1-4). Elevated number of white blood cells (WBCs) can be a consequence or complication of a number of diseases. So the accurate analysis of sterile body fluids is important for accurate and differential diagnosis (5). Although CsF samples account for the majority of sterile body fluids examined for the presence of WBCs in the clinical microbiology laboratory, other sterile body fluids such as PeF, PIF, SyF, PrF, bile, etc. can also be sent to the clinical microbiology laboratory for cell counting. The gold standard for examining sterile body fluids for the presence of WBCs is microscopic examination, it depends on the experience of the counting staff, is labor-intensive and time consuming and also this procedure has a high intra- and interobserver variability and does not supply any additional information on the predominant cell type (6,7). The use of automated systems in the clinical microbiology laboratories has recently gained attention as an alternative method to microscopic examination. For obtaining accurate, objective, reproducible and fast results, automated systems have been evaluated and proposed to be a solution for these purposes. Although automated systems are of much interest for sterile body fluids, currently they are widely used for blood and urine analyses. Due to the lack of literature data in this field, it is very important to evaluate the performance of different automated cell counting technologies (8). The Sysmex UF-1000i (Sysmex UF-1000i, Japan) is a fully automated flow cytometric system with validated urine and body fluid modules for the evaluation of cellular contents of urine and other sterile body fluids. In this study, we aimed to evaluate the performance of the body fluid module of Sysmex UF-1000i system in comparison with cell counting chamber results.

## MATERIALS and METHODS

*Samples*

We studied 71 routinely collected sterile body fluid samples from hospitalized- and out-patients. The type and number of evaluated body fluid samples are given in table-1. Each sample was collected in tubes coated with ethylene-diamine-tetraacetic acid (EDTA) and analyzed within an hour. Cell counts were simultaneously determined with both bright lined Neubauer Cell Counting Chamber® (Marienfeld, Germany) and Sysmex UF-1000i BF (Sysmex, Japan) according to the manufacturers' recommendations. Manual microscopic examination was performed with light microscope using an eyepiece set at 10X and a lens set at 40X, for a total magnification of 400X. The Sysmex UF-1000i was used in body fluid mode for the counting of cells.

**Table 1:** Type and Number of Samples

Sample	n	%
Cerebrospinal Fluid	35	50
Peritoneal Fluid	12	17
Pleural Fluid	11	15
Synovial Fluid	10	14
Drain Fluid	1	2
Bile	1	2
<b>Total</b>	<b>71</b>	<b>100</b>

*Statistical analysis*

The samples were chosen randomly. All the samples which were sent to the central microbiology laboratory for the determination of white blood cell number were analyzed by both methods without prior knowledge about the patient. The reliability was evaluated according to the "interclass correlation coefficient (ICC)". Results were evaluated with ICC (CI=95%) and related p-values were calculated. The median (minimum-maximum) values were used as descriptive statistics. SPSS 15.0 for windows was used for statistical analyses and p values less than 0.05 were considered as statistically significant.

## RESULTS

There were seven purulent samples (4 CsF, 3 SyF). Outliers were seen between microscopic examination and Sysmex UF-1000i results with purulent samples. The difference between the cell counts of the two methods was 1979 cells (cells/ml) with outliers, and 69 cells (cells/ml) without outliers.

The median Sysmex UF-1000i cell count was 160cells/ml (with outliers) and 134cells/ml (without outliers). The median cell counting chamber cell count was 107 cells/ml (with outliers) and 100 cells/ml (without outliers). In general, Sysmex UF-1000i was found to give higher values.

The ICC with and without outliers were 0.128 and 0.963, respectively. With outliers, correlation between the two methods was not statistically significant ( $p=0.139$ ). Without outliers, correlation between two methods was statistically significant ( $p<0.001$ ) and good agreement was observed (Table-2).

**Table 2:** The results of the statistical analysis

n	With outliers	Without outliers
Number of samples	71	64
Cell count difference	1979	69
ICC [95% CI]	0.128 [-0.104 – 0.348]	0.963 [0.939 – 0.977]
p value	0.139	<0.001*

\* $p < 0.05$  was considered as statistically significant

## DISCUSSION

Clinician sent a variety of sterile body fluid samples to microbiology laboratory. Examination of these fluids for cell counting is generally the first step for valuable clinical information (9).

Counting the number of white blood cells (WBC) in sterile body fluids is an essential parameter both for initial screening of abnormalities and preliminary and/or differential diagnosis of many inflammatory diseases (10). Cell counting chambers are still the gold standard method for determining the number of WBCs in a given body fluid. However, this technique is labor-intensive, time consuming and has wide interobserver variability and poor reproducibility (6).

Classical microbiology techniques are relatively slow in comparison to analytical techniques. The need for rapid and accurate methods has made flow cytometer instruments to be put into market. Since their acceptance for the use in blood and urine analyses, many studies evaluated a variety of automated analyzers which were initially developed for blood or urine analysis for counting cell numbers in other sterile body fluids. But, today FDA has approved many automated hematology analysers such as Siemens-Advia 120/2120 (Siemens Healthcare Diagnostics, Deerfield, IL, USA), Sysmex- UF-1000i BF mode (Sysmex, Japan), and Beckman Coulter- LH 750 BF mode (Beckman Coulter, USA) suitable for routine body fluid (BF) analysis is put into the market (5). Companies marketing automated analyzers with body fluid modes are trying to enhance the precision and accuracy of the instruments for cell counting in body fluids other than blood and urine (11).

There are several reports considering the performance of flow cytometer instruments for blood and urine analyses, however there is still need for evaluating the performance of different automated technologies for other sterile body fluids due to the lack of literature in this field (8,12). To contribute this need of literature, we evaluated various body fluids by using Sysmex UF-1000i (Sysmex, Japan) and compared the results with microscopic analysis. Instruments having body fluid mode have been reported as showing good agreement with manual cell counting (5). In our study all of the samples except macroscopically purulent ones showed good correlation with microscopic analysis and our results were compatible with other reports (10,13,14). As CsF is the most challenging body fluid related to low number of cells, it is the most frequently studied one (14-16). For CsF samples, contradicting results were reported for WBC counting with automated systems (8,10,14,16). In most of these studies, WBC counting correlation was found to be poor with automated systems for CsF samples (17,18). Low cell numbers in CsF is the major cause of poor correlation with manual cell counting methods (8,13).

Unlike other studies, our results showed that for macroscopically non-haemorrhagic CSF samples, the results obtained by Sysmex UF-1000i showed good correlation with counting chamber for samples even with very low WBC counts. In general, Sysmex 1000i shows good correlation with the counting chamber, however the Sysmex UF-1000i WBC counts were found to be higher than the counting chamber results (10,19). Our results are compatible with these reports; however attention should be paid for purulent samples. Although our results obtained by the Sysmex UF-1000i analyzer showed good correlation with the counting chamber for non-purulent samples, for purulent samples the correlation between the two methods is not statistically significant ( $p>0.05$ ).

Abnormal numbers of cells other than erythrocytes in the body fluids are indicative for various inflammatory diseases, and the total WBC count has a diagnostic value (5). Using only WBC count as diagnostic criteria Fleming C et al [10] have reported that the Sysmex UF 1000i BF mode has a 100% sensitivity and specificity, and thus negative results prevent inappropriate antibiotic usage; they also claim that false positive results are also possible. However the reports do not comment on the reason of this result. Both in our study and in other similar studies mentioned above, the Sysmex UF-1000i WBC count is found to be higher than the counts obtained by the counting chamber. According to our study results, we also highlight the adverse effect of macroscopically purulent samples which may account for the high counting values of automated systems. Despite its disadvantages such as errors made during pipetting, dilution and chamber loading, our results show that especially for macroscopically purulent samples, counting chambers would still be the best choice for WBC counting, for macroscopically non-purulent samples Sysmex UF 1000i is a good alternative instrument for rapid workflow especially in routine microbiology laboratory in sterile body fluids other than blood and urine.

Our findings suggest that the introduction of automated cell count systems will ease the microbiology work flow for sterile body fluid cell count at least for nonpurulent sterile body fluids.

#### Conflict of interest

No conflict of interest was declared by the authors.

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