Comparison of Body Fluid Cell Counting between Automate Hematology Analyzer Sysmex XT-4000i and Manual Microscopic Method.

Sirin Lohajaroensub MSc. (Medical Technology)[1*]
Prirayapak Sakoonwatanyoo MD., PhD (Molecular Genetics and Genetic Engineering)[1]
Suthida Sakun thaworn MSc. (Clinical Pathology)[1]
Dalad Pichanun MSc. (Clinical Pathology)[1]

[1] Department of Clinical Pathology Faculty of Medicine Vajira Hospital, Navamindradhiraj University
* Corresponding author, email address: sirinc7@hotmail.com

Abstract

Objectives: To evaluate the comparison of body fluid cell count between automated hematology analyzer Sysmex XT-4000i and the gold standard manual microscope method using improved Neubauer hemocytometer or counting chamber.

Methods: Two thousand and fifty-three body fluid specimens, i.e., 85 of cerebrospinal fluid, 42 of synovial fluid, 51 of pleural fluid and 75 of ascitic fluid), were collected at the Hematology Laboratory, Department of Clinical Pathology, Faculty of Medicine Vajira Hospital. For counting red blood cells (RBC) and white blood cells (WBC) of body fluid specimens, the automatic Sysmex XT-4000i was used for analysis to compare with counting chamber, which is the gold standard method.

Results: Comparison of RBC and WBC counts of the two methods were analyzed by using Spearman's rank correlation test (r_s). The acceptable value of 218 of red blood cells count was in the range of more than 1,000 cells/µL (RBC count $\leq$1,000 cells/µL with the *p*-value = 0.037). The correlation of RBC count were r_s = 0.857 (CSF), 0.748 (synovial fluid), 0.944 (pleural fluid) and 0.973 (ascitic fluid). The correlation of WBC count of 253 were r_s = 0.950 (CSF), 0.936 (synovial fluid), 0.956 (pleural fluid) and 0.967 (ascitic fluid). RBC and WBC count by two methods were indicated that the correlation were high and in the same direction in any specimen except for synovial fluid. The correlation of differential polymorphonuclear cell (PMN) (r_s=0.903) was better than mononuclear cell (MN) (r_s=0.894). The background count, accuracy, precision, linearity and carryover for RBC and WBC count were found to be in the range. The correlation of counting WBC was better than RBC.

Conclusion: There is a good agreement for body fluid, red blood cells, white blood cells count and differential for PMN and MN examination were obtained between Sysmex XT-4000i and the manual gold standard method using counting chamber with the exception of the synovial fluid. With some limitations, the automate analyzer should not be used for body fluid cell counting, especially in the case of RBC less than 1,000 cells/ µL.

Keywords: Manual method, Sysmex XT- 4000i, body fluid cell count, cerebrospinal fluid, synovial fluid, pleural fluid, ascitic fluid.

การเปรียบเทียบการตรวจนับเซลล์ในสารน้ำของร่างกายระหว่างเครื่องอัตโนมัติ Sysmex XT-4000i และการตรวจนับด้วยกล้องจุลทรรศน์

สิริน โลหะเจริญทรัพย์ วทม. (เทคนิคการแพทย์)[1]*

ไปรยาภัค สกุลวทัญญู พ.บ., ว.ว. พยาธิวิทยาคลินิก ปร.ด (อณูพันธุศาสตร์และพันธุวิศวกรรมศาสตร์)[1]

สุธิดา สกุลถาวร วทม. (พยาธิวิทยาคลินิก)[1]

ดาลัด พิชานันท์ วทม. (พยาธิวิทยาคลินิก)[1]

[1] ภาควิชาพยาธิวิทยาคลินิก คณะแพทยศาสตร์วชิรพยาบาล มหาวิทยาลัยนวมินทราธิราช

* ผู้ติดต่อ, อีเมลล์: sirinc7@hotmail.com

บทคัดย่อ

วัตถุประสงค์: เปรียบเทียบการตรวจนับเซลล์ในสารน้ำของร่างกายระหว่างเครื่องอัตโนมัติ Sysmex XT- 4000i และการตรวจนับด้วยกล้องจุลทรรศน์ โดยใช้ improved Neubauer hemocytometer หรือ วิธี counting chamber ซึ่งเป็นวิธีมาตรฐาน

วิธีดำเนินการวิจัย: เก็บสิ่งส่งตรวจจากหน่วยโลหิตวิทยา ภาควิชาพยาธิวิทยาคลินิก คณะแพทยศาสตร์วชิรพยาบาล เพื่อตรวจวิเคราะห์สารน้ำในร่างกายด้วยเครื่องตรวจวิเคราะห์เม็ดเลือดทางโลหิตวิทยาอัตโนมัติ Sysmex XT-4000i เปรียบเทียบการตรวจนับเซลล์ด้วยกล้องจุลทรรศน์ โดยใช้ counting chamber ซึ่งเป็นวิธีมาตรฐาน จากการศึกษา โดยการนับเซลล์เม็ดเลือดแดงและเม็ดเลือดขาว จำนวน 253 รายจากสิ่งส่งตรวจ 4 ชนิดคือ น้ำหล่อสมองไขสันหลัง 85 ราย น้ำไขข้อ 42 ราย น้ำจากช่องปอด 51 ราย และ น้ำจากช่องท้อง 75 ราย

ผลการวิจัย: จากการเปรียบเทียบการนับเม็ดเลือดแดงและเม็ดเลือดขาวทั้ง 2 วิธี โดยใช้ Spearman's rank correlation test (r_s) สำหรับการนับเม็ดเลือดแดง จำนวน 218 ราย โดยนับจำนวนนับเม็ดเลือดแดงที่อยู่ในช่วง มากกว่า 1,000 เซลล์ เนื่องจากเม็ดเลือดแดงที่มีจำนวนต่ำกว่า 1,000 เซลล์มีค่า p = 0.037 เปรียบเทียบ 2 วิธี ได้ค่า r_s = 0.857 (น้ำหล่อสมองไขสันหลัง), 0.748 (น้ำไขข้อ), 0.944 (น้ำจากช่องปอด) และ 0.973 (น้ำจากช่องท้อง) สำหรับการนับเม็ดเลือดขาวจำนวน 253 ราย เปรียบเทียบ 2 วิธี ได้ค่า r_s = 0.950 (น้ำหล่อสมองไขสันหลัง), 0.936 (น้ำไขข้อ), 0.956 (น้ำจากช่องปอด) และ 0.967 (น้ำจากช่องท้อง) และค่าความสัมพันธ์ของเม็ดเลือดแดงและเม็ดเลือดขาวใน 2 วิธีพบว่า การนับเม็ดเลือดในสารน้ำทุกชนิดยกเว้นน้ำไขข้อ ซึ่งมีค่า r_s ต่ำ ส่วนสารน้ำอื่น ๆ มีความสัมพันธ์กันสูงมากและเป็นไปในทิศทางเดียวกัน จากการเปรียบเทียบการนับแยกชนิดเม็ดเลือดขาว พบว่าค่า r_s ของ polymorphonuclear cell = 0.903 มีความสัมพันธ์กันมากกว่า mononuclear cell = 0.894 ค่า background count, accuracy, precision, linearity และ carryover ของเครื่องอัตโนมัติ Sysmex XT-4000i พบว่า มีค่าอยู่ในช่วงดีผ่านเกณฑ์ โดยมีค่าความสัมพันธ์ของเม็ดเลือดขาวดีกว่าเม็ดเลือดแดง

สรุป: การตรวจนับเซลล์ในสารน้ำของร่างกายระหว่างเครื่องอัตโนมัติ Sysmex XT-4000i และการตรวจนับด้วยกล้องจุลทรรศน์ โดยใช้ counting chamber สามารถใช้แทนกันได้ ยกเว้นการนับน้ำไขข้อ แต่การตรวจด้วยเครื่องมีข้อจำกัดบ้างในกรณีที่จำนวนเม็ดเลือดแดงต่ำกว่า 1,000 cells/μL

Introduction

Body fluid analysis in the clinical laboratory is an important procedure that provides valuable information for the diagnosis and treatment of numerous medical conditions. In cerebrospinal fluid (CSF), white blood cell (WBC) count more than 10 cells/μL in children suggests meningitis, encephalitis or a leukemic CSF infiltration as no red blood cell (RBC) are found in normal CSF.[1] CSF analysis is also useful to monitor the response to intrathecal chemotherapy.[2] Laboratory results of synovial fluid analysis can be used to determine the pathologic organ of arthritis. The beneficial tests most frequently performed on synovial fluid are WBC count and differential count.[3] The increasing of WBC, especially neutrophils in pleural fluid and ascitic fluid are indicative of bacterial infection such as pneumonia, pancreatitis, pulmonary infraction,[4-5] bacterial peritonitis and cirrhosis.[6]

RBC and WBC count of body fluid are routinely performed by the microscopic analysis or manual method. It has been the gold standard examination for determination of cell count and differential WBC in the most laboratories.[7] There are several disadvantages in the procedure: the analysis requires sufficient practical experience or skills of the technologists, delayed result may constitute a problem in emergency diagnostics, and the imprecision and wide inter-observer variability.[8]

In contrast, the reproducibility precision and accuracy of an automated hematology method by using function "BF" (body fluid mode) is more consistent because it is not subjective to the variation inherent in the manual method. A large number of cells can be analyzed. Several published studies had examined the performance of these automated methods for cell counts. [9-14]

The aim of this study is to compare two methods of body fluid examination entail quantification and differentiation of cell populations presenting in CSF, synovial fluid, pleural fluid and ascitic fluid. The first method was manual microscopic method using improved Neubauer hemocytometer and the second was an automate hematology analyzer, Sysmex XT- 4000i. The study has been approved by the Institutional ethical review board.

Methods
Patients and samples

Two hundred and sixty-five body fluid samples (CSF, synovial fluid, pleural fluid and ascitic fluid) were collected in either EDTA anticoagulant or no anticoagulant in a sterile container. After exclusion of unsuitable samples, there were 253 body fluid specimens. The rejected specimens were unsuitable for evaluation because of the presence of clots, crystals, high viscosity and error messages related to WBC and RBC parameters which may compromise results.[15] The samples were from 137 male (54.2%) and 116 female (45.8%) patients. The subject's ages were between 3 days to 91 years (mean = 57.7 years). The specimens were examined in Hematology Laboratory at Faculty of Medicine Vajira Hospital between June and September 2013. There were 85 CSF, 42 synovial fluids, 51 pleural fluids and 75 ascitic fluids. Samples were tested within two hours of receiving. The performance of four body fluid types were examined with the Sysmex XT-4000i and compared with the manual method with respect to RBC, WBC and differential for polymorphonuclear (PMN) and mononuclear cell (MN). RBC counts were evaluated from 176 body fluid specimens (42 CSF, 38 synovial fluid, 50 pleural fluid and 46 ascitic fluid samples). WBC counts were evaluated from 253 body fluid specimens and differential WBC count were from 205 specimens (57 CSF, 42 synovial fluid, 51 pleural fluid and 55 ascitic fluid samples) (WBC count > 10 cells/μL).

Study methods

RBC, WBC count and differential WBC were determined using the improved Neubauer hemocytometer in a manual method. The automatic method was done by using Sysmex XT-4000i Manual counting chamber method[15-17]

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The average number of RBC and WBC counted on both sides of improved Neubauer hemocytometer was placed into a formula that calculates the number of cells per microliter. For body fluids with low cell counts, RBC and WBC were separately counted in five squares on each side of the chamber. With high cell counts, the cells within one central square on each side were counted, and ten multiplied the average of the two counts. A 1:10 dilution of the fluid was counted with too high cell count to be accurately measured without a dilution. In this study, two experienced technicians performed the counting of RBC and WBC by manual microscopic methods. The differential WBC counts were performed on Wright-Giemsa's stained slides.

Automated method: automated hematology analyzer Sysmex XT-4000i[18-19]

The Sysmex XT-4000i is an automated hematology analyzer from Sysmex Corporation (Kobe, Japan). The analysis of the body fluids can be automatically performed. RBC and WBC can be specified even for a weak concentration with a lower limit of detection (> 1,000 cells /μL for RBC-BF (body fluid mode of RBC count) and 10 cell /μL for WBC–BF (body fluid mode of WBC count). The instrument is also able to distinguish the PMN from the MN cells (lymphocytes, monocytes). The Sysmex XT-4000i aspires 85 μL of the sample and in perform of hematology analysis according to the hydro dynamic focusing (DC detection) for RBC and flow cytometry method using a semiconductor laser and fluorescent measurement for WBC and differential. The instruments express the counts of WBC to three decimal places and RBC counts to four decimal places. The body fluid analysis mode of the Sysmex XT-4000i uses the 4-DIFF scattergram and the RBC distribution obtained from a specialized analysis sequence to calculate and display the WBC counts, mononuclear cell (MN)/ PMN counts and percentages, and RBC (RBC-BF) counts found in the body fluid. Startup was performed each day with close observation of background counts before specimens were analyzed. Three levels of quality control material (e-CHECK(XE)) were used (levels 1, 2, and 3) to check the instrument throughout the comparison study. The result throughput for body fluid analysis is approximate 30 samples/hour. The evaluation of carryover in body fluid mode, carryover analysis was performed with the procedure according to International Council for Standardization in Hematology.[18-19]

Statistical Analysis[19-22]

Statistical analysis was performed using excel and MedCalC-version 12.7.5.0. Data was analyzed, included the calculation of regression statistics. Agreement between automated cell counts and microscopic data was examined by Spearman's rank correlation coefficient test. The $p < 0.001$ considered statistically significant.

Results

Before the comparison of two methods, the background count, accuracy, precision, within-run and between-run precision, linearity and carryover of the Sysmex XT-4000i were study. The acceptable limit of them were the standard limit of ICSH (International Committee for Standardization in Haematology),[19] CLSI (Clinical and Laboratory Standards Institute)[7] and Sysmex Corporation (Kobe, Japan).[18]

Background Count

The acceptable background limits of automatic analyzer were ≤ 0.003×10^6/μL and ≤ 0.001×10^3/μL for RBC for WBC. In the study, the background limits of the Sysmex XT-4000i were in the acceptable limits.

Accuracy[18-19]

The acceptable accuracy for body fluids cell counts and differential by automatic analyzer are r = ≥0.80, ≥0.90, ≥0.70, ≥0.70 (RBC, WBC, PMN%, MN %) and the slope are 1 ±0.30 for RBC and WBC count and 1 ±0.50 for PMN and MN differential count. In the study, the accuracy of the Sysmex XT -4000i was in the acceptable limits.

Precision[15]

The acceptable precision (reproducibility) of automatic analyzer for body fluids counting are coefficient of variation (CV) ≤ 40 % for RBC count and ≤ 30 % for WBC count. In the study, the precision of the Sysmex XT-4000i were in the acceptable limits.

Within-run Precision[19] (Table 1)

With-in run precision: exhibits the coefficients of variation by repeating 20 times continuously of three levels specimens. Comparison of mean value (Mean), standard deviation (SD) and CV analysis were performed. The result of RBC, WBC count and differential WBC using Sysmex XT-4000i and the microscopic reference method were done. The results of CV were in the acceptable limits

Between-run Precision[19] (Table 2)

Two levels of XN-CHECK BF manufactured by Sysmex Corporation have been used as hematology control for BF mode of the Sysmex X series instrument system. They were measured continuously for 20 times in different days. The CV analysis was performed to compare the result of RBC, WBC count and differential for WBC by using Sysmex XT-4000i. The mean, SD and CV showed that CV of RBC were 1.67-2.38%, WBC were 3.13-5.30% and differential WBC for PMN and MN cell for low and normal level were 15.60, 15.10 and 4.90, 5.80% respectively. The results of CV were in the acceptable limits

Linearity[19] (Table 3)

Linearity of WBC and RBC count were evaluated by diluting nonspecific body fluid (in this

Table 1:

Within-run precision of Sysmex XT-4000i for RBC, WBC count, differential PMN and MN cell.

Parameter (n=20)	Mean	Minimum	Maximum	SD	CV %
RBC ($\times 10^6$/μL)					
Level 1	0.043	0.042	0.045	0.001	2.35
Level 2	0.098	0.096	0.010	0.002	1.07
Level 3	2.207	2.200	2.211	0.004	0.18
WBC ($\times 10^3$/μL)					
Level 1	0.043	0.035	0.051	0.005	12.87
Level 2	0.254	0.240	0.267	0.009	3.53
Level 3	2.730	2.704	2.762	0.020	0.75
PMN (%)					
Level 1	86.6	85.1	87.7	0.87	1.01
Level 2	61.7	54.3	70.3	5.11	8.28
Level 3	68.3	60.1	78.3	7.05	10.34
MN (%)					
Level 1	13.4	12.0	14.9	0.87	6.46
Level 2	38.3	29.7	45.7	5.11	13.35
Level 3	35.2	23.7	39.0	3.85	10.97

study, pleural fluid) samples to obtain reportable range and low levels of detection. The RBC and WBC correlation coefficient test (*r*) were 0.983 (slope=0.944; intercept = 0.005) and 0.974 (slope= 1.051; intercept= 0.012), respectively. Linearity of both WBC and RBC were good at low level of detection for WBC and high levels of detection for RBC.

Carryover (Table 4)

The evaluation of carryover rate in body fluid mode, carryover analysis was performed with the procedure of the International Council for Standardization in Hematology.[18-19] The nonspecific body fluid were used, pleural fluid for high and CSF for low count. They were analyzed three consecutive times (H1, H2, H3 and L1, L2, L3). The acceptable percentage of carryover of RBC and WBC automatic analyzer were less than 0.3%.[19] In the study, WBC and RBC %carryover results of high to low carryover testing were shown in the table 4.

Correlation (Table 5)

Evaluation of the correlation of manual and automate counting of RBC and WBC count in all specimen types.[21,22] The *p*-value of all level of RBC and WBC count were less than 0.001 except the range of RBC < 1,000 cells /µL, *p*=0.0367 (significant difference) which was limitation according to manufacturer of Sysmex XT-4000i. For WBC count *p* < 0.001, this showed that all of correlations were accepted.

Table 2:

Between-run precision: exhibits CV for the samples in the precision study by using two BF-controls material.

Parameter (n=20)	Mean	Minimum	Maximum	SD	CV %
RBC (x10^6/µL)					
Low	0.025	0.024	0.026	0.006	2.38
Normal	0.073	0.071	0.075	0.001	1.67
WBC (x10^3/µL)					
Low	0.083	0.074	0.089	0.005	5.30
Normal	0.321	0.305	0.339	0.005	3.13
PMN (%)					
Low	8.7	1.2	17.6	4.50	15.60
Normal	11.4	2.9	21.6	2.55	15.10
MN (%)					
Low	91.3	82.4	98.8	4.50	4.90
Normal	88.6	78.4	97.1	2.55	5.80

Table 3:

Linearity of RBC count and WBC count

Parameter	Range Tested	*r*	Slope	Intercept
RBC	0.001 - 0.880 x10^6/ µL	0.983	0.944	0.005
WBC	0.000 - 55.350x10^3/ µL	0.974	1.051	0.012

The correlation of manual counting in all specimens (CSF, synovial fluid, pleural fluid and ascitic fluid) of RBC count (r_s=0.995), WBC count ((r_s=0.932) of two technicians were excellent. In the same time of cell counting by the two technicians, the automated cells counting had been done by the third technician. The average number of cell counted by two technicians were compared with the automated method.

Evaluation of the correlation between manual method and Sysmex XT-4000i, samples was measured for RBC and WBC in CSF (42, 85),

synovial fluid (38, 42), pleural fluid (50, 51) and ascitic fluid (46, 75), in total 253 samples. The result of correlation (r_s) between manual method and Sysmex XT-4000i and the mean value were analyzed. The correlation for RBC count and WBC count were evaluated for CSF: r_s = 0.857, 0.950, synovial fluid: r_s = 0.748, 0.936, pleural fluid: r_s = 0.944, 0.956, ascitic fluid: r_s = 0.973, 0.967 and the differentiation for MN and PMN were : r_s = 0.894, 0.903, respectively. There was a good overall correlation of RBC and WBC count by two method especially RBC count in synovial fluid. The

Table 4:

Carryover analysis of RBC, WBC count

Parameter	H1	H2	H3	L1	L2	L3	Carryover %	Acceptable Performance %	Status
RBC ($\times 10^6$/µL)	0.099	0.099	0.097	0.044	0.043	0.042	0.275	≤ 0.3	Pass
WBC ($\times 10^3$/µL)	0.256	0.240	0.245	0.038	0.035	0.045	-0.350	≤ 0.3	Pass

Table 5:

Correlation of Manual vs. Sysmex XT-4000i RBC and WBC count for all specimen types.

Manual cell Range (cells /µL)	No. of Cases	r_s, 95% CI	p-value
RBC count			
0 - < 1,000	77	0.221(0.141-0.409)	0.0367
0 – 10,000	191	0.787 (0.728-0.834)	< 0.001
0 – 100,000	244	0.856(0.819-0.887)	< 0.001
0 – > 100,000	253	0.871 (0.836-0.898)	< 0.001
Rule out RBC < 1,000			
1,000 - 10,000	114	0.806(0.754-0.870)	< 0.001
1,000 - all	176	0.877(0.836-0.908)	< 0.001
WBC count			
0 - 10	29	0.758 (0.542-0.880)	< 0.001
0 - 20	55	0.856 (0.764-0.914)	< 0.001
0 - 50	84	0.881 (0.822-0.921)	< 0.001
0 - 100	112	0.932 (0.902-0.953)	< 0.001
0 - 1,000	186	0.969 (0.959-0.977)	< 0.001
0- > 1,000	253	0.966 (0.938-0.974)	< 0.001

Table 6:

Correlation of RBC, WBC count and differential WBC between manual and Sysmex XT-4000i. method

Fluid Type	Mean	Minimum	Maximum	Spearman's correlation r_s	95% CI
RBC ($\times 10^6/\mu L$)					
CSF (n=42)					
Manual	0.0153	0.0011	0.0988	0.857	0.782-0.908
Sysmex XT-4000i	0.0172	0.0012	0.1057		
Synovial fluid (n= 38)					
Manual	0.0077	0.0010	0.0373	0.748	0.521-0.877
Sysmex XT-4000i	0.0147	0.0006	0.0820		
Pleural fluid (n= 50)					
Manual	0.0278	0.0010	0.3125	0.944	0.895-0.970
Sysmex XT-4000i	0.0324	0.0015	0.3479		
Ascitic fluid (n=46)					
Manual	0.0465	0.0012	0.8803	0.973	0.947-0.986
Sysmex XT-4000i	0.0458	0.0012	0.7970		
Body fluid combined (n=218)					
Manual	0.0257	0.0010	0.8803	0.877	0.782-0.986
Sysmex XT-4000i	0.0287	0.0006	0.7970		
WBC ($\times 10^3/\mu L$)					
CSF (n=85)					
Manual	0.239	0.000	6.820	0.950	0.925-0.968
Sysmex XT-4000i	0.258	0.000	6.793		
Synovial fluid (n= 42)					
Manual	11.226	0.044	55.350	0.936	0.914-0.965
Sysmex XT-4000i	12.099	0.069	57.938		
Pleural fluid (n= 51)					
Manual	1.301	0.010	14.962	0.956	0.929-0.976
Sysmex XT-4000i	1.298	0.008	13.471		
Ascitic fluid (n=75)					
Manual	1.274	0.012	32.385	0.967	0.948-0.979
Sysmex XT-4000i	1.248	0.010	30.343		
Body fluid combined (n=253)					
Manual	2.584	0.000	55.350	0.966	0.938-0.974
Sysmex XT-4000i	2.727	0.000	57.938		
Differential WBC (%)					
MN (n=205)					
Manual	54.8	0	100	0.894	0.862-0.918
Sysmex XT-4000i	54.5	0	100		
PMN(n=205)					
Manual	44	0	100	0.903	0.874-0.925
Sysmex XT-4000i	46	0	100		

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Sirin Lohajaroensub, Prirayapak Sakoonwatanyoo, Suthida Sakunthaworn, Dalad Pichanun

differentiation of WBC count between two methods were in agreement. All of them were significant ($p < 0.001$)

The study showed slope and intercept of RBC count compared between manual and Sysmex XT-4000i. The slope of RBC count for each type of fluids (42 CSF, 38 synovial, 50 pleural, 46 ascitic fluid and 218 body fluid combined) were 1.100, 1.775, 1.092, 0.929 and 0.944 respectively. The intercept of RBC count for each types of fluids were 0.000, 0.001, 0.002, 0.003 and 0.944 for body fluid combined, respectively. The slopes of all specimen types were closed to 1.000 and intercept were closed to 0.000 except synovial fluid. All body fluids counting for RBC by two methods were correlated for CSF, pleural and ascitic fluid. The regression analysis R^2 for RBC count were 0.974, 0.652, 0.988, 0.995 and 0.983, respectively. There was an good overall correlation for two methods, exception for synovial fluid. (Figure 1)

The study showed slope and intercept of WBC count compared between manual and Sysmex XT-4000i. The slope of WBC count for each types of fluids (85 CSF, 42 synovial, 51pleural, 75 ascitic fluid and 253 body fluid combined) were 1.014, 0.856, 1.071, 0.932 and 1.05. The intercept of WBC count were 0.015, 0.183, 0.078, 0.061 and 0.012, respectively. The slopes of all specimen types were closed to 1.000 and intercept were closely to 0.000. All body fluids counting for WBC by two methods were correlated for CSF, pleural and ascitic fluid. The regression analysis R^2 for WBC count were 0.976, 0.885, 0.957, 0.996 and 0.974, respectively. The results suggested that all body fluids WBC counting by two methods were correlated for all types of body fluids, exception for synovial fluid. (Figure 2)

The slope and intercept of differential WBC of 205 body fluid samples (WBC count > 10 cells/ µL) for MN and PMN were 0.820, 0.846 and 9.395, 8.486 respectively. The regression analysis R^2 for differential WBC were 0.837 and 0.874. The results suggested that differential WBC counting for PMN was better than MN. (Figure 3)

Discussion

The correlations of RBC and WBC counting method between manual and Sysmex XT-4000i were good to excellent. The correlation (r_s) of RBC and WBC count in body fluid ranking from high to low were ascitic fluid, pleural fluid, CSF and synovial fluid. The overall correlation by Spearman 's correlation test (r_s) between the manual method and Sysmex XT-4000i was not agree quite well with the correlation for RBC. The correlation for RBC count and WBC count were evaluated for CSF: r_s = 0.857, 0.950, synovial fluid: r_s = 0.748, 0.936, pleural fluid: r_s = 0.944, 0.956, ascitic fluid: r_s = 0.973, 0.967 and the differentiation for MN and PMN: r_s = 0.894, 0.903 respectively. Presented data indicated that Sysmex XT-4000i count was trustworthy and could provide more precise and reliable information than manual method, except low synovial fluid. In RBC and WBC count, CSF, pleural and ascitic fluid, all correlation between automated and reference method were over 0.850, except for synovial fluid. There was a better correlation obtained for PMN count (0.903) than for MN count (0.894) (Table 6).

However, that manual differential review and an extensive examination of slides were needed for differential cell count, especially for detectection of abnormal cells. The studies of background count, accuracy, precision (Table 1, 2), linearity (Table 3) and carryover (Table 4) for RBC and WBC count were found to be in the range. Carryover was less than 0.3%. Precision results were less than 30% for WBC counts from 0.038 x 10^3/µL to 0.256 x 10^3/µL and for RBC counts from 0.042 x 10^6/µL to 0.099 x 10^6/µL. Linearity at low levels of detection was very good. The differentiation into PMN and MN from the DIFF channel (Figure 3) showed a good regression analysis with the reference microscopic differentiation (R^2= 0.874, 0.837). They showed that RBC, WBC and DIFF channel from the XT-4000i could be described as satisfactory to excellent. This allowed the operating technologists to evaluate the potential advantages of the

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automated method. Manual microscopy chamber of body fluid cell counts are time-consuming, labor-intensive and frequently imprecise, but remains the gold standard according to NCCLS.[11] Fully automated analysis meets time and quality

requirements and are objective in material handling. Conversely, laboratory technicians frequently must force microscopic chamber counting into tight laboratory schedules with increasing workloads, additionally needing to
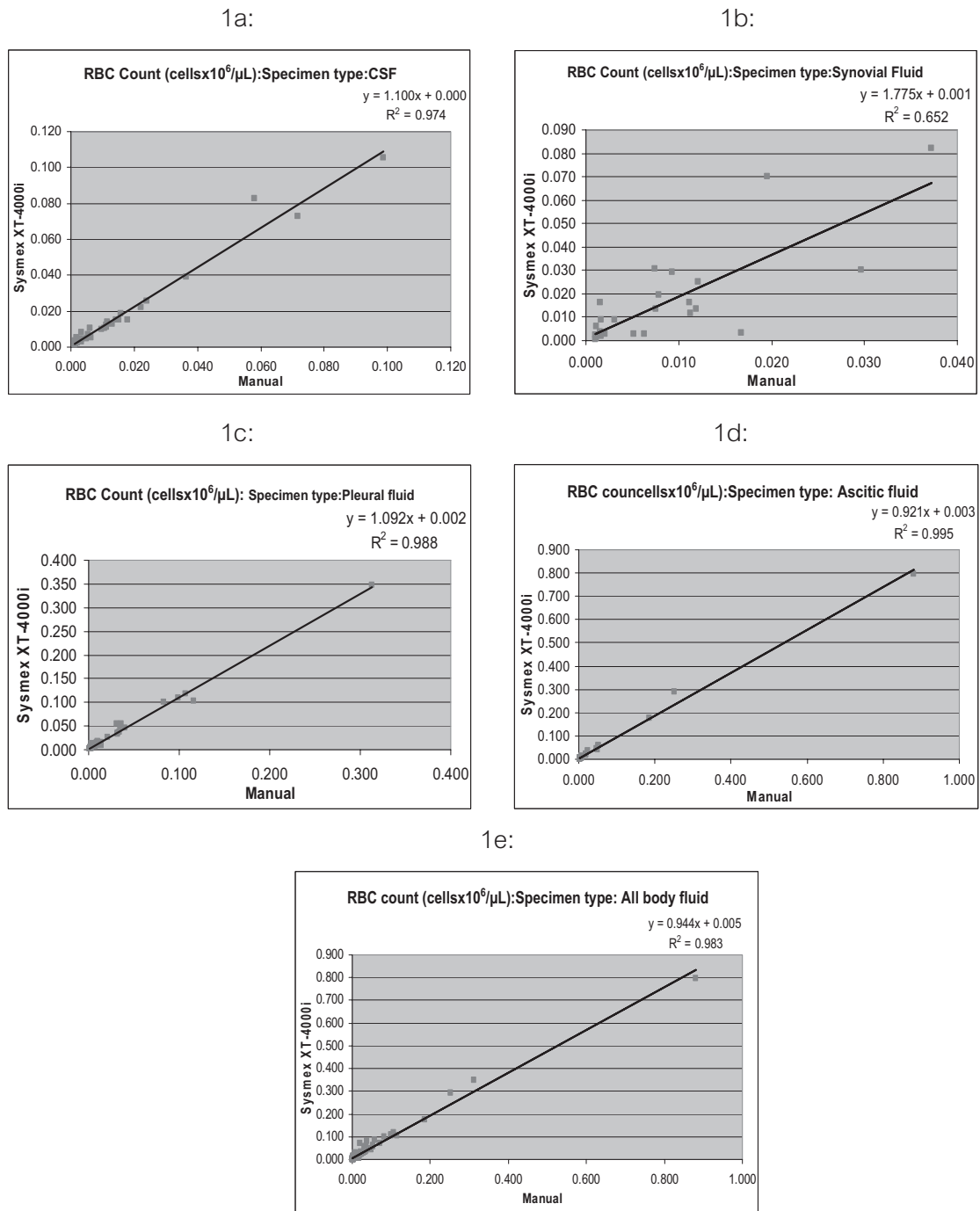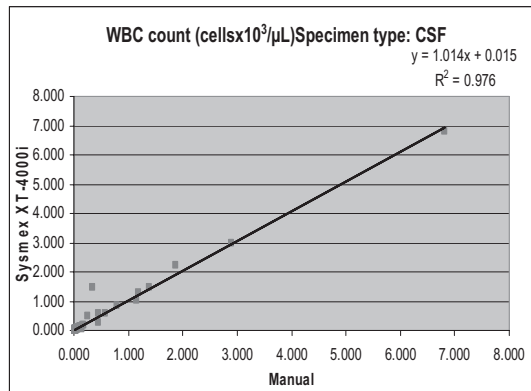
1a:

1b:

1c:

1d:

1e:

Figure 1: The regression analysis of RBC count between manual method and the Sysmex XT-4000i. 1a. CSF; 1b. synovial fluid; 1c. pleural fluid; 1d. ascitic fluid; 1e. all specimen types
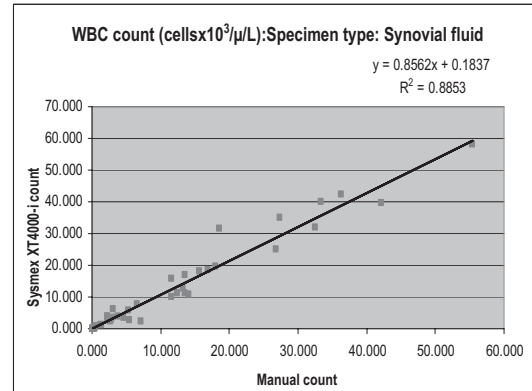
consider several other factors, e.g., number of samples and quality of cells. Furthermore, insufficient practical experience in microscopic counting chamber and the subjectivity of individual laboratory workers added to the unreliability of the results frequently observed. The reason for lack of more specific body fluid diagnoses often is insufficient sample volume and/or too few cells in a sample specific automated system for body fluid cell counting are not available at present.
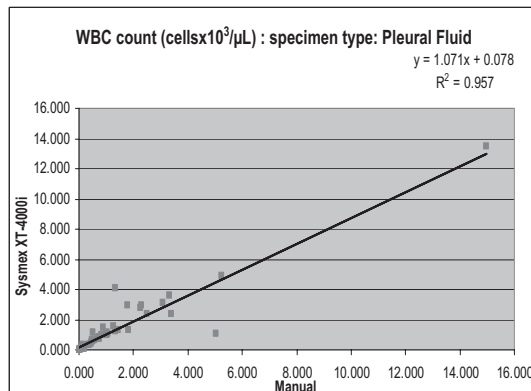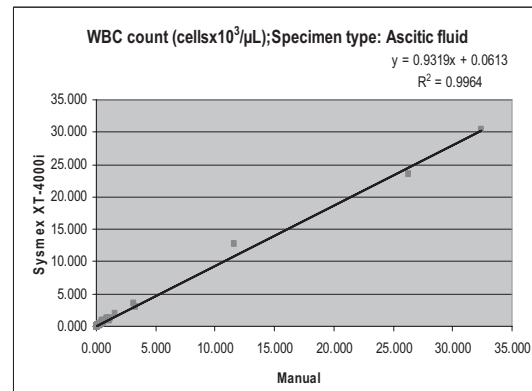
2a:

WBC count (cellsx10^3/μL)Specimen type: CSF

$y = 1.014x + 0.015$
$R^2 = 0.976$

2b:

WBC count (cellsx10^3/μ/L):Specimen type: Synovial fluid

$y = 0.8562x + 0.1837$
$R^2 = 0.8853$

2c:

WBC count (cellsx10^3/μL) : specimen type: Pleural Fluid

$y = 1.071x + 0.078$
$R^2 = 0.957$

2d:

WBC count (cellsx10^3/μL);Specimen type: Ascitic fluid

$y = 0.9319x + 0.0613$
$R^2 = 0.9964$

2e:

WBC count (cellsx10^3/μL):Specimen type:All specimen types
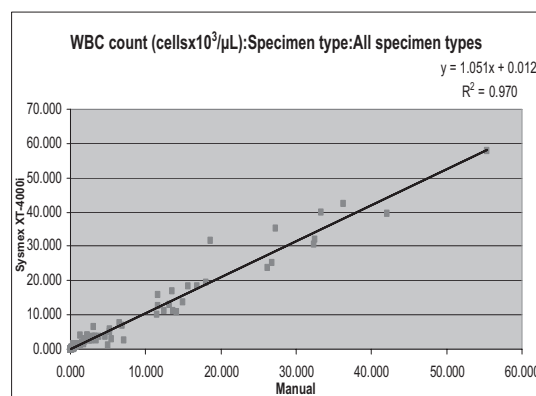
$y = 1.051x + 0.012$
$R^2 = 0.970$

Figure 2: The regression analysis of WBC count between manual method and the Sysmex XT-4000i 2a. CSF; 2b. synovial fluid; 2c. pleural fluid; 2d. ascitic fluid; 2e. all specimen types
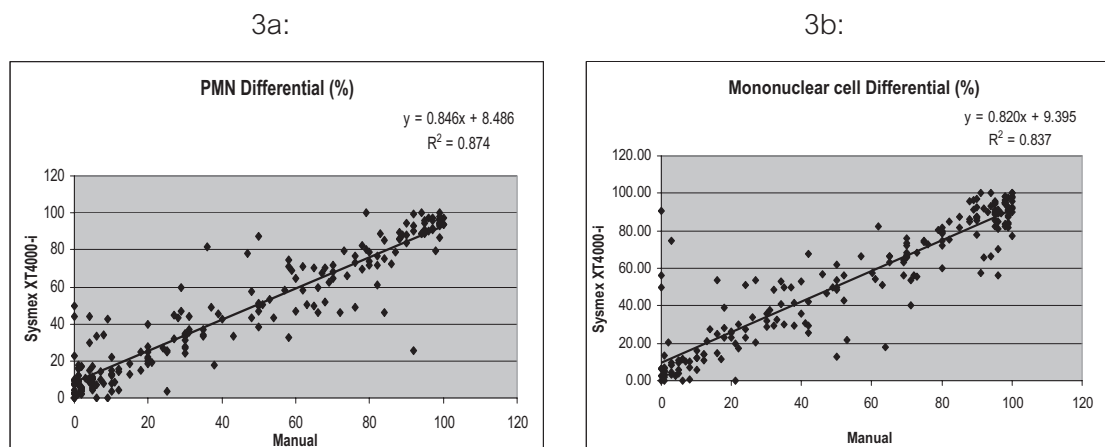
3a:

PMN Differential (%)

y = 0.846x + 8.486
R^2 = 0.874

3b:

Mononuclear cell Differential (%)

y = 0.820x + 9.395
R^2 = 0.837

Figure 3: Correlation of differential WBC between manual method and the Sysmex XT-4000i automated hematology analyzer. (3a. PMN differential, 3b. MN differential)

Conclusion

The Sysmex XT-4000i had an excellent, basic performance of body fluid examination except for synovial fluid. The Sysmex XT-4000i analyzer is a useful additional tool for cerebrospinal fluid, pleural fluids and ascitic fluids cell count examination, especially in the emergency setting. However, it is not proper for differential white blood cell. It provides the reproducibility, precision, accuracy, rapid and reliable data on RBC and WBC count, and is easier for quality control and standardization than manual method. Fully automated analysis meets time and quality requirements and are objective in material handling. Moreover, automated method would bring homogeneity between different laboratories. The use of automated systems may be limited by their poor sensitivity when used with samples with low cell counts (RBC count less than 1,000 cells/μL). Linearity in the WBC count less than 10 cells/μL has led to the reconsideration of these aspects.

The authors declare that there is no conflict of interest.

Acknowledgment

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