Automated Hematology Analyzer XE-5000 — Overview and Basic Performance

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XE-5000 which we developed has not only the same function of XE-2100 but also software to measure immature cells and body fluid mode. We can measure cerebrospinal fluid (CSF), pleural fluid, ascites and synovial fluid samples by XE-5000. We evaluated basic performance of XE-5000, reproducibility, linearity, carryover, correlation between XE-5000 and manual method, and the stability of samples at room temperature and 4°C.

Key Words Automated Hematology Analyzer, XE-5000, Body Fluid, Performance, Flow Cytometry

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INTRODUCTION

We developed a Automated Hematology Analyzer XE-5000 — an upgrade of XE-2100. In addition to the features of the XE-2100, the XE-5000 has a new feature "Body fluid mode" and immature cell measurement software (HPC master, IG master, RET master and IPF master) as a standard function. This article gives an overview of the system and presents some basic data.

TECHNOLOGIES

Principle of measurement

Flow cytometry method using semiconductor laser

Using the semiconductor laser technology, which was first established for the XE-2100, the XE-5000 applies a flow cytometry method for detecting fluorescent signals (***Fig. 1***).

From the resulting scattergrams different parameters can be obtained by means of side scatter, forward scatter, and side fluorescent signals. The different signals are used to obtain the following cell counts:

a. White blood cells/basophils (WBC/BASO): Side scatter and forward scatter signal
b. Four differential counts of white blood cells (DIFF) including neutrophils (NEUT), lymphocytes (LYMPH), monocytes (MONO), and eosinophils (EO): Side scatter and side fluorescent signal
c. Reticulocytes (RET): Forward scatter and side fluorescent signal
d. Nucleated red blood cells (NRBC): Forward scatter and side fluorescent signal

An example of the screen displaying the measurement results is shown in ***Fig. 2***.

RF/DC detection method

The XE-5000 uses the RF/DC detection method in order to measure hematopoietic progenitor cells (HPC) and to obtain information for the detection of immature white blood cells for IP messages and flags. The RF/DC detection method can simultaneously detect changes in the signal amplitude of radio frequency (RF) and direct current (DC) when a blood cell passes through an aperture of the detector. The DC component gives information about the volume, while RF detection technology uses high frequency alternating current to measure the cellular density.

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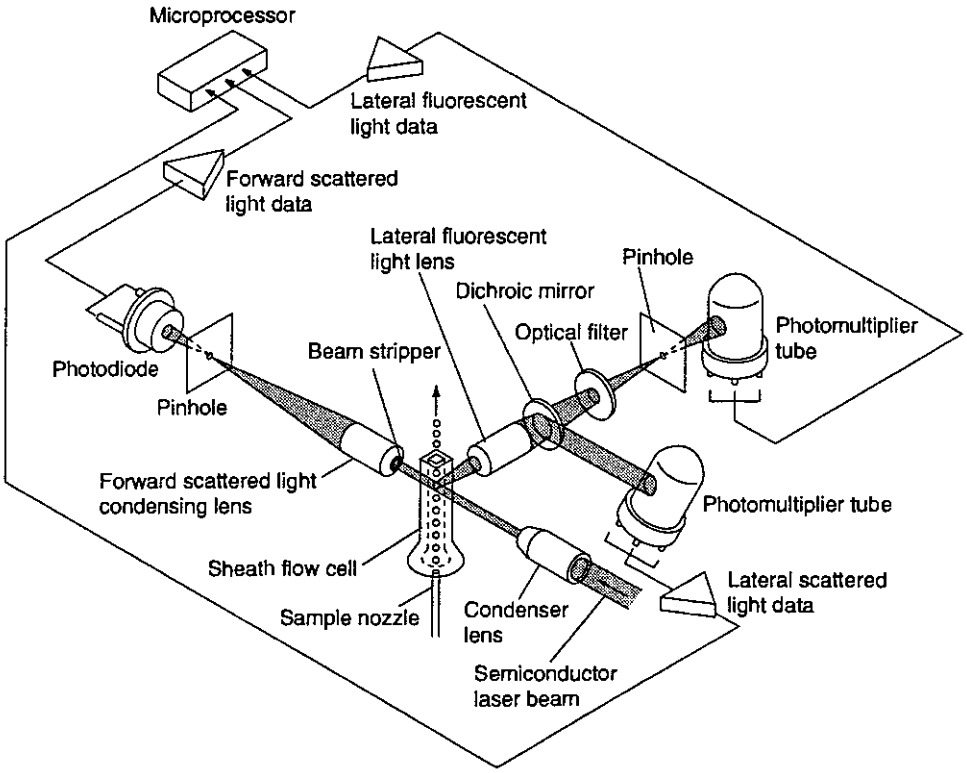

Fig. 1 *Flow cytometry method*

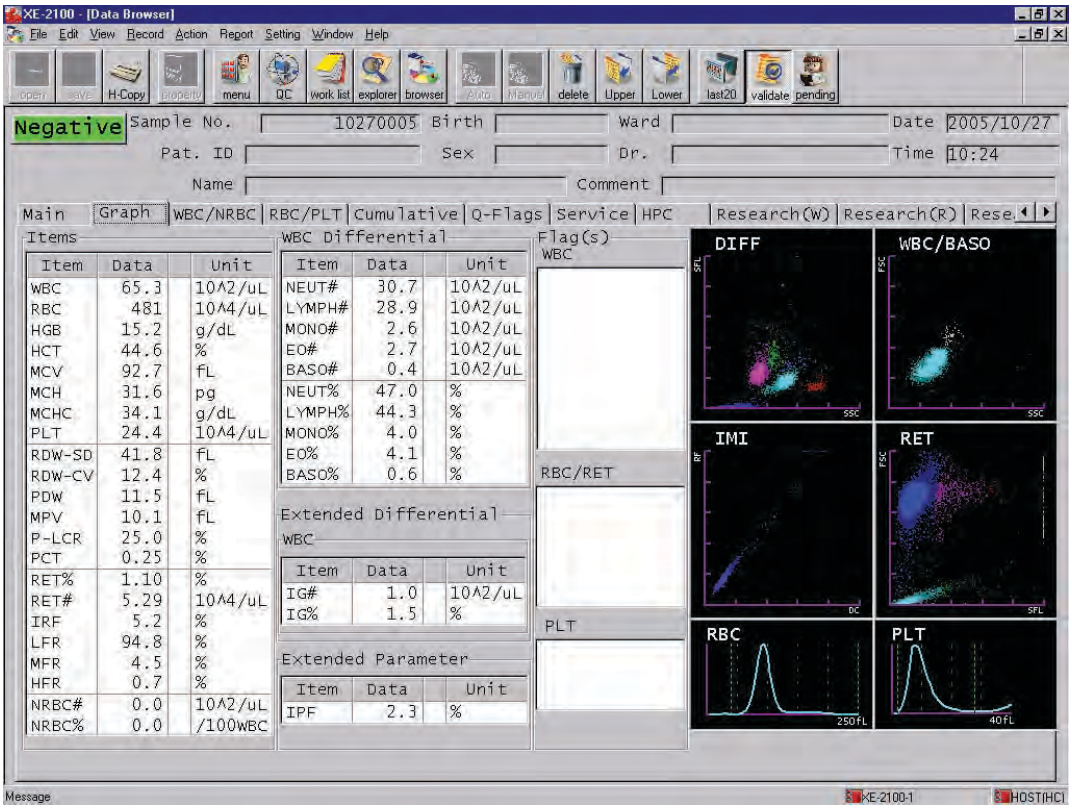

Fig. 2 *An example of the screen displaying the measurement results*

Sheath Flow DC detection method

For the measurement of red blood cell (RBC) count and platelet (PLT) count, the XE-5000 employs Sheath Flow DC detection method. With the Sheath Flow method, the sample flow is focused to the center of the aperture. It can then obtain precise size distributions of RBC and PLT by changing the generated electrical resistance into pulses, which provides highly precise results.

SLS hemoglobin method

The XE-5000 measures hemoglobin level (HGB) using colorimetry based on the SLS hemoglobin method. The SLS hemoglobin method utilizes sodium lauryl sulfate ($C_{12}H_{25}SO_4Na$: SLS), and the following reaction mechanism 1.) Hemolysis by reaction of erythrocyte membrane with SLS, 2.) Change in the three-dimensional structure of globin by SLS, 3.) Oxidation of heme iron by oxygen, and 4.) Formation of SLS-HGB.

This method is highly reliability and safe to use because of its cyanide-free content. Sysmex has used it on many generations of instruments.

Incorporation of immature cell measurement software as a standard function

The immature cell measurement software can detect hematopoietic progenitor cells (HPC) , immature granulocytes (IG), reticulocyte hemoglobin content ($RET\text{-}H_e$) immature platelets fraction (IPF) and other related parameters.

Addition of body fluid mode

The XE-5000 incorporates a newly added body fluid mode, that allows the measurement of body fluids in addition to peripheral blood. The body fluid mode, can measure, cerebrospinal fluid (CSF), pleura fluid, ascites, and synovial fluids, directly without any special treatment or pretreatment requirements.

EVALUATION OF BASIC PERFORMANCE OF THE BODY FLUID MODE

Within-run reproducibility (WBC)

Reproducibility at 10 successive measurements using pleura fluid is shown in ***Table 1***.

The result was excellent, with a CV of 13% at a count level of 27.90 WBC/μl.

Linearity

Using bronchoalveolar lavage fluid (BALF), we prepared a six-step dilution series and confirmed the linearity of the WBC count. The results are presented in ***Fig. 3***. In this analysis, the linearity was confirmed up to 500/μL of WBC-BF.

Carry over

Table 2 indicates the results of our test on carry over using pleura fluid. In this analysis, no carry over was observed using 9,000/μL of WBC-BF high-concentration sample.

Correlation

We analyzed correlations between the results obtained by XE-5000 and those by manual measurement, using pleura fluid, ascites, CSF, BALF, and pericardial fluid. The results are shown in ***Fig. 4***.

For the manual measurement, the WBC count in the body

Table 1 *Reproductivity*

Sample	WBC-BF /μL
1	22
2	25
3	35
4	30
5	30
6	28
7	28
8	25
9	30
10	26
SD	3.63
MEAN	27.90
CV%	13.03%

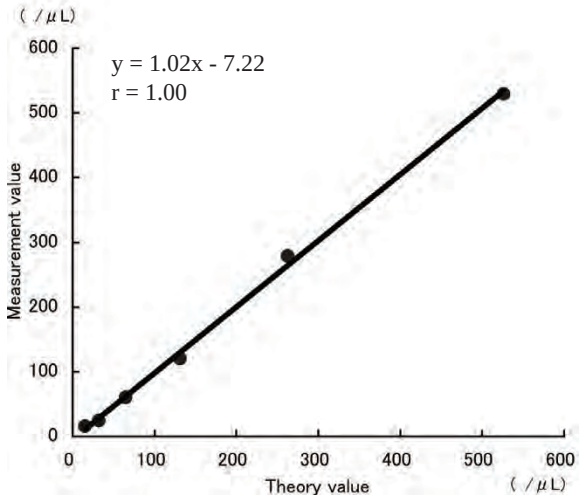

Fig. 3 *Linearity*

Table 2 *Carry over*

Sample	WBC-BF /μL	Sample	WBC-BF /μL	Sample	WBC-BF /μL
1	9449	1	9041	1	9432
2	9260	2	9289	2	9749
3	9323	3	9427	3	9469
4	1	4	0	4	0
5	0	5	0	5	0
6	0	6	0	6	0
Carry over (%)	0.0107	Carry over (%)	0.0000	Carry over (%)	0.0000
Ave = 0.0036					

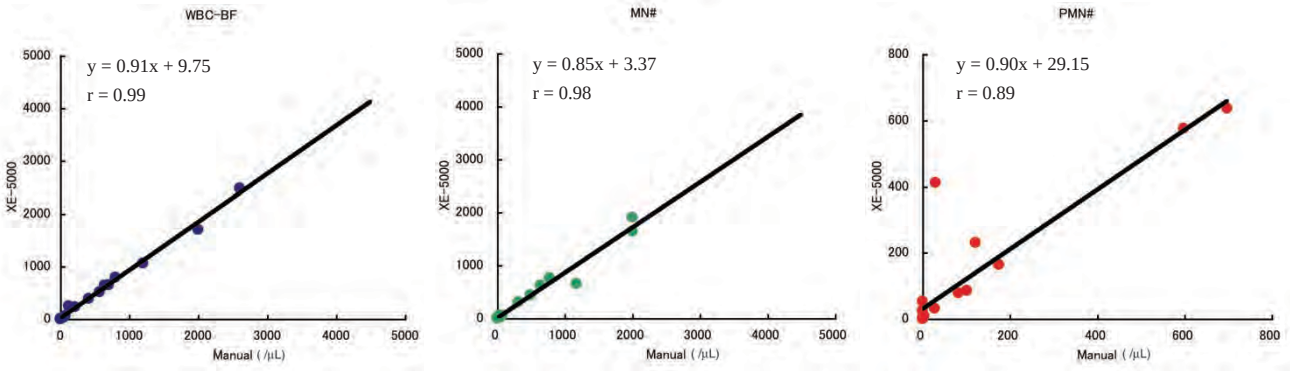

Fig 4 *Correlation*

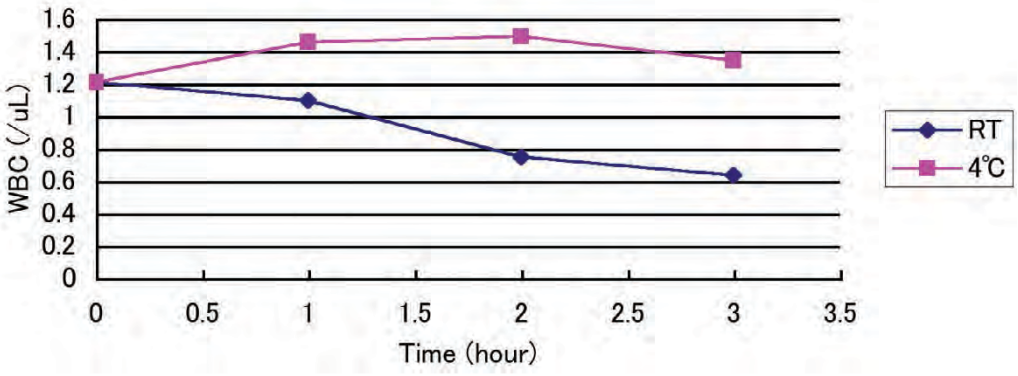

Fig 5 *Time stability*

fluid mixed with Samson fluid in the melangeur for the white blood cell counting was determined using the Fuchs-Rosenthal chamber. Cells included in the body fluid other than hematocytes such as histiocytes or mesothelial cells are included in the WBC count.
In the Body fluid mode of XE-5000, the data MN#/% corresponds to monocytes and lymphocytes, and PMN#/% corresponds to neutrophils, eosinophils, and basophils. However, in addition to these leukocytes, macrophages, mesothelial cells, histiocytes, cancer cells and others can be observed in body fluid, and they may have strong fluorescence intensity even beyond the range detectable by the DIFF scattergram. We analyzed the correlations after excluding the specimens that might include non-hematocyte cells.

WBC count

The correlation of WBC count between manual measurement and XE-5000 indicated more favorable results ($r = 0.99$, $y = 0.91x + 9.75$).

MN count and PMN count

The correlations for the concentration of MN and PMN between manual measurement and XE-5000 were good MN#: $r = 0.98$, $y = 0.85x + 3.37$; and PMN#, $r = 0.89$, $y = 0.90x + 29.15$) (***Fig. 4***).

Time stability

We examined the stability of WBC count at the following time intervals: at the sample submission to the laboratory (fresh) and 1, 2, and 3 hour(s) after the body fluid sampling. The results are presented in ***Fig. 5***.
In general, cells in body fluid are fragile, and thus it is desirable to determine them as soon as possible after sampling. In this examination, the WBC count started to decrease gradually from 1 hour after sample aspiration when stored at room temperature, while it was stable up to 3 hours later when stored at 4°C. The results suggest that the body fluid samples can be refrigerated to achieve measurement with good stability of data, if there is no way of performing measurement just after the sampling.

CONCLUSIONS

This article describes the newly developed Automated Hematology Analyzer, XE-5000, especially focusing on the evaluation data of the body fluid mode that had been newly added.
For immature cell measurement software that is incorporated in XE-5000 as a standard function, several clinical applications of the additional and of research parameters were reported.
In future, we will further explore the clinical utility of XE-5000 by combining parameter(s) of the immature cell measurement software with various new parameters yet available on the XE-5000.

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