Overview of Automated Hematology Analyzer XE-2100™

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The XE-2100 is a new automated hematology analyzer developed by Sysmex Corporation. With consideration of medical analyzer requirements for the 21st century, this unit was developed to satisfy the following concepts and to cope with diversified laboratory needs.

1) Electronic network communication

2) Presentation of high level value-added information

3) Contribution to test cost control

4) Pursuit of a user-friendly analyzer

The XE-2100 provides integrated reticulocyte analysis, previously available only in a separate device, and has the ability to enumerate nucleated red blood cells. The instrument's capacity for high throughput allows simultaneous determination of five-part white blood cell differential, nucleated red blood cell count at the rate of 150 samples per hour. These features contribute to a significant reduction in report turn-around-time (TAT).

In addition, the instrument is designed to provide remote maintenance support and on-line quality control reporting through an electronic network, thus improving laboratory management.

(Sysmex J 9 : 58 – 64, 1999)

Key Words Automated Hematology Analyzer, XE-2100, Network, Turn Around Time, Flow Cytometry

INTRODUCTION

Sysmex launched its automated multi-parameter hematology analyzers, the NE Series and SE-9000™, in 1988 and 1993, respectively. These analyzers employ a fully automated WBC 5-part differential and screening capabilities; since their release they have been used in a large number of clinical laboratories.

We launched the world's first automated reticulocyte analyzer, R-1000™, in 1988, followed by the R-3000™/ R-2000™ in 1991, and the R-3500™ and R-500™ in 1998, thus, continuing to advance in to clinical laboratory automation.

Most recently, we have developed an automated hematology analyzer XE-2100™ (***Fig. 1***) to meet the 21st century's medical demand. This was accomplished using a concept different from that used with the SE-9000.

This analyzer is capable of reticulocyte (RET) analysis, whose function has traditionally been analyzed using a separate analyzer, and also incorporates a new function for nucleated red blood cell (NRBC) analysis.

The XE-2100 has a high throughput of 150 samples per hour for blood cell counting and simultaneous WBC 5-part differential and NRBC counting. It also enables fully automated hematology analysis by means of an automatic barcode reader and a cap piercer. It contributes to a significant reduction in turn-around-time (TAT) and includes a newly added network function for being information-oriented in medical treatment in the future.

The XE-2100 is outlined below.

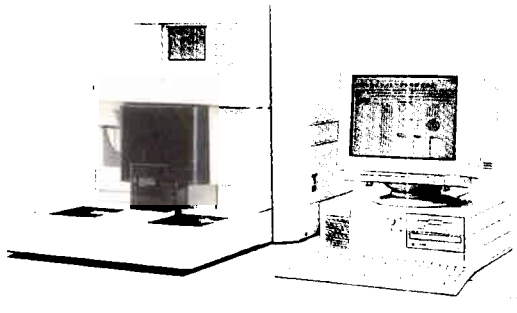

Fig. 1 *Sysmex XE-2100 automated hematology analyzer*

DEVELOPMENT CONCEPT

To meet the diversifying needs of the 21st century and create an ideal clinical laboratory analyzer, this instrument has been developed on the basis of the concepts shown below.

To meet network demands

1) Information network inside and outside of hospitals
2) Remote support using network
3) Quality control using network

To provide pertinent information

1) Introduction of NRBC analysis as a new item for measurement, to improve WBC counting accuracy with the presence of NRBC
2) Inclusion of RET analysis function
3) Improvement of platelet (PLT) analysis accuracy using two measurement methods (electric impedance and optical)
4) Inclusion of hematopoietic progenitor cell monitoring function

To reduce the cost of laboratory testing

1) Analysis time reduction by throughput improvement
2) Reagent reduction by enhancement of discrete analysis performance consumption
3) Efficient use of laboratory space through the use of a semiconductor laser for flow cytometry and consequent reduction of both system size, and power consumption

To improve user-friendliness

1) Improved operational ease by use of the WINDOWS-NT program
2) Unified data processing using a single data processing unit
3) Decrease in maintenance and simplification of maintenance procedures

TECHNOLOGY

Measurement principles

Flow cytometry using semiconductor laser

The term "cytometry" is defined as a measurement of physicochemical properties of cells and other biological particles. Flow cytometry offers measurements of cells and other particles flowing in thin streams. Generally, it detects optical information from cells or other particles flowing in a thin stream under irradiation of a laser beam. Such optical information sources include scattered light and fluorescence depending on the measurement objective.

Employing flow cytometry using a semiconductor laser, the XE-2100 detects three kinds of optical information, *i.e.*, forward scattered light, side scattered light and side fluorescence to obtain the optimum information for analysis for WBC 5-part differential, NRBC, RET, and optically measured PLT (PLT-O).

A diagram of the XE-2100 for the principle of flow cytometry using a semiconductor laser is shown in ***Fig. 2***.

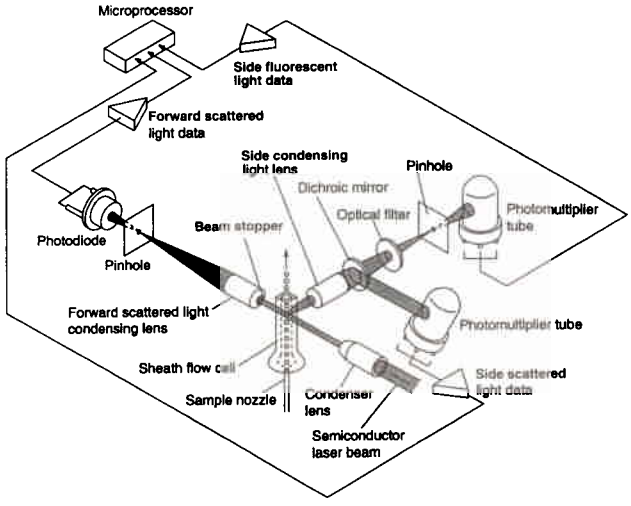

Fig. 2 *Flow cytometry method*

1) Forward scattered light

When an obstacle, such as a particle, is present in the light path, the light changes its orientation upon contact with the obstacle. This phenomenon is known as light scattering. Such scattered light is detected in order to obtain information regarding microparticle size and material. In particular, information regarding particle size can be obtained from forward scattered light. Similarly, light is scattered by the blood cells upon laser beam irradiation to the cells.

Based on the forward scattered light intensity, the XE-2100 achieves WBC/BASO measurement, NRBC measurement and RET/PLT-O measurements.

2) Side scattered light

From side scattered light, information regarding the inner components of particles can be obtained. In laser beam irradiation to blood cells, side scattered light intensity depends on intracellular structural complexity (nucleus shape, size, density, granular content). Based on this characteristic, the XE-2100 achieves WBC/BASO measurement and WBC 4-part differential.

3) Side fluorescence

Upon light irradiation, a fluorescent substance, such as a stained blood cell, emits light of a longer wavelength than that of the incident light. Fluorescence intensity increases as the degree of staining increases; thus, the measurement of fluorescent intensity provides information on the degree of blood cell staining.

The XE-2100 measures the WBC 4-part differential, NRBC, RET and PLT-O, on the basis of side fluorescent intensity of blood cells (mainly DNA and RNA) stained with fluorescent dye (polymethine dye).

RF/DC detection method

The RF/DC detection method simultaneously detects RF (radio frequency) impedance and DC (direct current) impedance changes when blood cells pass through the aperture. This occurs while both direct and radio fre-

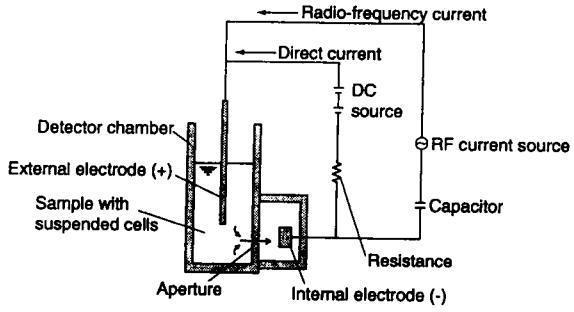

Fig. 3 RF/DC detection method

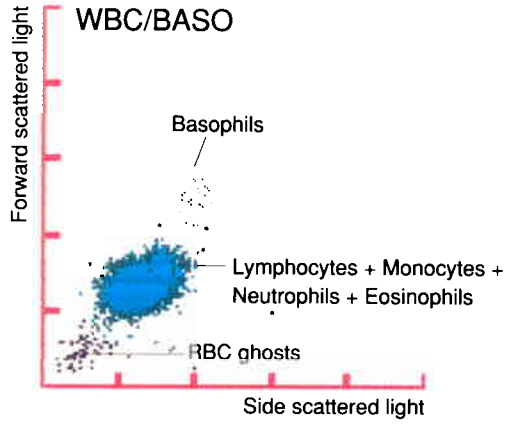

Fig. 4 WBC/BASO scattergram

quency currents being supplied to the detector (***Fig. 3***). The RF impedance change reflects intracellular information, whereas the DC impedance change is proportional to cell size (volume).

On the basis of this principle, the XE-2100 measures immature cell information in the IMI channel.

Measurement

Measurement of WBC

1) WBC/BASO

RBC are lysed with the acid hemolytic reagent STROMATOLYSER-FB. This reagent selectively suppresses the degranulation of BASO, resulting in their separation from other forms of WBC. In addition, because this reagent is strongly hemolytic and, hence, unlikely to be affected by PLT aggregation etc., it is also involved in WBC count.

After this reaction, the sample is analyzed by flow cytometry using a semiconductor laser to detect forward and side scattered light information, based on which a WBC/BASO scattergram (***Fig. 4***) is obtained. By analyzing this scattergram, WBC and basophil counts are taken.

2) WBC 4-part differential

RBC are lysed with STROMATOLYSER-4DL. At the same time, the reagent acts on the WBC membrane to allow dye passage. STROMATOLYSER-4DS (dying solution) is then added to allow the dye to enter the WBC at the damaged portion of its membrane and stain the DNA and RNA therein.

Following this reaction, the sample is analyzed by flow cytometry using a semiconductor laser to detect forward and side scattered light information, based on which a 4-DIFF scattergram (***Fig. 5***) is obtained. By analyzing this scattergram, 4-parameter counts (lymphocytes, monocytes, eosinophils, other granulocytes (neutrophils + basophils)) are taken.

3) IMI channel

Incorporated in the SE-9000, the IMI channel serves to obtain information on the presence of immature grans, blasts and hematopoietic progenitor cells. This information is used for producing IP messages (flags) and the HPC count.

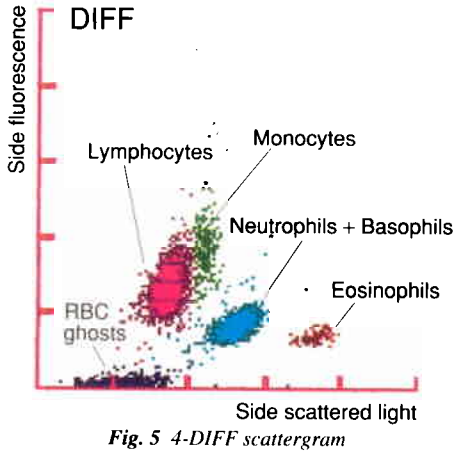

Fig. 5 4-DIFF scattergram

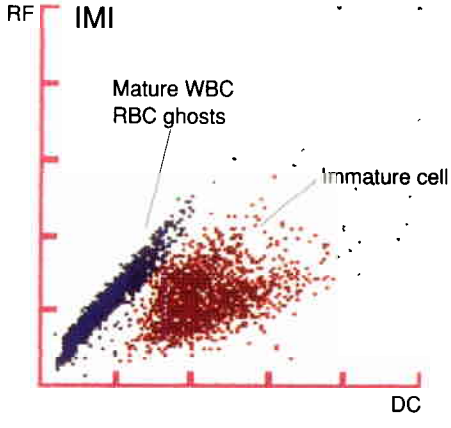

Fig. 6 IMI scattergram

RBC are lysed with STROMATOLYSER-IM, a hemolytic reagent that selectively protects immature cell, to expose the nuclei of non-immature WBC. After such reaction, the sample is analyzed by the RF/DC detection method to detect DC and RF impedance signals, based on which an IMI scattergram (***Fig. 6***) is obtained. By analyzing this scattergram, information on the onset of immature cell and hematopoietic progenitor cells is obtained.

Measurement of NRBC

RBC are lysed with the STROMATOLYSER-NR diluent to expose the nuclei of NRBC. The STROMATOLYSER-NR staining solution is also added to specifically stain WBC.

After two reaction has taken place, the sample is analyzed by flow cytometry using a semiconductor laser to detect forward scattering light and side fluorescence information, based on which an NRBC scattergram (***Fig. 7***) is obtained. By analyzing this scattergram, NRBC are counted.

Conventional hematology analyzers are incapable of discriminating between WBC and NRBC; it has been impossible to take accurate WBC counts unless data obtained are corrected with manual NRBC counts. Although the XE-2100 also does not enable differentiation between NRBC from WBC on the WBC/BASO scattergram, it is able to automatically report highly accurate WBC counts by subtracting the NRBC count obtained from the NRBC scattergram.

Measurement of RET and optical measurement of PLT

Blood is diluted with the RET SEARCH (II) diluent to a preset concentration. RET SEARCH (II) staining solution is also added to stain WBC DNA and RNA and RET RNA. This reaction is based on the same principle as our R Series.

The sample is then analyzed by flow cytometry using a semiconductor laser to detect forward scattering light and side fluorescence information, based on which an RET scattergram (***Fig. 8***) is obtained. By analyzing this scattergram, RET counts, RET ratios for individual fluorescence intensity zones (LFR, MFR, HFR), immature reticulocyte fraction (IRF) and PLT-O are determined.

The XE-2100 also achieves PLT measurement with the sheath flow DC detection method, as was done on the SE-9000. By counting a large number of platelets, this method offers increased accuracy. However, in cases where a large number of RBC fragments or large PLT are present, (*i.e.*, in samples showing cell size distributions with PLT-RBC overlaps) accuracy tends to decrease. For such samples, the PLT-O measurement ensures higher accuracy than that of the sheath flow DC detection method, because of the lower degree of overlapping associated with the principle.

Using these two measuring methods, the XE-2100 is capable of presenting PLT counts of high accuracy by adopting the results from either method that seems more accurate judging from the cell size distributions and scattergrams obtained.

Suspect messages and scattergrams

When abnormal WBC appear, the XE-2100 displays various suspect messages (Blast?, Immature Gran?, Left Shift?, Atypical Lympho?, Abn Lympho/L-Blast?, NRBC?) from the 4-DIFF scattergram pattern changes in addition to IMI scattergram pattern changes like the SE-9000.

Abnormal cell locations on IMI and 4-DIFF scattergrams are shown in ***Fig. 9***. Because the newly introduced 4-DIFF scattergram detects intracellular DNA and RNA information by side fluorescence, abnormal cells having higher RNA contents than those of normal cells are detected at positions of higher fluorescence intensities on the scattergram. This ensures specific detection of abnormal cells.

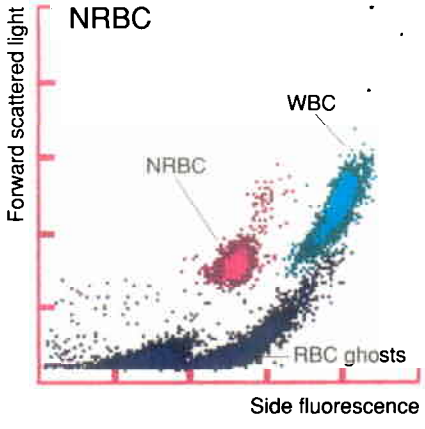

Fig. 7 *NRBC scattergram*

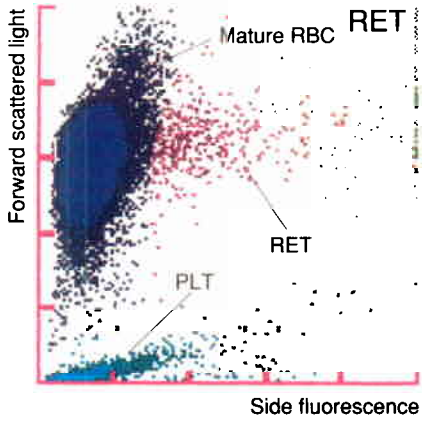

Fig. 8 *RET scattergram*

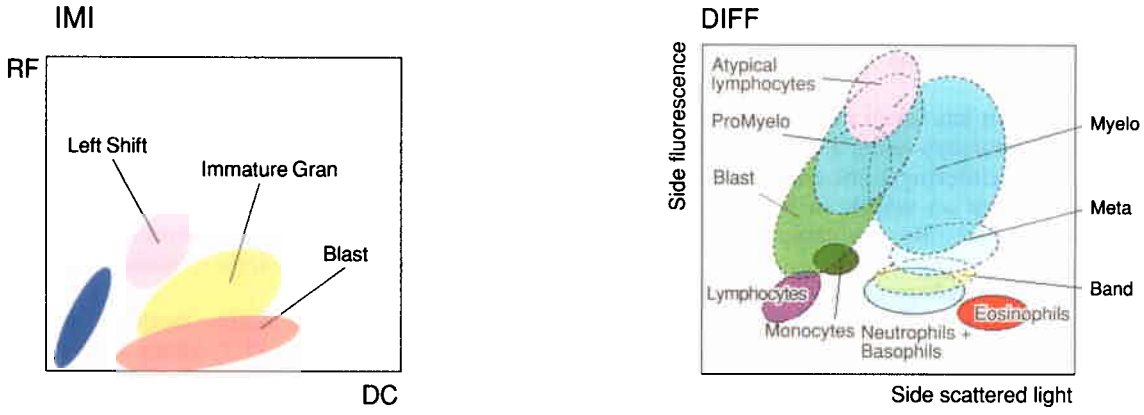

Fig. 9 Abnormal cell locations on IMI and 4-DIFF scattergrams

Table 1 *Reproducibility*

		WBC ($\times10^2/\mu L$)	RBC ($\times10^4/\mu L$)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT ($\times10^4/\mu L$)	NEUT (%)	LYMPH (%)	MONO (%)	EO (%)	BASO (%)	RET (%)	PLT-O ($\times10^4/\mu L$)			NRBC (%)
	Mean	44.6	443.8	13.7	40.5	91.3	30.9	33.8	23.1	67.3	21.0	6.3	4.3	1.1	0.9	21.6		Mean	0.7
1	SD	0.77	2.94	0.06	0.25	0.16	0.28	0.31	0.32	0.99	0.97	0.64	0.32	0.21	0.06	0.67	6	SD	0.06
	CV(%)	1.72	0.66	0.41	0.61	0.18	0.91	0.91	1.40	1.47	4.62	10.11	7.49	19.49	6.91	3.14		CV(%)	8.33
	Mean	39.5	396.5	13.2	38.2	96.4	33.3	34.5	18.7	54.0	29.8	8.7	5.3	2.2	0.9	19.1		Mean	89.6
2	SD	0.68	1.72	0.06	0.16	0.16	0.23	0.22	0.36	0.97	1.11	0.90	0.38	0.27	0.07	0.54	7	SD	6.26
	CV(%)	1.72	0.43	0.46	0.42	0.16	0.70	0.65	1.91	1.79	3.72	10.43	7.10	12.49	7.80	2.80		CV(%)	6.98
	Mean	44.0	452.8	14.9	42.2	93.2	32.8	35.2	23.9	55.8	32.2	6.7	3.4	1.9	1.0	23.1		Mean	0.8
3	SD	0.78	1.62	0.06	0.13	0.10	0.17	0.16	0.22	0.79	0.58	0.63	0.37	0.25	0.05	0.59	8	SD	0.11
	CV(%)	1.77	0.36	0.40	0.30	0.10	0.51	0.45	0.93	1.41	1.80	9.41	10.81	12.70	4.75	2.56		CV(%)	13.79
	Mean	44.6	413.0	12.6	38.3	92.7	30.5	33.0	24.7	64.6	24.0	5.7	4.5	1.2	1.0	24.5		Mean	5.0
4	SD	0.62	1.89	0.04	0.16	0.12	0.19	0.21	0.38	1.05	0.95	0.42	0.29	0.15	0.07	0.35	9	SD	0.31
	CV(%)	1.39	0.46	0.34	0.42	0.13	0.62	0.62	1.53	1.63	3.99	7.51	6.51	12.15	7.15	1.42		CV(%)	6.19
	Mean	47.3	382.5	12.4	36.8	96.1	32.4	33.6	25.4	54.7	31.6	5.1	7.6	1.0	0.7	25.1		Mean	1.6
5	SD	0.73	1.58	0.07	0.17	0.13	0.20	0.20	0.36	1.04	1.02	0.40	0.26	0.12	0.07	0.34	10	SD	0.21
	CV(%)	1.55	0.41	0.53	0.46	0.13	0.62	0.60	1.43	1.89	3.23	7.83	3.39	12.05	9.42	1.37		CV(%)	13.19

Exemplification during developmental stage

To exemplify the assessment of the XE-2100 during developmental stage, example assessment data on its reproducibility are shown in ***Table 1***.

For the parameters other than NRBC, simultaneous reproducibility data were obtained from 10 consecutive runs for human blood. The data on NRBC concern with simultaneous reproducibility for samples in which NRBC appeared.

Good reproducibility was obtained for all items examined.

Data processing unit

To add network capability and improve operational ease, the XE-2100 employs the WINDOWS-NT operating system for the data processing unit. Also, easy-to-use menu icons and tabs enabled the user to directly choose the desired task on the menu.

Fig. 10 shows the main page of the data presentation menu (data browser). In the upper portion of the screen are arranged menu icons, such as quality control and worklist, clicking the appropriate icon enables the user to choose the desired menu. In addition, the data presentation screen allows screen switching by clicking tabs (Main, Graph, WBC and other tabs arranged on the screen).

Fig. 11 shows the screen appearing when the Graph tab is clicked.

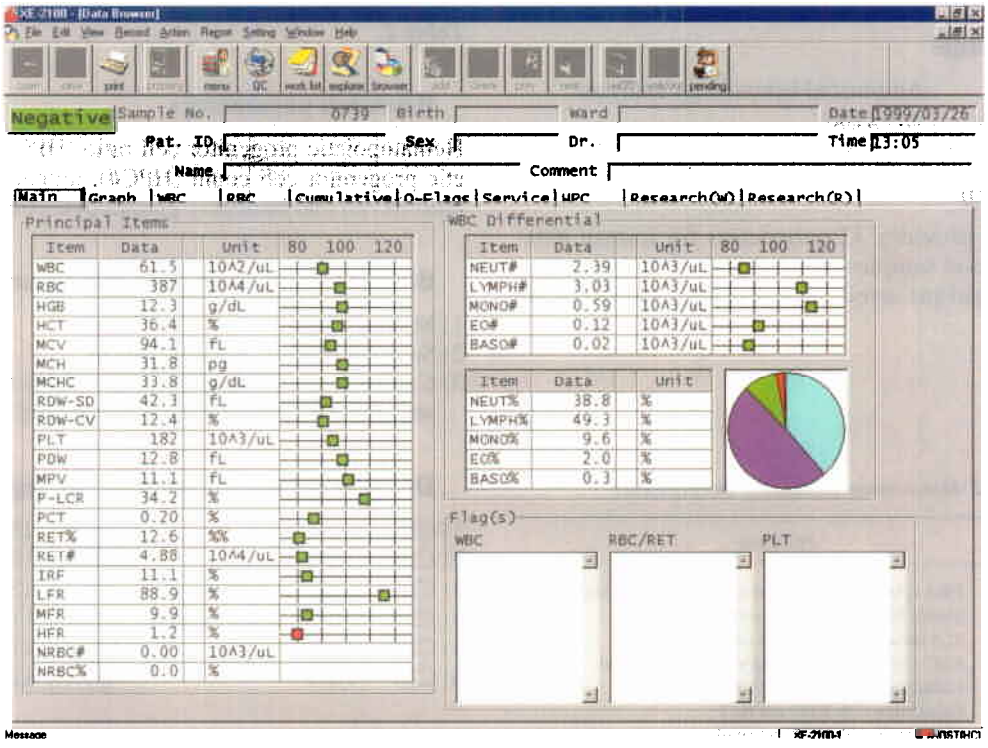

Fig. 10 *Main page of the data presentation menu (data browser)*

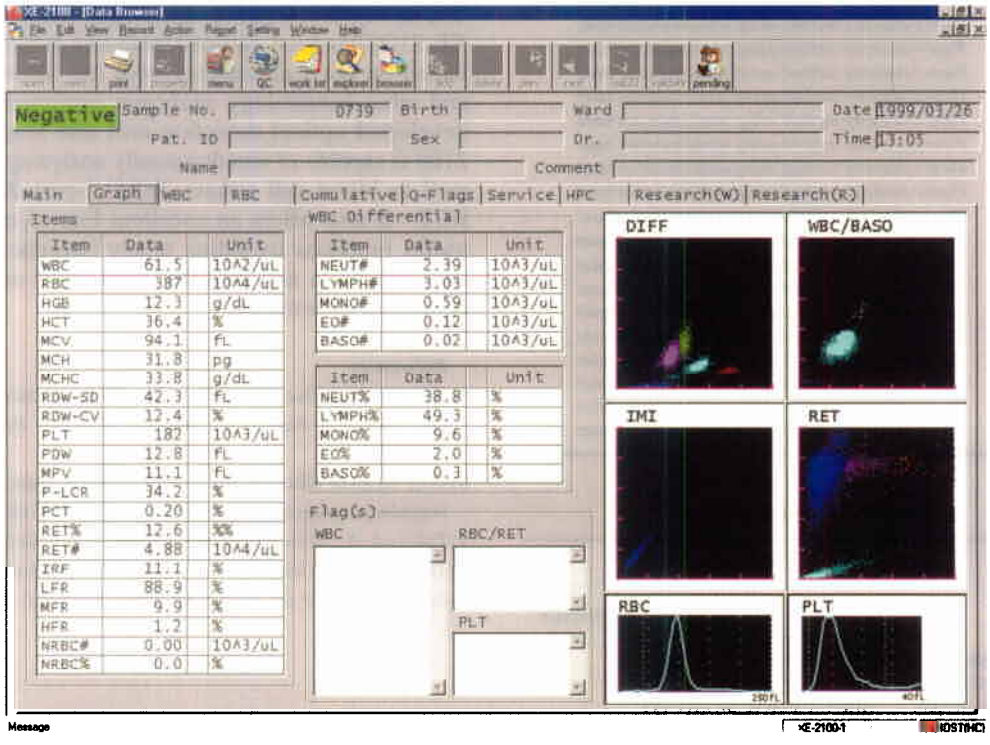

Fig. 11 *Graph page of the data presentation menu (data browser)*

PRINCIPAL SPECIFICATIONS

Product name

1) Name : Automated hematology analyzer
2) Model name : XE-2100

Application

The XE-2100 provides 32 parameters for human anticoagulated blood samples.
The anticoagulant used is K_2EDTA, K_3EDTA or Na_2EDTA.

Table 2 Measurement parameters and principles

Parameters	Principles
WBC	Flow cytometry method using semiconductor laser
RBC	Sheath flow DC detection method
HGB	SLS hemoglobin detection method
HCT	RBC cumulative pulse height detection method
MCV	Calculation with RBC and HCT
MCH	Calculation with RBC and HCT
MCHC	Calculation with HCT and HGB
PLT	Sheath flow DC detection method or Flow cytometry method using semiconductor laser
RDW-SD	Analysis from size distribution of RBC
RDW-CV	Analysis from size distribution of RBC
PDW	Analysis from size distribution of PLT
MPV	Analysis from size distribution of PLT
P-LCR	Analysis from size distribution of PLT
PCT	PLT cumulative pulse height detection method
NEUT%	Flow cytometry method using semiconductor laser
LYMPH%	Flow cytometry method using semiconductor laser
MONO%	Flow cytometry method using semiconductor laser
EO%	Flow cytometry method using semiconductor laser
BASO%	Flow cytometry method using semiconductor laser
NRBC%	Flow cytometry method using semiconductor laser
NEUT#	Flow cytometry method using semiconductor laser
LYMPH#	Flow cytometry method using semiconductor laser
MONO#	Flow cytometry method using semiconductor laser
EO#	Flow cytometry method using semiconductor laser
BASO#	Flow cytometry method using semiconductor laser
NRBC#	Flow cytometry method using semiconductor laser
RET%	Flow cytometry method using semiconductor laser
RET#	Flow cytometry method using semiconductor laser
HFR	Flow cytometry method using semiconductor laser
MFR	Flow cytometry method using semiconductor laser
LFR	Flow cytometry method using semiconductor laser
IRF	Flow cytometry method using semiconductor laser

Table 3 Reagents

Reagents	Parameters
CELLPACK	Diluent, Sheath solution for flow cytometry
CELLSHEATH	Sheath solution for RBC/PLT
STROMATOLYSER-FB	WBC, BASO
STROMATOLYSER-4DL	NEUT, LYMPH, MONO, EO
STROMATOLYSER-4DS	NEUT, LYMPH, MONO, EO
STROMATOLYSER-NR	NRBC
SULFOLYSER	HGB
STROMATOLYSER-IM	IMI
RET SEARCH (II)	RET, PLT-O

Parameters and principles

The measuring parameters and principles are shown in ***Table 2***.

Research parameters

Hematopoietic progenitor cell ratio (HPC%), hematopoietic progenitor cell count (HPC#), immature granulocyte ratio (IG%) and immature granulocyte count (IG#)

Blood sample volume requirements

1) Manual mode : Approx. 130 μL
2) Sampler mode : Approx. 200 μL
3) Capillary mode : Approx. 40 μL (Only 8 CBC parameters and RET are measured in capillary mode)

Discrete measurement and throughput

1) CBC : Approx. 150 samples/hour
2) CBC + DIFF : Approx. 150 samples/hour
3) CBC + DIFF + NRBC : Approx. 150 samples/hour
4) CBC + DIFF + NRBC + RET : Approx. 113 samples/hour
5) CBC + DIFF + RET : Approx. 113 samples/hour
6) CBC + RET : Approx. 113 samples/hour

Reagents

Information for the reagents is shown in ***Table 3***.

CONCLUSION

The newly developed an automated hematology analyzer XE-2100 has been outlined including actual assay data. Based on our technologies from the SE-9000, R Series, and other hematology analyzers equipped with a newly developed optical detection unit and reagents, the XE-2100 is capable of simultaneously analyzing for NRBC, as well as blood cell counts, WBC 5-part differential and RET. It also offers an excellent line of many clinically useful functions including newly introduced parameters, easy operation and provides high value-added measurements and information.

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