

Feature Article
特集論文

Pentra 120 Retic Hematology Analyzer
Principles of Analysis and Clinical Utility

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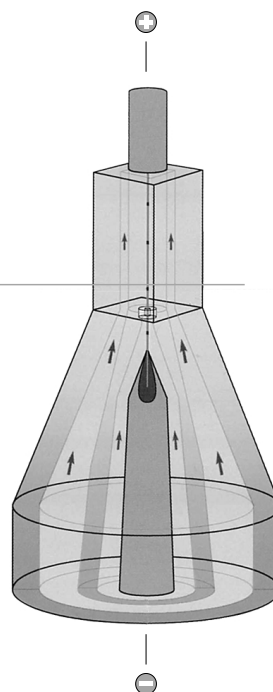


Pentra120

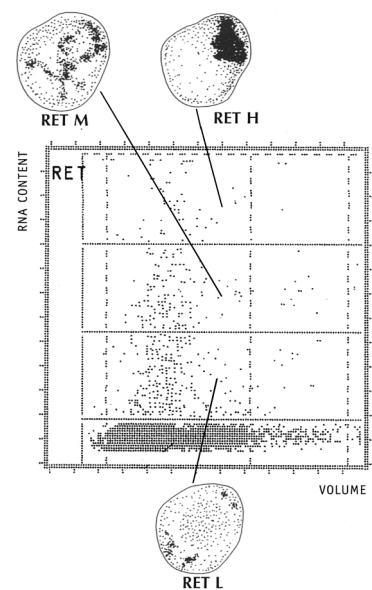
LIGHT ABSORBANCE
FOCUSED-FLOW
IMPEDANCE

Principles of Analysis

Double Hydrodynamic
Sequential System
(DHSS)



Results of Analysis



要旨

ABX社のPentra 120 Reticは血液分析計の最上位機種で、高い生産性と新しい機能を備えている。本装置は、細胞化学法と組合せて集光型フローインピーダンス法と光学吸収法により白血球分類を、集光型フローインピーダンス法と蛍光法により網赤血球をそれぞれ分析する。本装置は、高い測定精度を持っており、血液検査室に新たな視点を持ち込む血液診断装置である。白血球分類(LMNE matrix)から得られるLICとALYは、検査室の能力の拡大、とりわけ悪性血液性諸症の診断に有効である。新しい網赤血球指標である平均蛍光指数(MFI)と平均網赤血球容積は、化学療法や造血細胞移植手術を受けた患者の経過診断をするのに大いに助けとなる。

<Abstract>

The ABX Pentra 120 Retic is a top-of-the-range hematology analyzer associating high productivity and new analytical capabilities. The association of cytochemistry, focused flow impedance and light absorbance for the leukocyte differential analysis along with focused flow impedance and fluorescence quantification for the reticulocyte analysis gives an excellent accuracy to the analyzer as well as new insights into the blood cell biology to the laboratory. The enumeration of Large Immature Cells (LIC) and Atypical Lymphocytes (ALY) provided by the LMNE analysis increases the laboratory analytical capability, especially for the diagnosis of malignant hematological disorders. The new reticulocyte parameters Mean Fluorescence Index (MFI) and Mean Reticulocyte Volume are interesting tools for the hematologist in the follow-up of patients undergoing chemotherapy and/or hematopoietic stem cell transplantation.

1. Introduction

One strong trend outlines the high-end segment of hematology analyzers : the enhanced productivity for the laboratory. To achieve this goal, all major IVD companies have designed fully-automated hematology analyzers with Complete Blood Cell Count (CBC) combined with five-part differential (5DIFF) and fully-automated reticulocyte analysis (RET) capabilities. Among the various technologies which are currently available for these analysis, the ABX Pentra 120 Retic uses the most referenced and complementary ones : impedance for CBC, impedance with cytochemistry (Eosinofix™ containing Chlorazol Black E, and Basolyse™) and light absorbance measurement for 5DIFF, and impedance combined with fluorescence intensity quantification (following the blood cell incubation with RETIX™, containing the fluorescent probe Thiazole Orange) for RET. These features allow the Pentra 120 Retic to provide the laboratory with the most up-to-date hematological parameters, at a 120 samples per hour throughput. In this paper, we will focus on the unique aspects of the Pentra 120 Retic, i.e. 5DIFF and RET analysis.

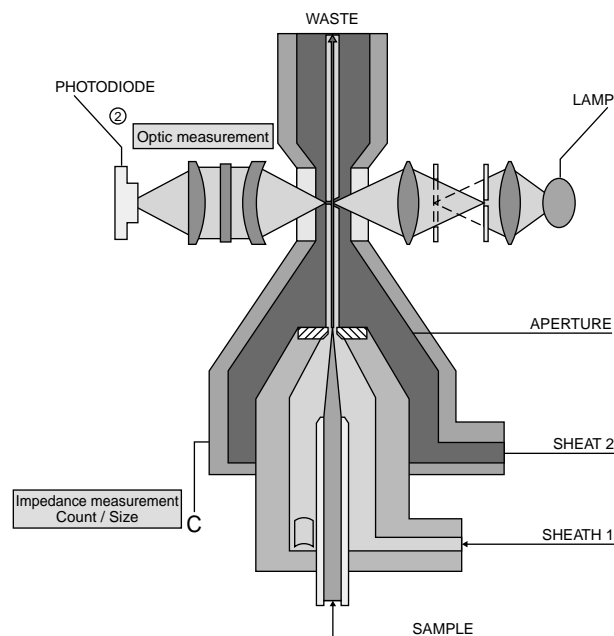


Fig.1 DHSS technology

2. Principles of analysis

2.1 5DIFF Analysis

The ABX Pentra 120 Retic carries out the 5DIFF analysis by using two different channels : the LMNE channel with the Eosinofix reagent for Lymphocytes, Monocytes, Neutrophils and Eosinophils, and the BASO channel with the Basolyse reagent for the basophil count. The Eosinofix reagent contains Chlorazol Black E. Following the action of Eosinofix, the analysis of leukocytes is carried out in a flow-cytometer by using the Double Hydrodynamic Sequential System technology (DHSS) (Fig. 1).

The Eosinofix reagent has three properties :

Red blood cell analysis

Leukocyte membrane fixation (inducing a post-draw stability up to 48 hours)

Differentiation of leukocytes with intracytoplasmic grains, according to Chlorazol Black E principle.

Then, each cell undergoes two different measurements differentiating and quantifying the five normal sub-populations of leukocytes :

Measurement of the cell volume (impedance variation principle),

An analysis of the cellular complexity (light absorbance principle).

< 全自動血液診断装置 Pentra 120 Retic >

1. はじめに

ABXの Pentra 120 Reticは、全血測定(CBC)はインピーダンス法で、白血球5分類には光吸収法で、そして網赤血球(RET)はインピーダンス法とレーザー蛍光法を組合せて、それぞれ分析する。これにより、120検体/時 という驚異的な処理速度を実現した。本稿では 検査室の生産性を格段に向上させている血液検査装置の最先端動向について述べる。

2. 分析原理

2.1 白血球5分類

白血球の測定系は2系統に分かれている。LMNEチャンネルではEosinofix™試薬を用いてリンパ球,単球,好中球,好酸球を、パゾチャンネルでは Basolyse™試薬を使い、DHSS用いたフローメトリー法で好塩基球を測定する(Fig.1)。

From these two measurements, a matrix (cell volume = x-axis /cellular complexity = y-axis) is obtained which gives the quantification of Lymphocytes, Monocytes, Neutrophils and Eosinophils. This matrix is the LMNE matrix (Figure 2). Due to its high resolution, the DHSS technology allows the instrument to detect and quantify two additional and pathological leukocyte subsets : LIC (Large Immature Cells) and ALY (Atypical Lymphocytes) (Table 1).

Table 1 LIC and ALY value taken by Pentra120 and Manual method

	LIC		ALY	
	Manual	PENTRA 120	Manual	PENTRA 120
TN	368	344 (93.5%)	395	372 (94.2%)
FN		24 (6.5%)		23 (5.8%)
TP	54	48 (88.9%)	27	24 (88.9%)
FN		6 (11.1%)		3 (11.1%)
Specificity	93.5%		94.2%	
Sensitivity	88.9%		88.9%	
NPV	98.3%		99.2%	
PPV	66.7%		51.1%	

The presence of LIC or ALY was checked by examining blood smears (reference method). Then, the corresponding samples were analyzed through the Pentra 120 Retic and the presence or absence of LIC or ALY was compared to the reference method results. The clinical sensitivity of LIC and ALY counting is then deduced from false negative, false positive, true negative and positive rates.

The Large Immature Cells (LIC) made up of immature granulocytes and large blasts....

The Atypical Lymphocytes (ALY) made up of large lymphocytes, stimulated lymphocytes, lymphoid cells

of the lymphoproliferative chronic syndromes other than the CLL small lymphocytes, and small sized blasts.

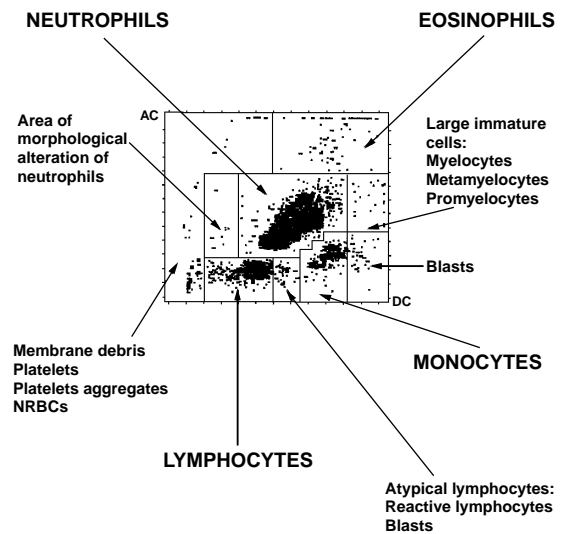


Fig.2 LMNE matrix

Eosinophils are located in the upper part of the optic axis because of their greater absorbance. Mature neutrophils, because of the presence of granules in their cytoplasm and their nucleus usually segmented, will absorb more light than immature granulocytes. Monocytes, large sized cells with a kidney-shaped nucleus, have a large cytoplasm with few granules and therefore diffract and absorb little light. This positions them towards the lower end of the optic axis and to the right of the volume axis. Lymphocytes, small sized cells of regular shape, are to be found at the lower end of the optic axis and to the left of the volume axis.

Basophils are detected in a specific channel. In this channel, blood cells are incubated with Basolyse™, which destroys their membranes with the exception of the basophils. Basophils are counted by impedance.

Eosinofix™試薬は1)赤血球の分析,2)白血球の細胞膜の固定,3)白血球の分類の三つの機能を持っている。それぞれの細胞は,2つの異なる測定が行われた後,分類し定量される。白血球は,細胞容積の測定(インピーダンス変化法)および細胞の透過度の測定(光学吸収法)の二種類の測定後,分類し定量分析される。細胞の大きさをX軸に,透過度をY軸にして得られたマトリックス(Fig.2)からリンパ球,単球,好中球,好酸球を分別定量する。この装置は分解能が高いため,さらに2つの特異的白血球である大型幼若細胞(LIC)と異型リンパ球(ALY)を検出することができる(Table 1)。好塩基球は,Basolyse™試薬により好塩基球以外の細胞膜を破壊し,インピーダンス法で計数される。

2.2 Reticulocyte analysis

Reticulocyte analysis is fully automated and performed in primary tubes by using the fluoro-flow cytometry reference dye : Thiazole Orange (TO).

0.8 μ L of the sample from the sampling chamber is incubated for 25 seconds at 35°C with 2.5 ml of Retix™ (the TO-containing proprietary reagent). The sample is injected into the fluoro-flow cytometer using the DHSS technology (ABX patent)

Two measurements are carried out :

- Cell volume by impedance variation
- Fluorescence at 90°

The fluorescence is detected and measured (green fluorescence at 530 nm) after stimulation with the Argon ion laser at 488 nm. Up to 32,000 cells are counted (Fig.3).

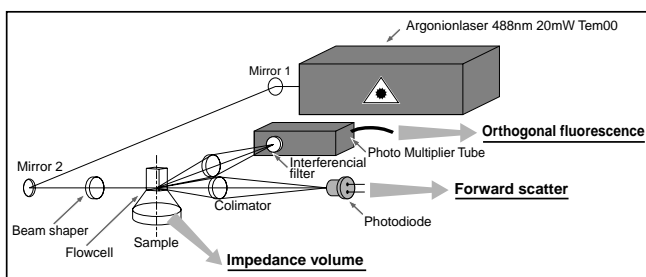


Fig.3 Measurement Principles of reticulocyte

The 2 parameters are plotted on the RETIC matrix (Fig.4).

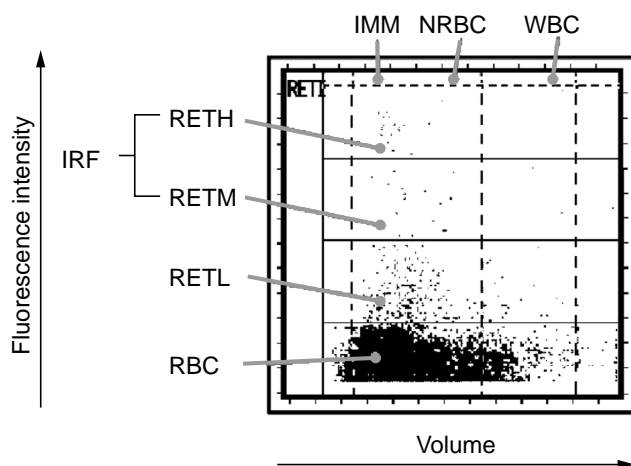


Fig.4 Reticulocyte Matrix

The x-axis represents the cellular volume and the y-axis the fluorescence intensity of each cell. The sensitivity of the reading gives the distinction between mature red blood cells and reticulocytes and differentiates the reticulocyte maturation classes.

The Pentra 120 Retic provides one 10 parameter reticulocyte analysis; including :

(1) Reticulocyte counting parameters

Red Blood Cells (RBC) Reticulocyte count in % (RET%), Reticulocyte count in absolute (RET#), Immature Cells in % (IMM%), Corrected Reticulocyte Count in % (CRC%),

(2) Reticulocyte Maturation parameters

Maturation Classes [Reticulocytes with low RNA content (RETL%), medium RNA content (RETM%), high RNA content (RETH%)], Immature Reticulocyte Fraction (IRF), Mean Fluorescence Index (MFI%) and Mean Reticulocyte Volume (MRV).

2.2 網赤血球分析

網赤血球は、0.8 μ l のサンプルに、チアゾールオレンジを含んだ試薬 (Retix™) 25ml を加えて 35 °C で 25 秒間保持後、フローサイトメータで蛍光分析する (Fig.3)。最高 32,000 個まで計数できる。

Fig.4 に、網赤血球のマトリックス (Retic matrix) を示す。

Pentra は網赤血球分析の 10 種類のパラメータを供給する。

- (1) **網赤血球数指標** : 赤血球数, 網赤血球数%, 網赤血球絶対数, 未成熟細胞%, ヘマトクリット補正した網赤血球数%
- (2) **網赤血球成熟度指数** : 成熟度分類「RETL%, RETM%, RETH%」, 成熟度指数 (IRF), 平均蛍光指数 (MFI), 平均網赤血球容積 (MRV)

3. Clinical utility of the PENTRA 120 RETIC

3.1 5DIFF Analysis :

Besides the usual 5DIFF parameters, ALY and LIC enumeration capabilities of the Pentra 120 Retic are extremely useful for diagnosing malignant blood cell disorders, as exemplified in Table 1.

Fig.5 are shown three examples of the ABX LMNE analysis on the Pentra 120 (DHSS and MDSS technology) with samples from patients with acute leukemia. Note the good

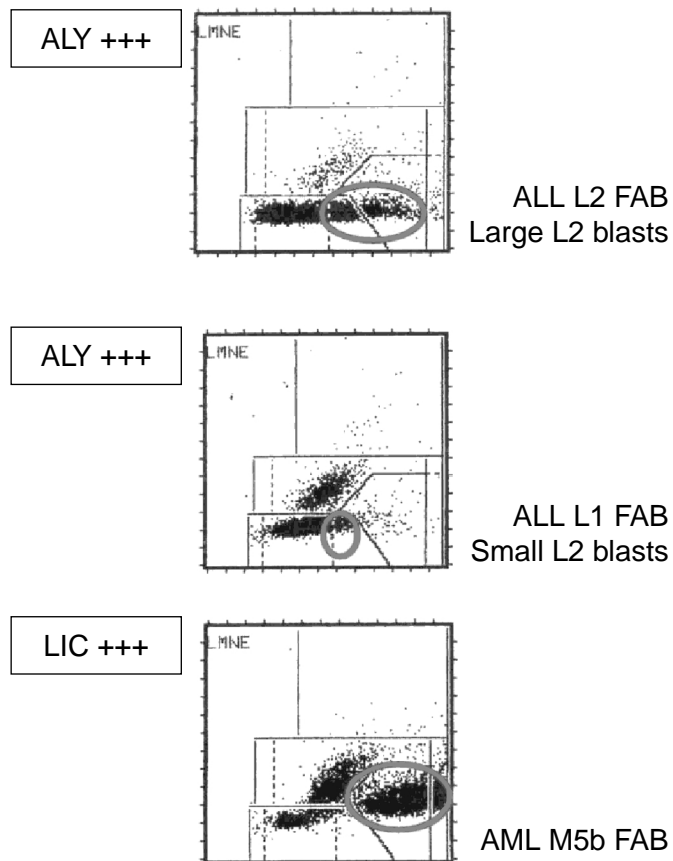


Fig.5 Effective screening using the 5-part diff. part analysis

correlation between the type (cell volume and granules) of blasts and the cell positioning on the LMNE Matrix. Furthermore, the alarms provided by the LMNE analysis combined with the reflex testing capability of the analyzer enhance the validation process for the laboratory, eliminating useless blood smear reviews.

3.2 Reticulocyte analysis :

Correlation between the New Methylene Blue method (NCCLS reference method) and the Pentra 120 Retic RET% values is excellent ($r = 0.95$). The clinical utility of the Pentra 120 Retic reticulocyte analysis has been demonstrated by several studies. Briefly, the CRC% is useful in improving the accuracy of the anemia classification (Fig.6).

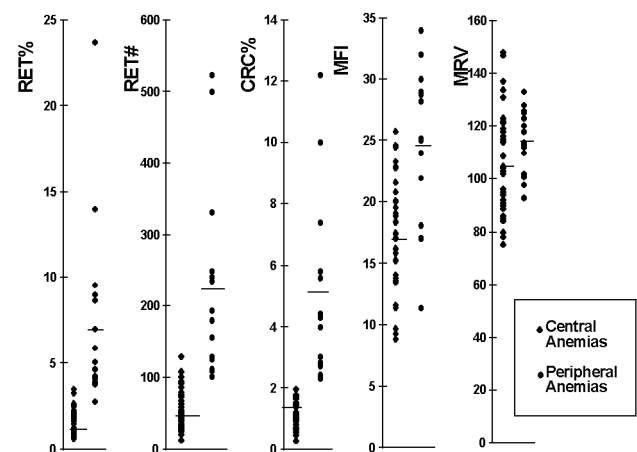


Fig.6 Accuracy of CRC% for the diagnosis of anemia

The comparison of reticulocyte parameters in anemic patients show that CRC% is the most accurate parameter for the differentiation between non regenerative and regenerative anemia.

3. 臨床応用

3.1 白血球5分類

ALYとLICの分画機能は、悪性血液性諸症の診断に特に有効である (Table 1)。急性白血病患者の検査例を Fig.5 に示すが、細胞の種類と位置の相関が良くとれている。さらに、反射作用検査と組合せた LMNE 分析により、検査の有効性が高まり、血液顕微鏡検査も省くことができる。

3.2 網赤血球分析

Pentra の RET% とメチレンブルー法の相関は良くとれており ($r=0.95$)、貧血症の分類の精度向上にも役立つ (Fig.6)。

さらに、Pentra 独自の2つパラメータ MRV と MFI は、骨髄移植患者の造血能力評価に有効で、化学療法や造血細胞移植手術を受けた患者の経過診断に推奨できる (Fig.7)。

Furthermore, two original Pentra 120 Retic reticulocyte parameters, MFI% and MRV, are useful for the early prediction of the hematopoietic recovery following bone marrow transplantation (Fig.7). Due to their excellent precision, MRV and MFI can be recommended as the first-line parameters for the follow-up of patients undergoing chemotherapy and/or hematopoietic stem cell transplantation.

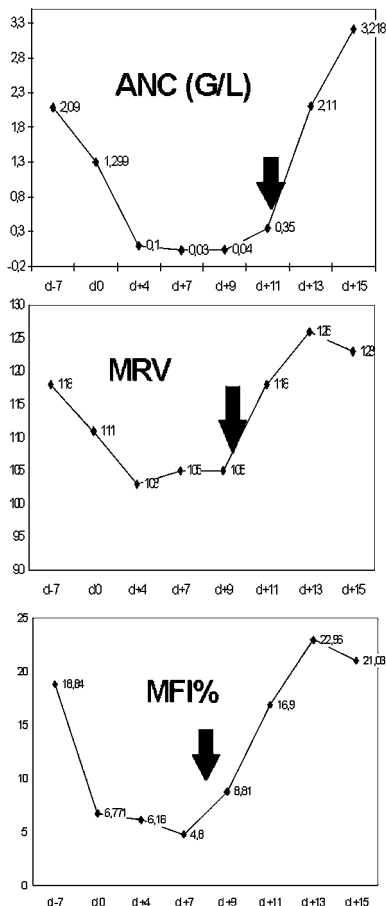


Fig.7 Hematopoietic recovery following bone marrow transplantation

MRV and MFI parameters are the earliest parameters for the appraisal of the hematopoietic recovery in bone marrow transplanted patients.

4. CONCLUSION

The ABX Pentra 120 Retic represents a real improvement for the laboratory. By combining user-friendliness, high throughput, total automation and proven high analytical capabilities, this high-end segment hematology analyzer illustrates what laboratory hematology has become : improved productivity with higher diagnostic performances. This strong trend was forestalled by ABX as demonstrated by the world wide success of the Pentra 120 range of hematology analyzers.



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4. おわりに

Pentra 120 Reticは、使い易く、処理速度が速く、完全に自動化されており、その上高い分析能力を備えた最高の血液検査装置である。世界中の検査室の生産性改善と診断能力の向上にお役に立つものと言えよう。