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HORIBA Technical Reports

特集 量から質へ臨床検査

March 1996 ■ No.12

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## The Franco-Japanese Co-Development of the Exclusive LC-220 Automated Blood Cell Analyzer

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# The Franco-Japanese Co-Development of the Exclusive LC-220 Automated Blood Cell Analyzer

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## <Abstract>

Horiba and ABX designed and co-developed the LC-220 Haematology Analyzer based on a simple basic operation for doctor's offices with 5 to 10 samples per day. The LC-220 can analyze the four parameters ; WBC, RBC, and Hct using electrical impedance method, Hgb using spectrophotometry. The concept of the system requires minimum knowledge of operation and very few maintenance.

## 1. History

Tests of Haematology are often used worldwide by laboratory technologists. Haematology refers to the study of human blood cells in term of quantity and quality. Quantity of cells is given as a concentration or number of cells per liter of whole blood. Quality of cells refers to their shape, size, and aspect.

Alteration of those two characteristics will diagnose many kinds of diseases or pathologies detected with the help of the microscope: The sampling is diluted and placed on a platform (Neubauer Haemocytometer) of ruled areas composed of 9 squares of 3 millimeters of equal size. Counting the cells is long, difficult and requires some specific skills and training. This method is still used as reference or confirmation of the pathology detected with an Automated Haematology Analyzer. Most of this alterations have been detected with the development of the Automated

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## LC-220 全自動血球計数装置の日・仏共同開発

### 1. 開発の経緯

血液学とは血液細胞を量と質の両面から研究する学問で、量とは血液中の血球数を、そして、質とは血球の形状や大小を対象とする。そして、この量と質とを測ることにより、種々の疾病の診断が可能となる。

血球計数は、希釈された血液を計算盤にのせ、顕微鏡にて計測することが基準であるが大変な手間がかかり、しかも熟練を必要とする。

1950年代に自動血球計数装置が開発されたが、当初は血液をあらかじめ希釈しておくなど手間のかかるものであった。

このたび(株)堀場製作所とABX社とが共同開発した自動血球計数装置LC-220(図1)は、診療所の医師が、1日5~6個の検体を処理するのに適した装置で、

\* ABX S.A.



Fig.1 Automated blood cell analyzer LC-220

Haematology Blood Cell Analyzer since the fifties. The first cell counters were semiautomated: the technologists had to per-dilute the sample before aspiration into the analyzer inducing extra time. Progressively, the technology was adapted to run the sample directly on whole blood right after the collection. The LC-220 is one fully automated system including automatic dilution and automatic rinsing with simple basic technology and few parts.

Horiba and ABX designed and co-developed the LC-220 Haematology Analyzer (Fig.1) based on a simple basic operation for doctor's office with 5 to 10 samples per day. The concept of the system requires minimum knowledge of operation and very few maintenance. The specifications of LC-220 and summarized in the Table 1.

Parameters:	WBC,RBC,HGB,HCT.
Measuring Principles:	Aperture-Impedance for WBC,RBC,and HCT Size of aperture:80 micrometers Cyanmethemoglobin for HGB at 555 nm LED
Sample Volume:	10 microliters Dilution ratio for WBC at 1/240 Dilution ratio for RBC at 1/30,000
Throughout	30 samples per hour
Reproducibility:	WBC: ≤ 3.0% at $10 \times 10^9/\mu\text{l}$ RBC : ≤ 2.0% at $5 \times 10^9/\mu\text{l}$ Hgt : ≤ 1.5% at g/l Hct : ≤ 2.0% at 45%
Linearity:	WBC: 0.5 to $80.0 \times 10^9/\mu\text{l}$ : $< \pm 0.3$ or $\pm 2.5\%$ RBC : 0.5 to $7.5 \times 10^9/\mu\text{l}$ : $< \pm 0.07$ or $\pm 2.0\%$ Hgb : 2.0 to 25.0 g/l : $< \pm 0.3$ or $\pm 2.0\%$ Hct : 10.0 to 59.9 % : $< \pm 2.0$ or $\pm 3.0\%$ 60.0 to 70.0 % : $< \pm 5.0$ or $\pm 5.0\%$
Carryover:	WBC: <2% RBC : <0.5% Hgb : <2% Hct : <2%
Display:	LED:Sample No. (1-99) Results (WBC,RBC,Hgb,Hct) States (Measuring,Ready,Operating Error)
Memory:	Last result only
Operating Temperature:	1.5-30 degrees Celcius
Humidity:	25-80%
Power Supply:	100V-50/60 Hz
Option:	External printer or RS232C

Table .1 LC-220 performance specifications

自動希釈や自動洗浄などが内蔵された容易に操作できる装置である。  
LC-220の主な仕様を表 1 に示す。

## 2. WBC, RBC, Hgb, Hctの測定手法

採血した血液は、まず抗凝固剤 (EDTA) を加えて凝固を防止する。

次に、正常な検体の場合には、赤血球 (RBC) が  $5,000,000$  個/ $\text{mm}^3$ 、白血球 (WBC) が  $7,000$  個/ $\text{mm}^3$  と高濃度のため等張性希釈液で希釈する。

LC-220は二段階の希釈をする。第一の希釈の後、溶血剤を加えて、1/240に希釈し、WBCの計数とヘモグロビン量 (Hgb) を測定する。

二回目の希釈比は1/30,000で、この試料を使ってRBCと、ヘマトクリット (Hct) すなわち赤血球容積比 (PCV) を測定する。

## 2. How do we determine the four parameters: WBC, RBC, Hgb, and Hct ?

Blood sample is collected from the vein of the arm into a tube and mixed with an anticoagulant (EDTA) to prevent coagulation. The normal concentration of the Red Blood Cell (RBC) or Erythrocytes is about 5,000,000 per  $\text{mm}^3$ . Normal White Blood Cells (WBC) or Leukocytes' Concentration is about 7,000 per  $\text{mm}^3$ . It will be then necessary to dilute this concentration to count the cells.

The reagent for dilution is composed of a filtered isotonic solution similar to a physiological serum. The LC-220 performs two dilutions successively. The final ratio for the first dilution is 1/240 including 0.4 ml of lysing agent.

This lysing agent is a solution derived from the Saponine and has two purposes: The potassium cyanide will free the hemoglobin from the RBC's cytoplasmic membrane to form the chromogenous cyanmethemoglobin compound. This compound is then measured by spectrophotometry through the optical part of the unique chamber with a wave length of 555 nm. The optical density is proportional to the concentration of hemoglobin.

This first dilution will then be used to count the WBC's and measure the Hemoglobin. The final ration for the second dilution is 1/30,000.

The second dilution will be used to count the RBC's and the Hematocrit or Packed Red Cell Volume (PCV).

## 3. Counting principle

The LC-220 presents the exclusive technical performance of counting the white and red blood cells in one unique chamber.

The counting principle is based on an impedance variation generated by the passage of cells (non conductive particle) through a calibrated micro-aperture of 80 micrometers. The sample is suspended in an electrolytic diluent (current conductor). The conductivity of the diluent differs considerably from the conductivity of the cells. This dilution is aspirated through the calibrated micro-aperture. Two electrodes are placed on each side of the aperture. Electric current passes through the electrode continuously. When the cell passes one by one through the aperture, it displaces its own volume of electrolyte. Electric resistance (or impedance) between the two electrodes increases proportionally with the cell's volume.

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### 3. 測定原理

計数原理は、血液細胞が $80\mu\text{m}$ の微細孔(アパーチャー)を通過したときに生じるインピーダンス変化の測定に基づいている。アパーチャーの両側には1対の電極が設けられており、非導電性の血液細胞がアパーチャーを通過すると細胞の体積に比例してインピーダンスの大きさ(パルスハイト)が変化する。パルスハイトごとのパルス数を計数するとWBCとRBCの数が求められ、Hctは全てのパルスハイトを合算して求める(図2)。

また、測定サイクルごとに洗浄剤を注入し、アパーチャーのツマリを防いでいる。

### 4. LC-220の主な特長

LC-220は、血球計数に必要なサンプリング機能、希釈機能、吸引排出機能がコンピュータ制御されたわずか3個のステッピングモータで実現している。

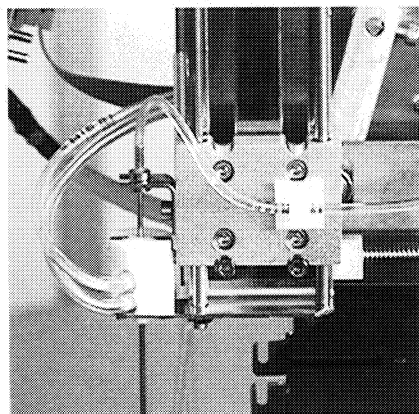


Fig.3 The overview of the needle system

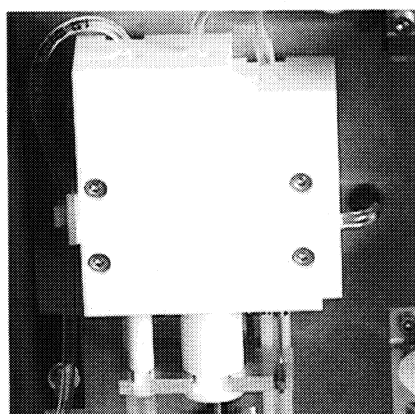


Fig.4 The overview of the bloc pump

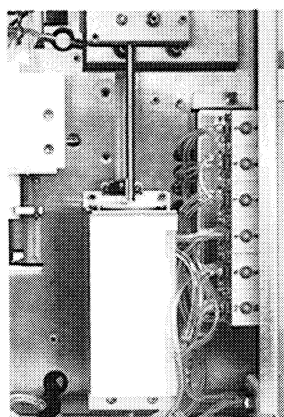


Fig.5 The overview of the air cylinder

While the number of pulses indicates red or white blood cell counts, the amplitude of the electrical pulse produced depends on the cell's volume. Each pulse is then sorted according to its size (Fig.2).

The hematocrit is measured by adding up all the pulse heights. A mathematical process is then applied to the sum obtained to compensate for coincidence passages in the aperture.

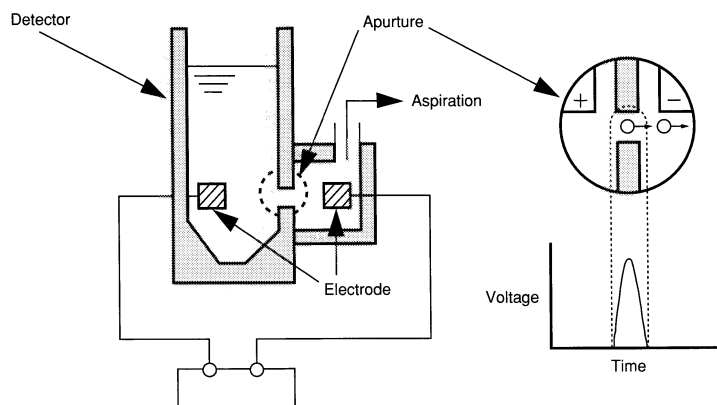


Fig.2 The impedance principle

We will prevent clogging of the 80 micrometers calibrated aperture with a specific reagent cleaner used during the automatic rinsing cycles. The cycle of the LC-220 will inject cleaner at every end of cycles to avoid pollution and clogging of the pneumatic circuit.

#### 4. Exclusive Specifications of the LC-220 Haematology Analyzer

The LC-220 uses only three microprocessor-controlled step by step motor to achieve the blood sampling, dilution and pressure or vacuum functions, all required for blood cell counting.

The needle carriage mechanism is to carry the blood sample (Fig.3). The first step by step motor is driving the carriage from the sampling position to the chamber with a brief stop for the inside rinsing of the needle.

The bloc pump (Fig.4) is composed of three pumps: the dilution pump, the lyse pump, and the blood sample aspiration pump. The LC-220 uses the second step by step motor to activate the three pumps together. This will guarantee precision and reliability.

更にこれらの機構であるニードル移動兼洗浄ユニット、三種の定注器を一体にした定注器ブロック、注射器タイプの空圧源エアシリンダ等どれも、少部品でコンパクト設計である。

これらの凝縮した特長ある技術が、大きさ300(W)×410(H)×360(D)、しかも13Kg(試薬を除く)と軽量で、高精度、高信頼の装置を生み出した。

また操作面において、前面の三種のスイッチにて、測定、リンス、洗浄等の基本操作が行える簡単な装置である。

図3は、検体吸引機構、図4は定注器、図5は被検液移動用エアシリンダーをそれぞれ示す。

A piston or syringe will replace the usual compressor to input pressure or vacuum into the pneumatic circuit. This pump will be activated up (vacuum) and down (pressure) by the third step by step motor triggered by a micro-processor. Additionally, the air cylinder (Fig.5) will drain the counting chamber, the vacuum in the counting head, and the backflush and bubbling. Reliability, precision, silent light and ease of use reflects from the choice of this air syringe.

The quality control (QC) data of LC-220 are shown in Fig.6 and Fig.7.

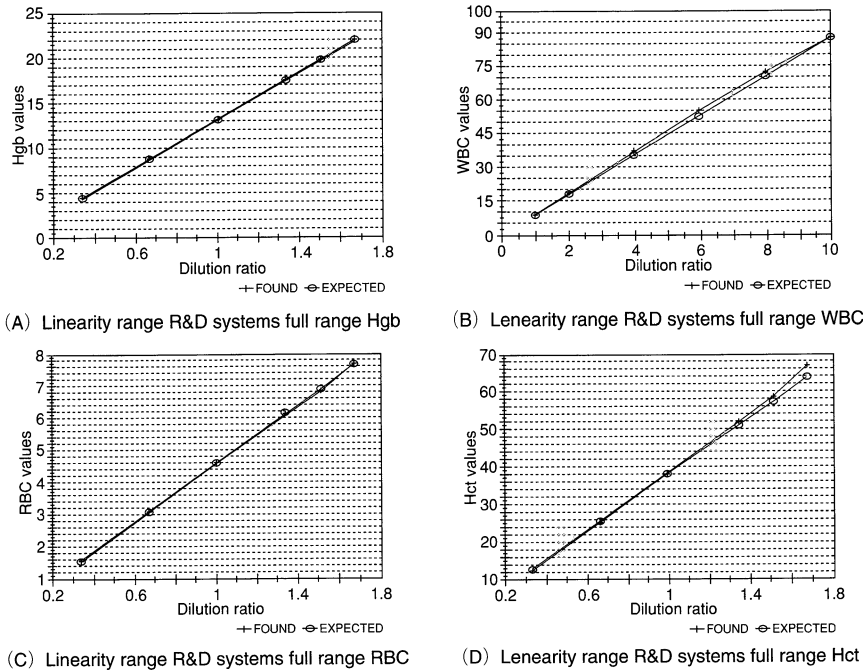
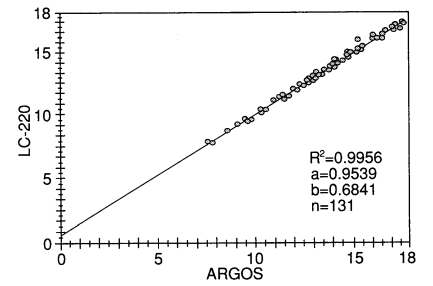


Fig.6 Linearity

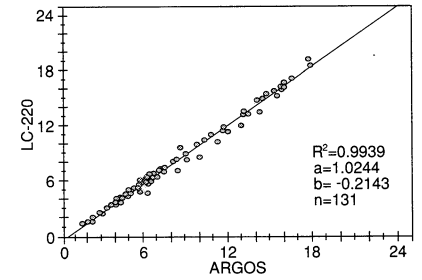
These highly sophisticated, unique technologies have yielded a reduction in both size and number of parts, and have assured high precision and reliability. The LC-220 is 300 (W) × 410 (H) × 360 (D) in overall size and is as light as 13kg (excluding the weight of reagent). It is designed for quiet operations.

The LC-220 is so constructed that measurement, rinsing, washing and other basic operations can be performed by use of three switches on the front panel. This analyzer requires no special training for effective handling.

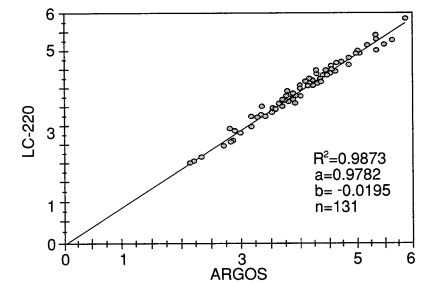
The LC-220 technology has been realized by ABX and Horiba through their Franco-Japanese joint development efforts.



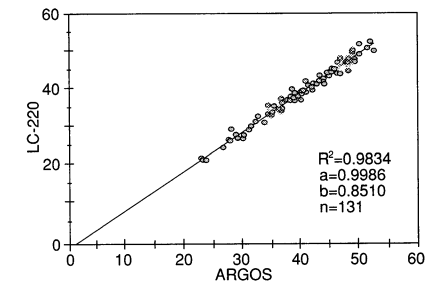
(A) Haemoglobin



(B) White blood cells



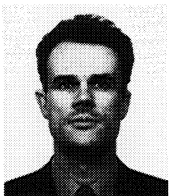
(C) Red blood cells



(D) Haematocrit

Fig.7 Correlation between LC-220 and ARGOS

図6は、LC-220における各測定項目の直線性を、図7はLC-220と白血球分類付血球カウンターARGOSとの相関を示す。



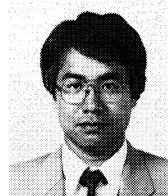
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