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Present Status of Hematology Analysing Systems

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特別寄稿

Guest Forum

Present Status of Hematology Analysing Systems

1. Introduction

It has nearly taken two handreds years since the recognition by VESALE of the blood as a fluid circulating through the body until the description of the two phases, one liquid and the other solid, under the microscope in the mid-eighteenth century. The solid phase is in fact the blood cells and another hundred years was necessary to classify the blood cells into populations thanks to the staining of blood films in the late nineteenth century by Giemsa, adapting the original Romanovsky stain.

Apart from the count of cells for each population, red blood cells (RBC), white blood cells (WBC), platelets (PLT), some more parameters were added; either measured like the pack cell volume (PCV) or hematocrit (Ht), hemoglobin concentration (Hb) or calculated like mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and mean cell volume (MCV).

If, at least in a normal patient, the RBC and PLT represent a quite homogenous population the WBC are classified in three populations as lymphocytes, monocytes and granulocytes. The latter is divided into three sub-populations as neutrophils, eosinophils and basophils. There were then five different populations of WBC described under the microscope according to their morphology and staining characteristics, the so-called white blood cell differentiation or 5 population differential.

So, until the 1950's, what we call the blood cell count (CBC) and the white blood cell differentiation were performed manually. As the CBC became one of the most common tests required to judge the status of a patient, it became obvious that the

	DEFINITION	UNITS	NORMAL RANGE ADULT
MCHC	$\frac{Hb\,(g/l)}{Ht\,(l/l)}$	g/l	32-36
MCH	$\frac{Hb\,(g/l)}{RBC(\chi 10^{12}/l)}$	pg	27-33
MCV	$\frac{Ht\,(l/l)\ \chi 1000}{RBC(\chi 10^{12}/l)}$	fl	80-100

Table.1 Calculated parameters of CBC

	UNITS	NORMAL RANGE ADULT	
		MALE	FEMALE
RBC	$\chi 10^{12}/l$	4.5-5.4	3.8-5.2
Hb concentration	χ g/l	131-165	120-150
Ht	l/l	0.40-0.50	0.35-0.45
PLT	$\chi 10^{9}/l$	150-400	
WBC	$\chi 10^{9}/l$	4-6	

Table. 2 The basic measured parameters of CBC

自動血球計数装置の現状

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1. はじめに

約200年前ヴェザリウスによって血液の体内循環が確認されて以来，18世紀には血液が液相と固相(血球)とから成ることが見いだされ，19世紀後半には血液塗膜染色法の開発に至り，血球の分類が可能となった．現在では，赤血球(RBC)，白血球(WBC)，血小板(PLT)さらにヘマトクリット値(Hct)やヘモグロビン量(Hgb)，また，これらの計数値から算出されるウイントローブ恒数と呼ばれる平均赤血球ヘモグロビン濃度(MCHC)，平均赤血球ヘモグロビン量(MCH)，平均赤血球容積(MCV)が計数されている．これらの項目は全血算(CBC)と称し，病態診断の基本項目となっている(表1，2)．WBCはリンパ球，単球，顆粒球の3種に，さらに顆粒球は好虫球，好酸球，好塩基球に，トータル5種類(5-diff)に分類される．

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＜Experience＞
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1976-81:M.D.Resident in ClinicalMedicine, Paris Hospitals, France.
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manual method was time-consuming and would no longer be applicable to large scale screening. The time for automation became mandatory.

In order to describe the present status of hematology analyzing systems, we should divide this article into three chapters: automation in blood cell counting, automation in WBC differential count and automation in sample processing and data management.

2. Automation in Blood Cell Counting

Automation has naturally taken as reference the manual methods to desigh and manufacture the system needed. Referring to blood cell counting, the first step is to know what blood cells can be compared to. The reference was particles for which we need to count whether it will be red cells, white cells or platelets. Then to differentiate between those three populations, ther is a need not only to count but to size the particles as well. At present, there are two different technologies which can be used: electrical impedance and light scattering. Hemoglobin, on the other hand, is measured with a classical spectrophotometric method.

Referring now to the concentration of cells in the blood which differ in a 1/1000 ratio from RBC to WBC, there is a need for dilution. Dilution of the samples is then a basic requirement on blood cell counting automation.

Whatever the technology used, the analyzers are working on the same basis: they have at least two channels, one for RBC, PLT and Ht and another one for WBC and

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1950年代までは血球は手作業で計数されていたが，CBCが臨床検査の基本項目になるに従い，検査装置の自動化が渇望されるようになってきた．

2．血球計数の自動化

自動化には，複数種の血球を検出する点と，RBCとWBCの濃度比が３桁も異なる点が問題になる．前者は，血球の大きさの違いを利用して電気抵抗式か光散乱式で，ヘモグロビン量は分光光度法で計測することで対応する．後者は２チャンネルの前処理法で対応する．すなわち，RBC, PLT, Hct用のチャンネルには(大)希釈液を，WBC, Hgb用には(小)希釈液と溶血剤を注入する(表３，４)．

電気抵抗式は調整が不要だが干渉を受けやすいのに対し，光散乱式は干渉を受けないが厳密な調整が必要になる．

Hb. There are different ways to process the sample (see table 4) but the results are the same at the end. In one channel, a diluent is added and RBC and PLT are counted. In the second one, diluent and a lytic agent are added. The purpose of the lytic agent is to lyse the RBC, leaving the WBC intact and to provide a solution for Hb measurement. In that case, the lysing agent comprises the regent needed for hemoglobin measurement.

Hb	Cyanmethemoglobin	in the presence of potassium cyanide, Hb is converted into cyanmethemoglobin measured at 540 nm by means of a photometer or colorimeter
Ht	Centrifugation	Ht is the proportion of a column of centrifuged blood which is occupied by the red cells

Table.3 Reference methods for Hb and Ht (ISCH &NCCLs recommendations)

2.1 Analyzers depending on electrical impedance

The principle refers to the very poor conductivity of blood cells. When placed in a conducting medium, cells are aspirated through an aperture accross which an electrical current is applied. When the cell is going accross the aperture, there is an increase in the electrical impendance. This increase is measurable and proportional to the size of the cell. Thus, the electrical pulse generated corresponds to one count and its height corresponds to the size of the cell counted. So, cells are counted and sized, giving the opportunity to differentiate the populations.

	PRINCIPLE	CHARACTERISTICS
ASPIRATION NEEDLE	Aliquot of blood is transferred to dilution chambers With multi-step dilution to the counting chambers	*Low sample volume (12 μl on LC 220) *Slow *Easy cleaning
SHEAR VALVE	The blood column is cut into aliquot Aliquot directly flows to the counting chamber by diluent	*High sample volume (100 μl at least) *Quick *Sensitive to blood clot, needs regular maintenance

Table.4 Sample processing device for dilution

In the RBC and PLT channel, Ht is measured by adding all the pulses produced by all the cells, given then the total volume of cells. Another way is to calculate the MCV from the mean pulse and then deduct the Ht from the formula on Table 2.

In a normal sample, the effect of WBC present in the RBC channel is negligeable but has to be taken into consideration when there is a marked increase like in leukemia. Also, the difference in size between PLT and RBC in a normal sample gives a big enough gap to differentiate the two populations. In pathological samples with either

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2.1 電気抵抗式

血球の低導電性を利用する．通電した生理食塩水などの電解質溶液に血球を浮遊し，小さな孔から吸引する．血球が孔を通過するときに生じる電気パルス信号（数と高低）から血球の数と大きさ（種類）を測定する．Hctは，RBC,PLTチャンネルで得られた全パルスを合算し体積換算するか，パルスの平均値からMCVを得てRBC値を掛けて算出する．白血病などの異常検体では，RBC , PLTチャンネルでのWBCの干渉が無視できないので注意を要する．

2.2 光散乱式

血球が光束を通過するときに生じる吸収や散乱を利用する．血球サイズ（種類）には散乱が，数には吸収が主に適用される．この方式では血球を一個づつ流すシースフロー機構のチャンバを備えている．Hct, Hgbの計測・算出は電気抵抗式と同じである．

large PLT or small RBC, there could be an overlapping of the two populations. Several electronic flags can now alert the user of such overlapping.

The WBC channel is also used to measure the Hb through a spectrophotometer, which is most of the time placed behind the counting chamber. In a normal sample, RBC and PLT are lysed, which then gives no interference with the WBC count. In case of presence of NRBC, the remaining nucleus are counted and interfere with the WBC count. Electronic thresholds are able to fix the limits and to give flag in such conditions.

2.2 Analyzers depending on light scattering

The principle refers to both absorbance and light scattering of a particle when passing through a light beam. The light source may be white or laser light, or both. Absorbance is mostly used to count the cells as indicating that a cell is passing through the light beam. Scattered light is used to size the cell. Depending on the design of the optical bench used, there is an optimum angle to measure the size of the cell.

All the instruments using light scattering technology have a chamber design including sheath flow in order to avoid coincidence. Otherwise, counting and sizing the RBC, PLT and WBC gives comparable results as with impedance technology. The measurements of Ht and Hb remains the same.

Comparing the two technologies, the impedance needs no adjustment but is subject to more interferences. To the contrary, there is less interferences with light scattering but the adjustment is critical to get quality results and the heat of a laser when

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2.3 血球の粒度分布

血球(例えばRBC)のサイズと数から粒度分布を表示し，血球粒度分布幅(RDW)が算出される．血球粒度の均一性が判断でき，病態診断の指標となる．

3. 白血球分類の自動化

用手法(顕微鏡)による白血球分類は，血球数が少ないため正確度が劣り時間がかかる．正常な白血球の場合は，単球＞顆粒球＞リンパ球の順の大きさで，好中球と好酸球と好塩基球とでは極端な差はない．そこで，画像パターン認識法とフローサイトケミストリ法の2種類の自動識別方法が開発された．

3.1 画像パターン認識法

スライドマーカに拡散・染色した血液膜の顕微鏡像をテレビカメラで読み取り，コンピュ

used is still not convenient. It is foreseen that the use of laser diode will greatly improved this aspect.

2.3 Automated blood cell counting and cell distribution

As far as the analyzers are sizing the cell, it is then possible to classify within the same population (i.e. RBC) cells having the same volume. It is then possible to visualize cell volume distribution through a histogram. On the X axis, volume is represented, on the Y axis, relative number of cells for a defined volume (see Fig.1).

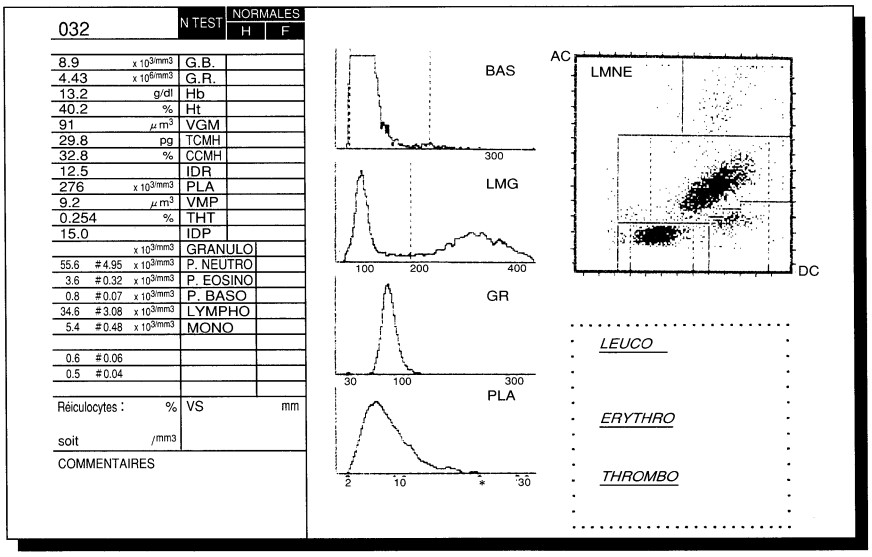

Fig .1 Exemple of result sheet
*Data given by COBAS ARGOS 5 DIFF showing the distribution curves as well as the matrix. Flow cytometry, cytochemistry and light absorbance are combined to obtain the matrix.

ータ画像処理によってWBCの分類・計数を行う．現在では120検体/時間の速度で精度よく処理することができ，主に大規模な検査センタで使われている．

3.2 フローサイトケミストリ法

血球と試薬とを呈色反応させて分類する方法で，好中球と好酸球とを他の3種類の白血球と識別するのに使われる．本方式と電気抵抗法や光散乱法によるサイズ計測とを組み合わせることにより，白血球の5分類が可能となる（図1）．

3.3 サイズ分離

溶血剤の濃度とpHによってWBCの溶解度が異なる性質を利用して，目的とする血球種だけを残して電気抵抗法で計測する．光散乱法でも可能で，血球の形状や内部構造を反映しており，WBCの5分類ができる．

The shape of the histogram is then a good indicator of the homogeneity of the population. It has then been possible to define new parameters for RBC and PLT, so-called red blood cell distribution width and platelet distribution width respectively.

3. Automation in WBC Differential Count

As already mentioned, manual WBC differential counting is time-consuming and inaccurate. The inaccuracy comes mainly from the small number of cells (100) counted under the microscope and the quality of the blood film and its stain.

Automated counters overturned this inconvenience, being able to count quickly a large number of cells, they are thus far more accurate. In addition to the count, the WBC differentiation is based upon the morphological characteristic of the cells. Size is one of those characteristics and we have seen above, that size between PLT, RBC, WBC at least in a normal sample, differs greatly enough to separate these three populations.

The problem becomes more complex with the WBC differentiation. In a normal sample, the lymphocytes are the smallest, the monocytes the biggest and the granulocytes are in between in terms of size. But granulocytes, considering neutrophils,eosinophils and basophils, are almost the same size. There is thus a need to define another characteristic being measurable to differentiate. Furthermore, in pathological samples, you may find large lymphocytes or cells which normally should not be present in the blood stream and which would have a size comparable to the normal size.

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4. 検体処理とデータ管理の自動化

血液検査室では，人件費の点から，血球計数と同時に検体の前処理とデータ管理の自動化が大きな課題となる．検体の検査装置への搬送，装置へのセット，検体IDの読み取り，検体の吸引，測定結果の伝送などそれぞれの工程の自動化が進んでいる．

(1) 検体の自動搬送

試験管をラックに入れ，ベルトコンベアで搬送後，計数装置に乗せ換える．広い設置スペースが必要で価格も高くなるため，主に大規模病院で採用されている．

(2) 検体の自動セット

中・大型計数装置はサンプラを備えている．ラックやトレイで運ばれてきた検体は，サンプラに自動的にセットされる．計数結果が正常範囲から外れている場合には，再度計測ラインに戻す機能を持つものもある．

All those conditions have led to the point that different or added technologies are now used in WBC differential automation. Two different ways were explored in the 70's: image of pattern recognition analyzers and flow cytochemistry instruments. During the 80's, improvement in the analysis of the signals given by cell sizing and light scattering gave the way to another generation of instruments. At present, those three approaches are still in use and even sometimes, combined in an attempt to give even more accurate results.

3.1 Pattern recognition instruments

Progress in computerization has eventually put that technology back into fashion. From a blood film, for which spreading and staining are standardized by a robotic slide-maker, the WBC differential is performed under a camera-equipped microscope. The image is then compared to the one which is memorized in a computer. Up to 250 characteristics of each possible cell to be found in the blood stream are memorized and there is the possibility to virtually identify each kind of WBC and count them.

Now, with the memory capacities and the speed of computer, up to 120 blood films with very accurate results are processed with these analyzers. But there is still the need for a blood film, which means either labor costs or investments in a blood film-maker robot. Nevertheless, for large laboratories, it is eventually a good additional option to the other instruments.

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(3) 検体IDの読み取り

ラック・トレイラベルや試験管ラベルをバーコードリーダで読み取る.

(4) 検体の吸引

最近は，安全面から（バイオハザードなど），栓付試験管から直接キャップ・ピアス方式によって必要量が吸引される.

(5) 計測結果の伝送

最近の計数装置はすべて計測結果をメインフレームに伝送することが可能である．大型の装置では双方向通信機能を備えており，ホスト側からID番号とワークシートを入力すると，所定の検体の指定項目を計測し，結果をメインフレームに伝送することができる.

3.2 Flow cytochemistry instruments

Using the right chemical, advantage is taken of a specific fixation of this chemical to a population of cells. WBC differential count is part of the full CBC given by the same instruments by adding one or more channels. The sample is then processed only once, blood film is not required and CBC and WBC differential count are given simultaneously. In order to compley with the requested productivity, the chemical used should react quickly with the cell and give a measurable signal. Flow cytochemistry is mainly used to differentiate eosinophils and neutrophils from the other three populations. Combining this technology with light scatter or volume, it is possible to build-up a matrix which shows a good separation of the different WBC populations (Fig.1).

3.3 Cell-sizing & light scattering instruments

The WBC react differently to lysis, depending on the strength of the lysing agent and as well to the pH of the solution. These characteristics are used to lyse some of the population and then count and size the remaining ones. The same occurs with light scattering and light absorbance which is partly due to, not only the size and shape of the cell, but also to its internal structure. Analyzing the scattered light at different angles and combining the different measured signals lead to the differentiation of the WBC. Again, in combining the two technologies, it is possible to build-up a matrix as for the flow cytochemistry instruments.

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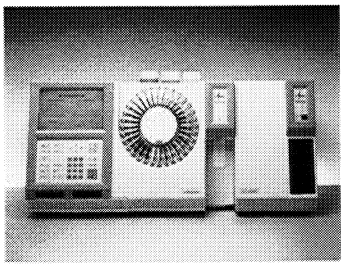
5. 結論

血球計数装置は，他の計測装置と同様，ロボット・エレクトロニクス・コンピュータ技術を駆使して，検査の効率，生産性，信頼性の向上をすすめてきた．

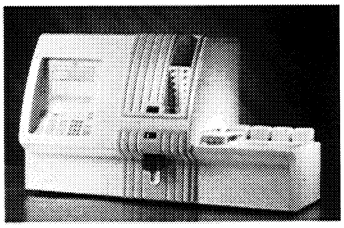
一方，血球の検出・分類などの基礎技術は，医療費の抑制や多額の投資が必要なため，近未来的には大きな進歩は期待しにくい．

（抄訳 編集部）

4. Automation in Sample Processing & Data Management



(A) COBAS ARGOS using rotating trays as an auto sampler



(B) COBAS VEGA using rack system

Fig .2 Two examples of fully automated high-through put CBC+5DIFF analyzers.

Up to know, we did describe automation in counting the cells. We have pointed out that the actual instrument can perform up to 120 CBC and WBC differential simultaneously per hour. It is then obvious that sample processing and data management would become the most important labor cost of a hematology workstation.

Automation of these two items is then mandatory but has not developped the same way. The problems to be solved are in the forthcoming order: bring the sample to the instrument, present the sample to the instrument, identify the sample, aspirate the sample, recall the results, transfer the right results of the sample to the prescriber. Except for the first step, all others are now fully automated on large instruments.

(1) Bring the sample to the instrument

Humen resources are still the most common way. Manufacturers start to develop conveyor lines with computer-driven rack-systems which bring the samples automatically to the various work stations where the samples are transferred to the instruments. Two obstacles reserve such kind of organization to very large laboratories: it is space-consuming and the cost is still expensive, still more expensive than the earning in personnel. But at least, the concept will be valid when these two obstacles are erase.

(2) Present the sample to the instrument

On small instruments, samples are presented by hand and one by one under the aspirating needle. Medium or large size instruments are now fitted with autosumplers. Tubes are positioned on a tray or racks, held by clips, the tray or racks being placed on the instruments which process the samples automatically. The instrument does not need someone to feed it and has more than one hour's autonomy. They are called "walkaway" systems.

Some instruments include an automatic rerun capabilities if the results are outside normal range, giving even more "walk away".

(3) Identify the sample

It is always possible to enter the ID number or the name manually through the front panel keyboard. On most of the instruments, this is now done with a barcode reader with identification of the tray or rack number, the position of the tube in such and if labelled with barcode, the ID number of the sample.

(4) Aspirating the sample

For evident safety reasons, all new generations of instruments are proposed with a closed tube sampling device, whether they are fitted or not with an autosampler. A needle pierces the rubber cap of the tube and with the help of a level detector, goes down to aspirage the required quantity of blood.

(5) Transfer of the results

All instruments are now fitted with a computer output allowing transmission of data to a main frame. In such case, sample identification is mandatory. The most sophisticated instruments have on board, or beside, a PC and work on two-way communications, allowing random access and selectivity. The work list is trasferred to the instrument with the ID number and the requested parameters for each sample. When the barcoded sample is recognized by the instrument, it is processed and the results directly sent to the main frame. In such case, no intervention has been necessary.

5. Conclusion

Like in other fields in laboratory equipment, hematology has greatly taken advantage of automation. Robotic, electronic, computerization have given all the necessary tools to give efficiency, productivity and reliability to the labs. The tendancy is now to consolidate the different available technologies and to combine several instruments to enhance productivity.

Major improvement in cell detection and differentiation are not foreseen in the near future, partly because the cost constraints in healthcare programs do not allow the development of expensive technology. Progress in automation will then rely more on robotic and computerization for the next years.