

Automatic Blood Cell and CRP Counter with Three-Part Differential Measurement of White Blood Cells, The LC-170 CRP

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LC-170CRP

WBC, RBC, Hct

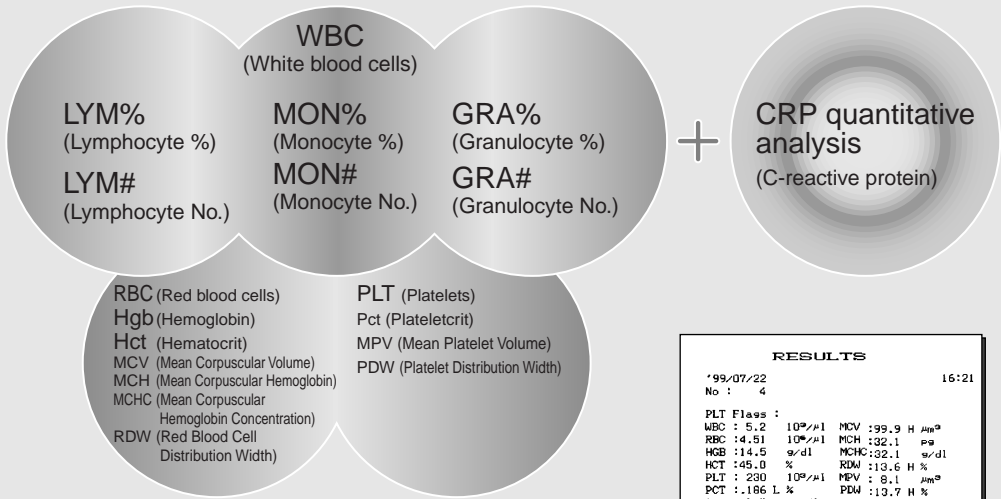
Electrical impedance method

Hgb

Cyanmethemoglobin method

CRP

Latex immunoturbidmetry



Example of results

RESULTS			
'99/07/22			16:21
No :	4		
PLT Flags :			
WBC :	5.2 $10^9/\mu\text{l}$	MCV :	99.9 H μm^3
RBC :	4.51 $10^{12}/\mu\text{l}$	MCH :	32.1 Pg
HGB :	14.5 g/dl	MCHC :	32.1 g/dl
HCT :	45.0 %	RDW :	13.6 H %
PLT :	230 $10^9/\mu\text{l}$	MPV :	8.1 μm^3
PCT :	.186 L %	PDW :	13.7 H %
CRP :	0.7 H mg/dl		
WBC Flags :			
DIFF :			
%LYM :	26.6 H %	#LYM :	1.3 H $10^9/\mu\text{l}$
%MON :	5.8 L %	#MON :	0.2 L $10^9/\mu\text{l}$
%GRA :	67.6 H %	#GRA :	3.7 H $10^9/\mu\text{l}$

Abstract

The LC-170 CRP automatic blood cell and CRP counter, developed by Horiba, is capable of simultaneously measuring all 19 C-reactive protein (CRP) density parameters and counting red blood cells, platelets, and three types of white blood cell: lymphocytes, monocytes, and granulocytes. As clinicians demand ever-higher precision measurements, a need has developed for clinical test machines having excellent operational and cost performance. This compact machine should make a powerful tool for initial diagnosis of inflammatory and infectious diseases, especially at small- and mid-size medical institutions.

1 Introduction

To prevent an explosion of medical costs as Japanese society ages and fewer children are born, the Ministry of Health, Labor, and Welfare is pursuing a thorough reform of the medical insurance system, including “preventing over-prescription of medicine to obtain marginal profit and excessive examinations due to fee-for-service payment systems,” as well as a “review of the insurance system for the elderly.”

For example, the ministry is studying the introduction of a medical diagnostic fee system that combines the compensation for diagnosis and treatment, and the Diagnosis Related Group-based Prospective Payment System (DRG/PPS), whereby payment for diagnosis and treatment is determined according to each disease. At the same time, when we visit a clinic or hospital for a health problem, it is essential that the doctor quickly obtain the necessary testing results during diagnosis in order to make the correct diagnosis and quickly determine the type of treatment. This also lightens the burden on the patient and is very effective in cutting costs. For this reason, there is a strong demand for an examination system that provides the required test at the required time, with speed and accuracy, and which anybody can operate.

In response to this need, Horiba, Ltd. and ABX Diagnostics S.A. jointly developed the LC-270 CRP automatic blood cell and CRP counter, and launched the product in April of 1998¹⁾. Easy to operate, this product requires only that the clinician place a whole blood sample in the sample holder, and it features quick measurement results for CBC including white blood cell count (WBC) and quantitative analysis of C-reactive protein (CRP), which are effective indicators in the diagnosis of inflammatory and infectious diseases. The instrument is currently enjoying wide clinical use.

As the LC-270 CRP comes into wide use, we are seeing a rapidly increasing desire for an instrument based on the LC-270 CRP with advanced functions, including white blood cell differentiation. We have therefore developed an advanced version of the LC-270 CRP, the LC-170 CRP Automatic Blood Cell and CRP Counter with Three-Part Differential of White Blood Cells. In a short time of only 4 minutes and 20 seconds, the LC-170 CRP performs easy measurement of 18 parameters including three-part differential of white blood cells, as well as quantitative analysis of CRP for a total of 19 parameters.

In this report I will explain the measurement principle of the LC-170 CRP and its functions, and introduce several examples.

2 Measurement Principles

The LC-170 CRP uses the electrical impedance method to count blood cells, the cyanmethemoglobin method to measure hemoglobin concentration, and latex immunoturbidimetry to measure CRP concentration.

2.1 Measurement of White Blood Cells, Red Blood Cells, and Platelets

White blood cells (WBC), red blood cells (RBC), and platelets (PLT) are measured by the electrical impedance method (Fig.1).

The blood sample is suspended in a highly conductive diluent solution, and then made to pass through an aperture between two electrodes. As the sample passes between the electrodes, it causes a change in the impedance between the electrodes according to the volume and number of blood cells. The number of blood cells can be determined from the number of pulses as the impedance changes, and the volume (type) of the cell can be determined from the height of the pulse.

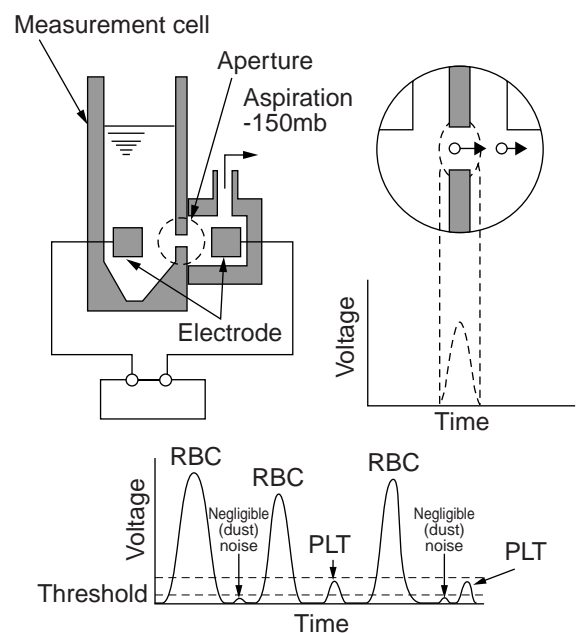


Fig.1. Measurement Principle (electrical impedance method) of RBC, WBC, and PLT.

2.1.1 Blood Cell Size Distribution

The shape of blood cells is not necessarily fixed according to the type of blood cell, and the shape of a cell may even change during the course of measurement. To correct for these effects and obtain an accurate measurement, the obtained pulse heights are divided into multiple channels according to each blood cell and measured in the optimum pulse height range.

Measurement conditions are set as follows in the LC-170 CRP:

- *WBC : 0 to 430 fL are divided into 256 channels and the measurement range is approximately 30 to 430 fL.
- *RBC : 0 to 300 fL are divided into 256 channels and the measurement range is approximately 25 to 300 fL.
- *PLT : 0 to 33 fL are divided into 128 channels and the optimum threshold value is automatically set in a measurement range of approximately 18 to 27 fL.

2.1.2 Three-Part Differential of White Blood Cells

White blood cells serve the body’s defense system, and the white blood cell count is essential to the diagnosis of a variety of infectious diseases. White blood cells consist of five types of cells: neutrophils, eosinophils, basophils, monocytes, and lymphocytes. The first three types are commonly grouped together under the term granulocytes.

Differentiating and counting each type of white blood cell provides extremely useful information for diagnosis and treatment. The LC-170 CRP is able to measure the 3-part differential of monocytes, lymphocytes, and granulocytes; these are especially important among the five types. Fig.2 shows the threshold values of the three types.

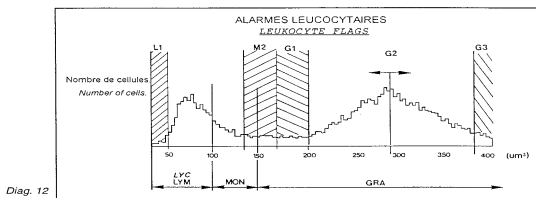


Fig.2. Threshold Values of Three-Part Differential WBC Analysis.

2.1.3 Principle of Differentiation of White Blood Cells and Reagents

The differentiation of white blood cells is carried out using a special diluent solution and hemolyzing agent for three-part differentiation, and is based on the difference in the change in volume of each type. The diluent solution protects the cell membranes for the differentiation reaction. The hemolyzing agent acts according to the cell type. For lymphocytes, the hemolyzing agent causes release of water from the cytoplasm, making the cell membrane contract (nucleate). For monocytes and granulocytes the degree of nucleation is progressively smaller.

2.2 Hemoglobin Concentration

The hemoglobin concentration (Hgb) is measured using the cyanmethemoglobin method, which has been established as an international standard by the International Council for Standardization in Hematology (ICSH) (Fig.3).

When the hemolyzing agent is added to the blood sample, the red blood cells are lysed and hemoglobin is released. The cyan in the reagent joins together with the hemoglobin to form cyanmethemoglobin. The cyanmethemoglobin absorbs green (540 nm) light well and thus the Hgb concentration can be determined from the degree of light absorption.

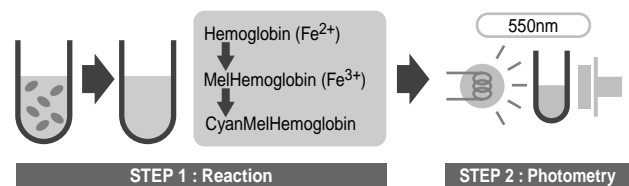


Fig.3. Measurement Principle for Hemoglobin Concentration.

2.3 CRP Measurement

The CRP concentration is measured by latex immunoturbidimetry. A special hemolyzing agent is used to lyse the blood sample, and then a latex reagent that is sensitized to anti-human CRP antibodies is added to make the CRP antigen in the blood cause an antigen-antibody reaction which results in agglutination (Fig.4). The CRP concentration in blood plasma is calculated, using a correction equation, from the amount of light absorption at 850 nm that occurs due to this reaction and from the hematocrit value (Hct) obtained from the blood cell count.

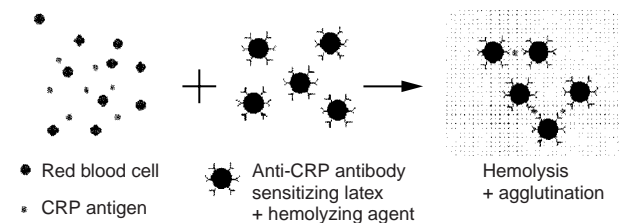


Fig.4. Antigen-Antibody Reaction of CRP.

3 Measurement Method

The measurement flow of the LC-170 CRP is shown in Fig.5.

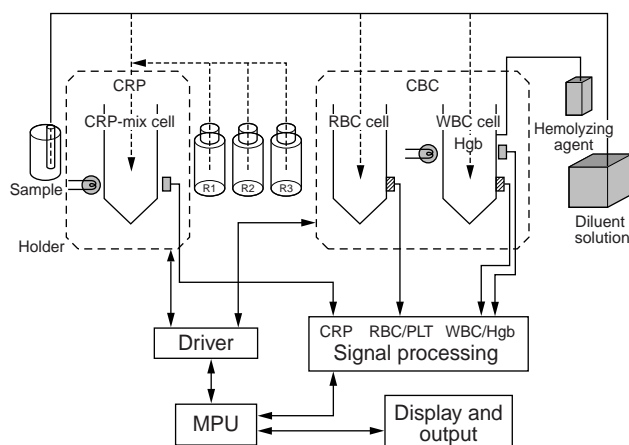


Fig.5. LC-170 CRP Measurement Flow.

The clinician performing the examination draws blood into the blood sample tube, which contains an anti-agglutination agent, and places the sample tube in the sample holder on the analyzer. The sampling probe samples 100 μ L of the reagent for CRP measurement (R1) and 8 μ L of the blood sample for CRP, and these are mixed together in the CRP-MIX cell. Next, 10 μ L of the blood sample for the blood count is placed in the WBC cell and diluted to 1/210 with an isotonic diluent solution. In the RBC cell, the sample is diluted in two stages (1/18, 400). In the WBC cell, a hemolyzing agent is added and the WBC count and hemoglobin (Hgb) concentration are measured. In the RBC cell, the RBC count and PLT count are measured. In the CRP-MIX cell, 100 μ L of CRP measurement reagent (R2) and 200 μ L of CRP measurement reagent (R3) are successively added and mixed. The CRP concentration is then measured based on the change in turbidity that occurs due to the antigen-antibody reaction.

4 Features

Based on the development concept of providing doctors with the test data that they need when they need it, with accuracy, convenience, and easy operation, the LC-170 CRP has the following features.

1) Simultaneous measurement of 3-part WBC differentiation and CRP

The LC-170 CRP can measure a total of 19 parameters, including complete blood cell count such as WBC, RBC, PLT, Hct, and Hgb, as well as three-part differentiation of white blood cells and quantitative analysis of CRP. This allows a wide range of diagnostic and treatment information to be obtained, including whether an inflammatory condition is bacterial or viral in nature.

2) Quick measurement

The time required for measurement is extremely short: 75 seconds in the case of only blood count parameters, or 4 minutes and 20 seconds when CRP is included. This means that testing can be completed while the doctor is seeing the patient, helping to achieve quick diagnosis and treatment. This also alleviates the burden on the patient, as he or she does not need to make a separate visit to learn the results of the test.

3) Automatic testing of whole blood

Separation by centrifuge and other preprocessing of the sample are not necessary. The clinician simply places the blood sample tube in the sample holder, after which all testing takes place automatically. When finished, the results are printed out. Daily maintenance such as cleaning the sampling probe and tube system are also performed automatically by simply pressing keys.

4) Small sample volumes

Using a special blood sample tube, the minimum sample volume is 10 μ L for CBC and 8 μ L for CRP, for a total of only 18 μ L. This makes the LC-170 CRP a powerful tool for the testing of infants and small children.

5) Compact and quiet

Unit dimensions are a compact 300 mm wide, 400 mm deep, and 410 mm high. A syringe-type air supply is used, enabling the unit to be installed in a corner of a clean and quiet consultation room without disturbing the consultation.

Table 1 shows the main specifications of the LC-170 CRP and Fig. 6 shows an example of a printout of the test results.

Measurement Mode	CBC, CBC+CRP, Control blood measurement
Measurement parameters	WBC, LYM%, LYM#, MON%, MON#, GRP%, GRP#, CRP, RBC, Hgb, Hct, MCV, MCH, MCHC, RDW, PCT, MPV, WBC 3-part differential distribution, RBC distribution, PLT distribution
Sample volume	CBC mode : 10µL, CBC+CRP mode : 18µL, Control blood measurement mode : 10µL
Throughput	CBC mode : 75 seconds, CBC + CRP mode : 260 seconds, Control blood measurement mode : 75seconds
Sample holder	Vacuum blood sample container, Micro-volume blood sample container, Control blood container

Table 1. Specifications of the LC-170 CRP.

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RESULTS
'99/07/22          16:21
No : 4

PLT Flags :
WBC : 5.2 109/µl  MCV : 99.9 H µm3
RBC : 4.51 106/µl  MCH : 32.1 pg
HGB : 14.5 g/dl    MCHC : 32.1 g/dl
HCT : 45.0 %      RDW : 13.6 H %
PLT : 230 109/µl  MPV : 8.1 µm3
PCT : 1.186 L %   PDW : 13.7 H %
CRP : 0.7 H mg/dl

WBC Flags :
DIFF :
%LYM : 26.6 H %   #LYM : 1.3 H 109/µl
%MON : 5.8 L %    #MON : 0.2 L 109/µl
%GRA : 67.6 H %   #GRA : 3.7 H 109/µl
    
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Fig.6 LC-170 CRP Printout Example

5 Evaluation

In order to evaluate the basic performance of the LC-170 CRP, we verified within-run reproducibility and performed correlation tests between WBC 3-part differential and CRP tests using other models. We reported the results of this evaluation at the 31st meeting of the Japan Society for Clinical Laboratory Automation.

1) Reproducibility

We verified reproducibility by performing repeated measurements on the same sample, and calculated the mean and coefficient of variation (CV) of the results. In this test, we performed ten repeated measurements on each of three samples. As shown in Table 2, the results confirmed high reproducibility.

		WBC	LYM	MON	GRA	CRP
		10 ³ /mm ³	%	%	%	mg/dl
Sample A	Mean	5.7	25.1	5.9	69	0.4
	C.V.	1.3	2.7	4.3	1.2	0
Sample B	Mean	4.4	25.7	6.7	67.6	1.87
	C.V.	1.4	1.9	8.8	1.1	2.6
Sample C	Mean	4.9	20.1	4	75.9	8.2
	C.V.	1.5	2.7	7.1	1	2.8

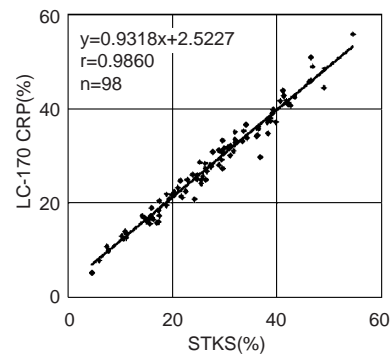
Table 2. Within-Run Reproducibility of the LC-170 CRP.

2) Correlation of WBC 3-part differentiation

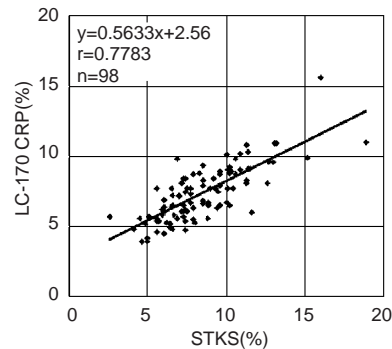
Three-part differential measurement of white blood cells was performed on samples from 98 patients using the LC-170 CRP and an instrument from another manufacturer (Coulter STKS), and correlation was evaluated by calculating correlation coefficients from the results of both.

As indicated in Fig.7, LYM, MON, and GRA all showed good correlation.

a) LYM



b) MON



c) GRA

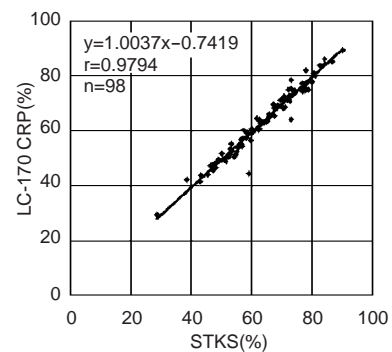


Fig.7. Correlation of Three-Part Differential WBC Results from the LC-170 CRP and Another Instrument.

- a) Correlation of LC-170 CRP and STKS for Lymphocytes
- b) Correlation of LC-170 CRP and STKS for Monocytes
- c) Correlation of LC-170 CRP and STKS for Granulocytes

3) Correlation of CRP

We evaluated CRP correlation using samples from 69 patients. For the correlating method, we measured serum samples with a Hitachi-7600 Biochemistry Analyzer, using Eiken Chemical’s LZ Test Eiken CRP for the reagent. The results were compared to the results of measurement of whole-blood samples with the LC-170 CRP. As shown in Fig.8, a good correlation was obtained.

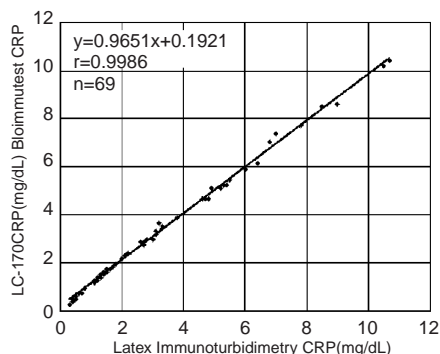


Fig.8. Correlation of CRP Measurement Results from the LC-170 CRP and Another Instrument.

6 Conclusion

Building on the main feature of the widely used LC-270 CRP—quick measurement of both blood cell count and CRP with a single instrument—the LC-170 CRP makes it possible to measure a total of 19 parameters—18 blood count parameters including 3-part differential of WBC, plus CRP— in a short four minutes and 20 seconds.

As reform of the medical system progresses at a breakneck pace, Horiba’s position is to further enhance the LC series, developing examination systems that provide the required test at the required time, with speed and accuracy, and which anybody can operate. We hope that this report will help in its own small way to further the advance of medicine.

Acknowledgements

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References

- 1) Yamao, et al.: Readout, No. 16, p. 11-15 (1998).
- 2) Yamao, et al.: Journal of the Japan Society for Clinical Laboratory Automation.



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