Feature Article

Automatic Blood Cell and CRP Counter LC-178 CRP

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Automatic Blood Cell and CRP Counter LC-178 CRP has been receiving high commendation from users as a powerful diagnostic tool of inflammatory and infectious diseases. The reputation is based on its unique feature of simultaneously measuring white blood cells, red blood cells, platelets, hemoglobin, and CRP simply and immediately in front of patients, from a blood sample tube installed at a medical examination room. The measurement functions have been added to allow three-part differential of white blood cells, and the LC-178 CRP, newly developed, expanded the measurement range of the CRP in whole blood from 0.2 - 10.0 mg/dL to the double, 0.2 - 20.0 mg/dL with the same usability and speed. This has been realized by the improved version of CRP reagent, and the expectation grows for the even wider range of diagnosis in inflammatory and infectious diseases.

Introduction

In many clinics or hospitals, the system that provides "required tests at required time and place, with speed and accuracy, and which anybody can operate" is under discussion. This is called as POCT, Point of Care Testing, which is inevitable for doctors to perform correct diagnosis and speedy judgment of medical therapy. POCT also contributes to alleviating physical and economical burden of patients. This is why the inspection system realizing POCT with low cost is strongly demanded.

In response to this need, HORIBA and HORIBA ABX jointly developed the FL-270 CRP automatic blood and CRP counter, and launched the product in April 1998 [1]. Easy to operate, this product only requires for a clinician to set a whole blood sample in the sample holder, allowing quick measurement result including red blood cells, hemoglobin, platelets, as well as white blood cells (WBC) and C-reactive protein (CRP), which are effective indicators in the diagnosis of inflammatory and infectious diseases in less than 5 minutes. Furthermore, as an advanced model of FL-270 CRP, we have launched LC-170 CRP featured with three-part differential function of white blood cells in March 2000, and LC-175 CRP, the improved model of LC-170 in March 2002, both enjoying wide use in clinical sites.

The newly developed LC-178 CRP (Figure 1), applying the improved CRP reagent, widens the CRP measurement range from the previous 0.2 - 10.0 mg/dL*1 to double, 0.2 - 20.0 mg/dL*2. This article reports the measurement principle and features of LC-178 CRP.

*1: Blood plasma and serum: 0.2 to 7.0 mg/dL
*2: Blood plasma and serum: 0.2 to 15.0 mg/dL

Figure 1 LC-178 CRP

Measurement Principle

In LC-178 CRP, the impedance method is used to count blood cells, the cyanmethemoglobin method to measure hemoglobin concentration, and latex immunoturbidimetry to measure CRP concentration (the same principles with the conventional models).

Measuring WBC/RBC/Platelet

White blood cells (WBC), red blood cells (RBC), and platelets (PLT) are measured by the impedance method. The blood sample is diluted to a suitable concentration using a highly conductive diluents (Minoton 3D) mainly composed of physiological saline, and then made to pass through an aperture between two electrodes. As a blood cell passes between the electrodes, it causes a change in the impedance between the electrodes (Figure 2). 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.

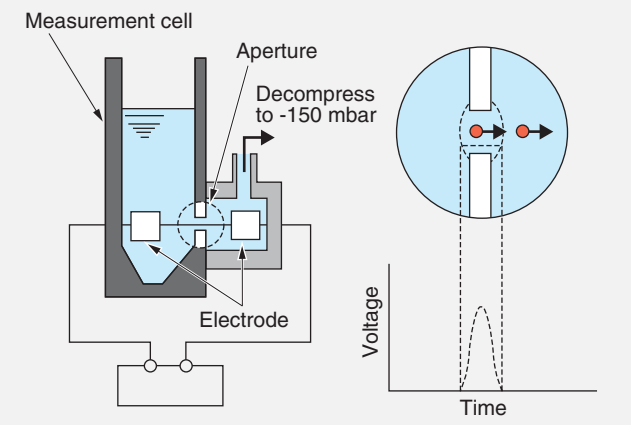

Figure 2 Measurement principle of WBC/RBC/PLT (Impedance Method)

- Particle Distribution of Blood Cell

  The shape of blood cells is not always fixed according to the type of blood cell, and the shape of a cell may even change during the course of measurement. To correct this interference and obtain an accurate blood cell counting, the obtained pulse heights (blood volume) are divided into multiple channels according to each blood cell and measured in the optimum pulse height range.

  The following measurement conditions are set for LC-178 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 measurement range is automatically set in approximately 1 to 27 fL.

- Three-part Differential of WBC

  White blood cells serve the body's defense system, and counting white blood cells 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-178 CRP is able to measure the three-part differential of monocytes, lymphocytes, and granulocytes; these are especially important among the five types.

  Figure 3 shows the threshold values of the three types with the typical histogram.

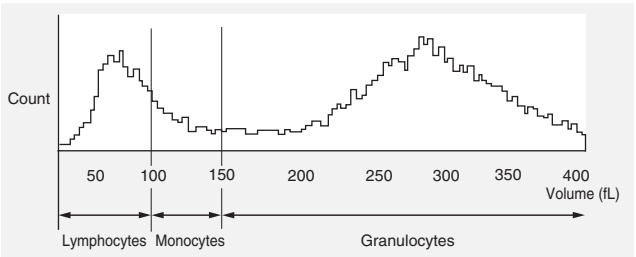

Figure 3 Threshold Values of Three-part Differential WBC Analysis

- Principle and Reagent of WBC Differentiation

  The differentiation of white blood cells is carried out using special diluents (Minoton 3D) and hemolyzing agent (Minolyse 3D) for three-part differentiation, and is based on the difference in the change in volume of each type. The diluents protect the membranes of white blood cells. The hemolyzing agent gives shrinking action to the three types of white blood cells, after breaking red blood cells and platelets. For lymphocytes, the hemolyzing agent causes release of water from the

cytoplasm, making cells contract (nucleate). For monocytes and granulocytes the degree of nucleation is progressively smaller.

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) (Figure 4).

When the hemolyzing agent (Minolyse 3D) is added to the blood sample, the red blood cells are lysed and hemoglobins are released. The hemoglobins are changed to methemoglobins by oxidants in Minolyse 3D, and then cyans in Minolyse 3D join together with the methemoglobins to form cyanmethemoglobins. The cyanmethemoglobin absorbs green light (540 nm) well and thus the Hgb concentration can be determined from the degree of light absorption.

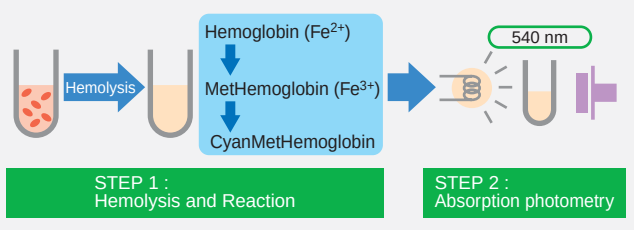

Figure 4 Measurement Principle of Hemoglobin Concentration (Cyanmethemoglobin Method)

CRP Measurement

The CRP concentration is measured by latex immunoturbidimetry. A special hemolyzing agent is used to lyse the blood sample, and then latices with anti-human CRP antibodies are added to cause antigen-antibody reactions with CRP antigens in the blood, which result in agglutination (Figure 5). 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 agglutination and from the hematocrit value (Hct) *3 obtained from the blood cell count. (The measurement method combining the above was patented by HORIBA (No. 3249919 in Japanese patents, No. 6030845 in USA patents, and under application for EU patents), and awarded with the Prize from the President of the Japan Patent Attorneys Association in 2004).

*3: RBC volume ratio in blood

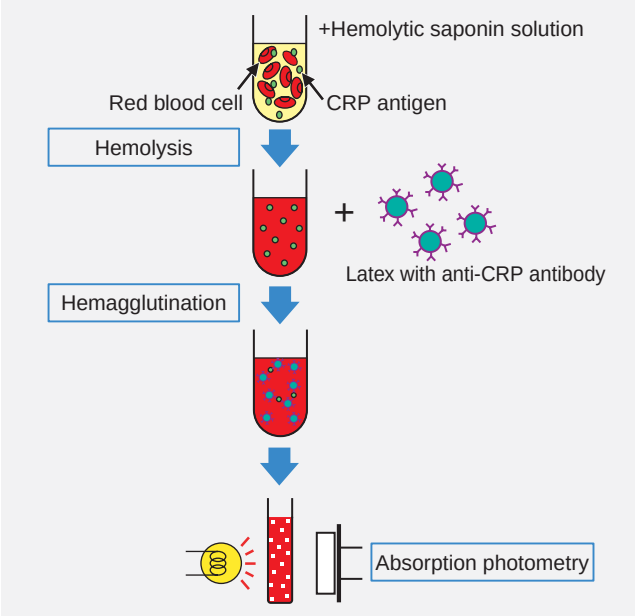

Figure 5 Measurement Principle of CRP Concentration

Measurement Procedure

The sample, reagent and signal flow of the LC-178 CRP is shown in Figure 6.

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. This starts the measurement.

The reagent R1 for CRP measurement (hemolyzing agent: hemolytic saponin solution) 100 µL and the blood sample in the sample tube 8 µL are mixed in CRP-MIX Cell. Next, 10 µL of the blood sample for the blood count is placed in the WBC Cell and diluted to 1/300 with diluents (Minoton 3D). A part of this diluted sample is further diluted to 1/20,000 in the RBC Cell. In the WBC Cell, a hemolyzing agent (Minolyse 3D) 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 µL of CRP measurement reagent R2 (buffer solution) and 200 µL of CRP measurement reagent R3 (coagulant, anti-CRP antibody immobilized on latex solution) 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.

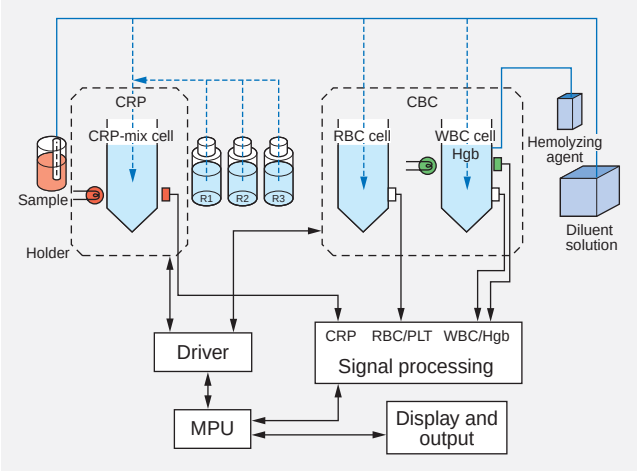

Figure 6 Sample, Reagent and Signal Flow of LC-178 CRP (Pattern Diagram)

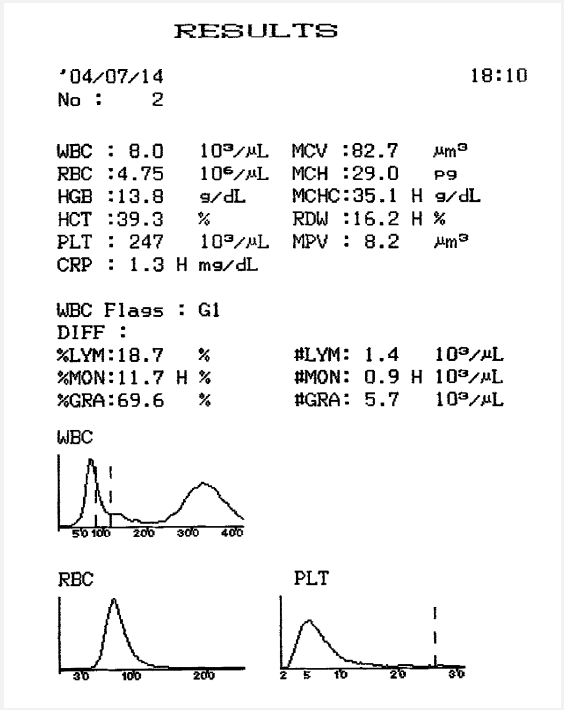

Figure 7 Printout Example of LC-178 CRP

Features

With the advantage of simultaneous, speedy measurement of total 19 items including blood count, three-part differential of WBC, and quantitative CRP simply by setting the sample to the sample holder without pre-treatment, the LC-178 CRP has been further improved to allow the following features:

(1) Widening CRP measurement range

The previous model allows the CRP measurement range in whole blood of 0.2 to 10 mg/dL (for plasma and/or serum, 0.2 to 0.7 mg/dL). LC-178 CRP dramatically improves the linearity of high concentration range due to the improved CRP reagents. As a result, the measurement range has been widened to the double, 0.2 to 20 mg/dL in whole blood, and 0.2 to 15 mg/dL for plasma and/or serum. This attains a growing expectation for a wider range of inflammatory diagnostics.

(2) Sample memory function

With an optional card attached, the LC-175 CRP is now available to record measurement data as many as of 50 samples, and to record/calculate accuracy control data as many as of 30 samples. All of the LC-178CRP models are equipped with the card as a standard specification, allowing the flexible data management necessary for the current and future users.

Table 1 shows the basic specification of LC-178 CRP, and Figure 7 shows the printout example of measurement result.

Table 1 General Specifications of LC-178 CRP

Measurement Mode	CBC mode (blood count only) CBC + CRP mode (blood count + CRP) QC (CBC) mode (quality assurance of blood count) QC (CRP)mode (quality assurance of CRP)
Measurement Item (Including computing and histogram)	WBC (white blood cell count), LYM# (lymphocyte cell count), MON# (monocyte cell count), GRA#(granulocyte cell count), RBC (red blood cell count), Hgb (hemoglobin concentration), Hct (hematocrit value),PLT (platelet count), CRP (C-reactive protein concentration), LYM% (lymphocyte %), MON% (monocyte %), GRA% (granulocyte %), MCV (mean red blood cell volume), MCH (mean red blood cell hemoglobin), MCHC (mean red blood cell hemoglobin concentration), RDW (red blood cell distribution width), MPV (mean platelet volume), PDW (platelet distribution width), PCT (platelet crit value) WBC 3-part diff distribution, RBC distribution, PLT distribution
Measurement Principle	WBC, RBC, Hct, PLT: Impedance method Hgb: Cyanmethemoglobin method CRP: Latex immunoturbidimetry
Sample Volume	CBC mode : 10 μL CBC+CRP mode: 18 μL QC (CBC) mode: 10 μL QC (CRP) mode: 18 μL
Measurement Time	CBC mode : Approx. 75 s CBC+CRP mode : Approx. 270 s QC (CBC) mode : Approx. 75 s QC (CBC + CRP) mode : Approx. 270 s
Size	300 (W) × 400 (D) × 410 (H) (mm)
Mass	Approx. 18 kg (main body)

Evaluation

To confirm the correlation in the widened CRP measurement range of LC-178 CRP, the correlation test of CRP concentration with the previous models was performed in the whole blood. For blood plasma and serum, the correlation test was performed for CRP concentration with a general automatic bio-chemical analysis system as a reference.

(1) CRP concentration correlation with previous model (using conventional CRP reagent)

The same sample was measured by the previous model (LC-175 CRP) and by the new model (LC-178 CRP), to confirm the correlation of CRP concentration. Since the LC-175 CRP uses the conventional type of CRP reagent, the CRP measurement range for the whole blood sample is 0.2 to 10.0 mg/dL. Accordingly, we apply dilution measurement to the whole blood sample for the sample more than 10.0 mg/dL, and multiply the dilution magnification to obtain the measurement value. This process realized a good correlation as in Figure 8, thus verifies the valid measurement value in the widened CRP measurement range of LC-178 CRP.

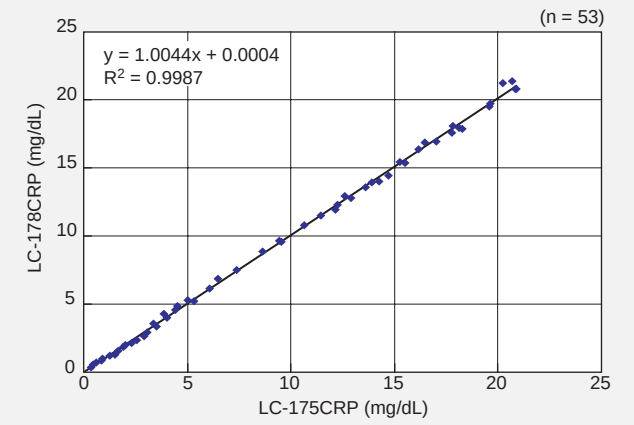

Figure 8 Correlation in CRP Concentration (Whole Blood) for LC-178 CRP and LC-175 CRP (Previous Model)

(2) CRP concentration correlation with reference model

The same whole blood, serum and plasma sample were measured by LC-178 CRP and HITACHI 7170 automatic bio-chemical analysis system (reference model) using the same improved type of CRP reagent, and the correlation in CRP concentration was observed. As shown in Figure 9, 10, and 11, a good correlation was obtained.

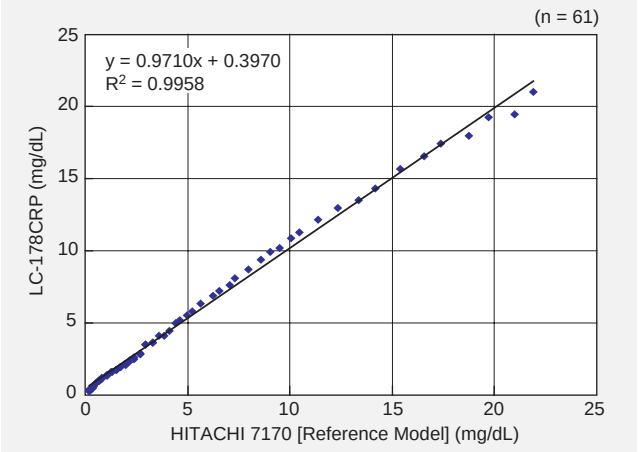

Figure 9 Correlation of CRP Concentration in Whole Blood between LC-178 CRP and HITACHI 7170 (Reference Model)

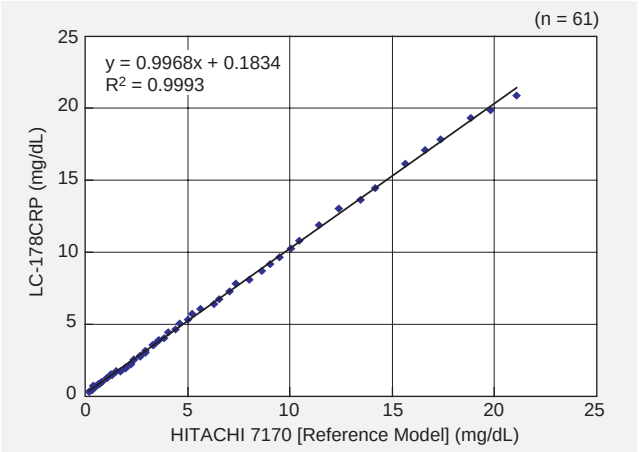

Figure 10 Correlation of CRP Concentration in Plasma between LC-178 CRP and HITACHI 7170 (Referrence Model)

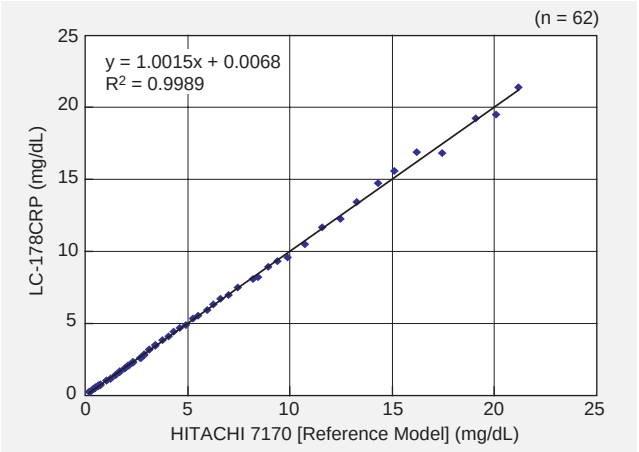

Figure 11 Correlation of CRP Concentration in Serum between LC-178 CRP and HITACHI 7170 (Reference Model)

Conclusion

The LC-178 CRP widened the CRP measurement range to the double of the previous model, LC-175 CRP, popularly used in the clinical sites, with the same excellent feature of simultaneous, speedy measurement of total 19 items including blood count, three-part differential of WBC, and quantitative CRP simply by setting the sample to the sample holder without pre-treatment.

As reform of the medical system progresses at a breakneck pace, and the test system satisfying POCT at a lower cost is strongly demanded, HORIBA's position is to further enhance the LC series, developing examination systems that provides "the required test at the required time and place, 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.

Reference

[1] Yasuo Yamao, Narihiro Oku, Henri Champeix, Automated Blood Cell Count and C-reactive protein Measuring Instruments, Readout No.16, 11-15 (1998)

[2] Yasuo Yamao, The LC-170CRP Automatic Blood Cell and CRP Counter with Three-Part Differential Measurement of White Blood Cells, Readout No.20, 27-31 (2000)

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