

# Evaluation of the rapid CBC and CRP analyzer using small amount of whole blood sample

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## Introduction

C-reactive protein (CRP) was firstly identified in 1930 in patients with pneumonia. Its name was derived from the fact that it reacted with the pneumococcal C-polysaccharide in patients' plasma during the acute phase of pneumococcal pneumonia. Afterwards it has been recognized as an acute-phase protein synthesized by the liver consisting of a cyclical arrangement of five identical subunits (Fig.1). CRP is produced rapidly in response to inflammation and tissue damage, with levels increasing dramatically in the circulation within 24-48 hours of the provoking event.

The early determination of serum concentration of CRP is a well-established laboratory test for the diagnosis and monitoring of different acute inflammatory processes, especially when it is combined with the measurement of leukocyte cell count. In particular their utility is well acknowledged to evaluate possible infection diseases and sepsis.

This is especially important in emergency department and a paediatric patients, where only small amounts of blood should be drawn and rapid and reliable responses are crucial in order to take actions.

Only one system in the market provides these two parameters together. Here we present the results of the performance evaluation of the HORIBA Medical Microsemi CRP, the novel version of this family (Fig.2).

## Methods

This analyser, unique for its features, needs only 18 µL for measurement of blood cell count, with a 3-part differential and CRP performed in 4 minutes.

In the study, a total of 244 samples (145 normal and 99 abnormal) were obtained at the Osaka Medical College Hospital, treated with EDTA-2K anticoagulant and measured for CBC; 120 out of these samples were also tested for CRP.

For the correlation test, the results obtained on the Microsemi CRP were compared to the routine method used in the laboratory for CBC and CRP analysis, the ABX Micros CRP 200.

The repeatability tests were conducted on blood controls (low, normal, high), whole blood patient samples for the CBC and CRP parameters and on serum samples for CRP results.

## Results

The repeatability was evaluated on 5 fresh whole blood samples with increased CRP, in 3 whole blood controls and in 5 serum samples. Each sample was run 10 times in CBC mode and in CBC+CRP mode. Table 1 shows the repeatability in whole control blood (low, normal and high levels) with the parameter CV for each level and the mean CV. Table 2 shows the CV (%) obtained for each parameter in each repeatability series, the range of CV and the mean CV calculated on the 5 repeatabilities. Finally, Table 3 presents repeatability of CRP measurements in 5 serum samples with elevated level of CRP across the all CRP range: minimum and maximum values, mean values, standard deviation and CV are reported. The observed CV were all in agreement with the manufacturer specifications.

The linearity range of CRP has been studied on plasma and whole blood samples. The Microsemi CRP has been tested on a series of increasing concentration of CRP. The graph in Fig.3 shows the correlation of the measured values with the expected values of CRP in plasma samples, where the graph in Fig.4 shows the same type of experiment performed in whole blood samples.

The results confirmed to be in agreement with the manufacturer specifications : 0-150 mg/L for plasma samples and 0-200 mg/L for whole blood samples.

No carry-over was found when high range samples and low range samples were run one after the other following the CLSI guideline (data not shown).

The results of the 244 samples were used to correlate the performances of the Microsemi CRP with the ABX Micros CRP 200 and provided satisfactory results. In particular the CRP measured on the novel generation correlated perfectly with the reference instrument. Table 4 shows the R<sup>2</sup> and the least square regression with the intercept and the slope for the main parameters.

## Conclusions

The Microsemi CRP is the latest generation of the Micros CRP family that can rapidly and reliably measure 3-differential WBC and CRP, uses small volume of whole blood sample (18 µL) and provides results in only 4 minutes. In addition, it presents a wide range of CRP detection, going from 0 to 200 mg/L in whole blood samples and up to 150 mg/L in plasma samples.

The control blood integrates the CRP protein avoiding the need of two separate tubes. The study showed good repeatability for all parameters. No carryover was detected and the linearity, performed on wide concentration range, was in agreement with manufacturer specifications. The comparison of Microsemi CRP with the routine method, the ABX Micros CRP 200, showed very good correlation. The interest of measuring CRP together with CBC is collecting growing approval all over the world. The association of the two parameters, performed in the initial stages of a disease, helps in the differentiation among viral and bacterial infection because of the different kinetic of expression of CRP face to pathogens (low CRP concentration in viral infections with normal or decreased WBC, high CRP concentration in bacterial infections with increased WBC). This differentiation is fundamental to initiate or not antibiotic therapy and to define more specific tests. Consequently, this contributes to make an early diagnosis and to support the disease monitoring. Finally, the optimization of the laboratory tests and a more appropriate antibiotic administration are cost-effective and improve patient healthcare.

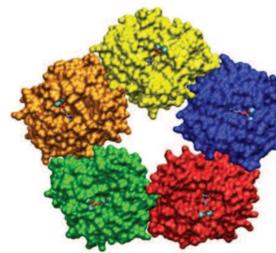


Fig.1. Crystal structure of CRP constituted of 5 subunits of 206 aminoacids each, non covalently assembled. Thompson D et al. Structure (1999)



Fig.2. Microsemi CRP

	WBC	RBC	HGB	HCT	PLT	LYM%	MON%	GRA%	CRP
Low control CV	1.59	1.06	0.52	0.96	4.33	1.45	3.19	2.48	3.81
Normal control CV	1.52	0.64	0.84	0.68	3.02	1.17	3.34	0.50	2.53
High control CV	0.85	0.92	0.37	0.97	1.16	1.24	3.08	0.32	3.21
mean CV	<b>1.32</b>	<b>0.87</b>	<b>0.58</b>	<b>0.87</b>	<b>2.84</b>	<b>1.29</b>	<b>3.21</b>	<b>1.10</b>	<b>3.18</b>

Table 1. Repeatability on whole blood controls

	WBC (CV %)	RBC (CV %)	HGB (CV %)	HCT (CV %)	PLT (CV %)	LYM% (CV %)	MON% (CV %)	GRA% (CV %)	CRP (CV %)
Sample A	1.91	1.49	1.11	1.34	4.66	3.59	4.61	2.58	3.57
Sample B	1.89	1.10	0.86	1.27	2.18	3.15	6.69	1.62	1.87
Sample C	1.17	1.46	0.74	1.57	3.55	3.66	8.07	1.10	1.80
Sample D	2.43	0.64	0.49	0.74	2.30	3.30	3.65	1.18	3.65
Sample E	2.08	0.96	0.68	0.96	3.99	3.61	9.00	1.03	3.78
CV ranges	1.17-2.43	0.64-1.49	0.49-1.11	0.74-1.57	2.18-4.66	3.15-3.66	3.65-9.00	1.03-2.58	1.80-3.78
Mean CV	<b>1.89</b>	<b>1.13</b>	<b>0.78</b>	<b>1.18</b>	<b>3.33</b>	<b>3.46</b>	<b>6.40</b>	<b>1.50</b>	<b>2.94</b>

Table 2. Repeatability on whole blood samples

	CRP mg/L				
1	5.58	19.0	38.0	80.0	138.0
2	5.42	19.0	38.0	76.0	133.0
3	5.27	18.0	37.0	76.0	134.0
4	5.20	19.0	37.0	74.0	137.0
5	5.08	19.0	36.0	75.0	130.0
6	5.10	19.0	37.0	75.0	133.0
7	5.13	19.0	37.0	76.0	130.0
8	5.02	19.0	37.0	77.0	128.0
9	5.30	19.0	37.0	74.0	134.0
10	5.02	19.0	38.0	74.0	130.0
Max	5.6	19.0	38.0	80.0	138.0
Min	5.0	18.0	36.0	74.0	128.0
Mean	5.21	18.90	37.20	75.70	132.70
SD	0.18	0.35	0.63	1.83	3.23
CV	<b>3.54</b>	<b>1.87</b>	<b>1.70</b>	<b>2.42</b>	<b>2.44</b>

Table 3. Repeatability on serum samples

Parameters	R <sup>2</sup>	Least square regression
WBC	R <sup>2</sup> = 0.9993	y = 0.9608x - 0.377
RBC	R <sup>2</sup> = 0.9936	y = 0.9941x + 0.024
HGB	R <sup>2</sup> = 0.9982	y = 0.9749x + 0.148
PLT	R <sup>2</sup> = 0.987	y = 1.0374x - 6.2571
LYM	R <sup>2</sup> = 0.9933	y = 0.9906x + 0.0542
GRA	R <sup>2</sup> = 0.9929	y = 0.9804x + 2.3917
MON	R <sup>2</sup> = 0.9533	y = 0.8935x + 0.0597
CRP	R <sup>2</sup> = 0.9977	y = 1.0671x + 0.0033

Table 4. Correlation of the Microsemi CRP versus the routine method, the ABX Micros CRP 200.

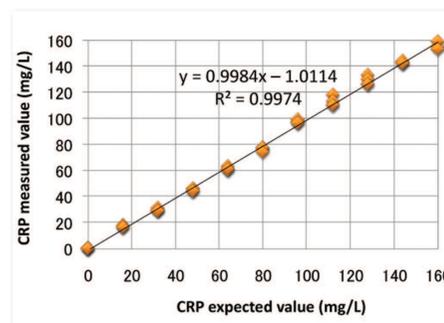


Fig.3. CRP linearity on plasma samples

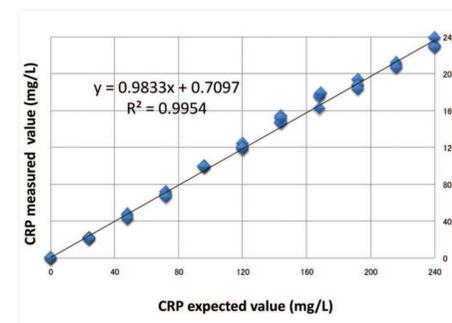


Fig.4. CRP linearity on whole blood samples