Adaptation and Evaluation of the Randox Full-Range CRP Assay on the Olympus AU2700[®]

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The implementation of a high-sensitivity CRP (hs-CRP) assay as a routine laboratory parameter may be necessary. A single CRP method that could yield reliable results for the whole concentration range (0.1–200 mg/L) would be the most practical solution for the laboratory setting. The aim of this study was to assess the Randox full-range CRP assay on the Olympus AU2700[®] biochemistry analyzer and evaluate its analytical performance on serum and heparin plasma samples. The Randox CRP turbidimetric assay was compared with the existing CRP assay used routinely on the Olympus AU2700[®]. The analytical performance of the Randox CRP with both Olympus CRP reagents (CRP for normal application and

hs-CRP) was good. We found that the Randox CRP method in the range of 0.5–160 mg/L was closely correlated to the Olympus CRP and hs-CRP for serum samples. According to a Bland-Altman analysis, the serum and heparinized samples showed an excellent agreement in CRP concentrations throughout the entire range (mean difference = -0.035 ± 1.806 mg/L) as well as in CRP levels <10 mg/L. Our data indicate that Randox full-range CRP measurements using an immunoturbidimetry assay on Olympus systems perform as well for routine diagnostics as other high-sensitivity applications using serum or heparin plasma. *J. Clin. Lab. Anal.* 21:34–39, 2007.

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Key words: full-range CRP; immunoturbidimetry; heparin plasma

INTRODUCTION

As an acute phase protein, C-reactive protein (CRP) has long been recognized as a useful indicator of acute inflammatory events (1). The normal CRP plasma level is around 1.5 mg/L (2) and can rapidly and markedly rise as much as 1000-fold or more after an acute inflammatory stimulus. Traditional CRP tests with an expected range of 5–200 mg/L (3) were designed to monitor acute infection and inflammation. Epidemiological data from recent studies have highlighted an association between a slight CRP elevation and future major cardiovascular events (2,4), leading to a recommendation by the American Heart Association that high-sensitivity CRP (hs-CRP) should be monitored for cardiovascular risk assessment (5). The determination of low-grade inflammation requires an hs-CRP method covering a range of 0.1–5 mg/L. A single CRP method that could provide reliable results for the whole concentration range (0.1–200 mg/L) would be the most practical solution for the laboratory setting.

We evaluated new CRP reagents that cover both low and high concentration ranges. Since many laboratories

(especially in Europe) use plasma rather than serum for many or most of their clinical assays, it appeared critical to evaluate the use of heparinized plasma samples, in comparison with serum, for CRP determination on a biochemistry analyzer. The use of plasma allows minimal collection of blood samples, optimizes the delivery of results, and satisfies most requirements of clinicians and biologists.

The aim of this study was to assess the Randox full-range CRP assay on the Olympus AU2700[®] biochemistry analyzer (Olympus, Rungis, France) and evaluate analytical performances on serum and heparin plasma samples. The Randox full-range CRP turbidimetric assay was compared with the existing hs-CRP and high-concentration-range CRP used routinely on the Olympus AU2700[®].

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Received 5 January 2006; Accepted 28 September 2006

DOI 10.1002/jcla.20160

Published online in Wiley InterScience (www.interscience.wiley.com).

MATERIALS AND METHODS

CRP Methods for Olympus

CRP for Olympus was used as the reference method. The analytical performance was previously assessed by Roberts et al. (6) and validated on serum and heparin plasma in our laboratory (7).

CRP levels were determined by an immunoturbidimetric method applied on the Olympus AU2700[®] biochemistry analyzer. Serum protein multicalibrator and system reagents for CRP (OSR 6147) and CRP latex (hs-CRP and OSR 6185) were obtained from Olympus (Rungis, France). Reagents and calibrators were used according to the manufacturers' recommendations with analytic measuring ranges of 5–200 mg/L for normal applications and 0.5–20 mg/L for the hs-CRP test. Calibrations for CRP and hs-CRP were performed once a week and once a fortnight, respectively. When the CRP level was <5 mg/L, hs-CRP was performed.

Randox Full-Range CRP Method

In the Randox full-range CRP method the sample includes a buffer and anti-CRP-coated latex. The formation of the antibody-antigen complex during the reaction results in an increase in turbidity, the extent of which is measured as the amount of light absorbed at 570 nm. By constructing a standard curve from the absorption of the standards, one can determine the CRP concentration of the sample. The assay buffer and antibody-latex reagent are supplied ready for use and are stable on board the instrument for approximately 28 days. For Randox CRP (CP 3849) on the Olympus AU2700[®], we used a 1/10 initial sample dilution automatically. The sample volume is 3 µL. The analytical measurement range is 0.3–160 mg/L with automatic dilution for results up to 160 mg/L. Sample concentration is determined from a stored six-point calibration curve (values = 0, 2.5, 10, 20, 80, and 160 mg/L) using the Randox CRP Calibrator Series[®] (CP2499). A multipoint calibration is recommended every 28 days with a change of reagent lot/bottle.

Analytical Performance of Randox-CRP

We assessed the analytical performance of Randox-CRP according to the Valtec protocol (8). To estimate the imprecision values (coefficients of variation (CVs)), we prepared pools from six serum and plasma patient samples. This allowed us to determine samples with low, medium, and high values. We determined within-day imprecision through 20 replicated analyses of samples, and between-day imprecision with low, medium, and high values using the same samples divided into aliquots stored at –20°C to prevent potential degradation. For 20

days, one aliquot of each concentration was thawed for analysis at two different times per day on the basis of a single calibration.

Serum and plasma samples with CRP concentration of 150 mg/L were used to test the linearity of CRP. These samples were diluted at concentrations of 150, 75, 37, 18, 9, 4.7, 2.3, 1.2, and 0.5 mg/L.

We checked the recoveries by adding purified CRP (Sigma, St. Quentin Fallavier, France) to serum and heparin plasma containing CRP <0.1 mg/L in order to increase the concentration from 0.1 to 2 µg/L on the range 0.3–2 mg/L, and to 2, 5, 10, 25, 50, 100, and 150 mg/L on the range of 2–160 mg/L. Recovery tests were performed for both the Randox and Olympus CRP methods. Samples were assayed in duplicate on five different days with the use of both methods.

Clinical Samples, Comparison Studies, and Statistical Analysis

CRP determination covering the analytical range from 0.1 to 160 mg/L was performed simultaneously on both heparin plasma and serum samples obtained from 128 consecutive patients admitted to different clinical departments of Lapeyronie Hospital.

A linear regression analysis was used to compare the Olympus CRP method with the Randox method. We estimated the slope, intercept, and correlation coefficient (r^2). For each method we examined the plots $Y = f(X)$, where X represents the data obtained from the comparison, and Y represents the data obtained from the evaluation. In addition, according to recommendations for method-comparison studies, the scatter of differences was visualized by means of Bland-Altman plots (9,10). A statistical comparison was performed with the use of Student's *t*-test. A *P*-value <0.05 was required for statistical significance.

RESULTS AND DISCUSSION

CVs, Linearity, and Recoveries

Table 1 shows the total intra-/interassay and total imprecision CVs for serum and plasma Randox CRP. The CV in the range of 0.5–5 mg/L was very low (<5.2%). Assay imprecision is a critical performance parameter when hs-CRP is used to assess cardiovascular risk. It is clearly demonstrated that for risk stratification for cardiovascular disease, CRP imprecision should be <10% at a concentration of 0.2 mg/L (4–6,11). Therefore, Randox-CRP can be used to estimate the risk of cardiovascular events.

The coefficients of linearity (r^2) obtained for serum and plasma with Randox-CRP were 0.998 and 0.999, respectively (Fig. 1). The results from the linearity

analyses of serum samples were comparable to those obtained with heparin plasma.

When CRP was added to serum samples to increase their concentrations, as described in Materials and Methods, the mean recoveries varied from 91% to

101% with the Olympus and Randox assays, respectively. However, recoveries varied from 75% to 87% for the range of 0.3–2 mg/L, and from 106% to 115% for the range of 2–160 mg/L with the Olympus and Randox assays, respectively. Details of the dilution recoveries are summarized in Table 2. The same data were obtained using heparin plasma samples with the two assays. Values observed in the high range led to overestimation of both Olympus and Randox CRP, whereas in the low range an underestimation was observed. This could be attributable to the CRP used in this study, which was a synthetic molecule in concentrated solution marketed by Sigma. We believe the unsatisfactory data obtained in the recovery studies are due to an inadequate environment for both of the immunoturbimetric methods. Despite this slight limitation, we assume that the performance of the CRP reagent assay was acceptable in the dilution recovery studies.

TABLE 1. Summary of Imprecision data on serum and heparin plasma samples with Randox-CRP full range

	Mean concentration (mg/L)	CVs intra-assay (%)	CVs inter-assay (%)	Total CVs
Serum	1.39	4.1	5.2	5.7
	6.31	1.4	2.8	3.2
	14.61	2.5	2.2	5.3
	38.37	2.0	1.6	2.5
	77.76	1.1	2.5	2.5
	146.53	2.2	3.5	4.1
Heparin plasma	0.78	4.5	4.4	7.8
	6.38	1.7	1.8	9.1
	16.69	1.7	2.2	3.1
	39.62	1.4	2.2	5.1
	81.88	1.7	1.3	7.2
	153.44	1.3	1.5	2

Comparison Analysis

We found that the Randox CRP method in the range of 0.5–160 mg/L was closely correlated to the Olympus

Fig. 1. Randox-CRP full-range linearity at concentration < 160 mg/L. The high pool was diluted to 100%, 50%, 25%, 12.5%, 6%, 3%, 1.5%, 0.75%, and 0.5%. Serum (A) and heparin (B) samples were assayed in triplicate in one analytical run at each CRP level. The plotted points represent the mean of three replicates. The correlation coefficient of linear regression is shown.

TABLE 2. Mean of recoveries of Randox-CRP and Olympus-CRP assays according serum or heparin plasma used

	Observed values (% of recoveries) ^a			
	By Randox-CRP on serum	By Olympus-CRP on serum	By Randox-CRP on heparin plasma	By Olympus-CRP on heparin plasma
Recoveries on range from 2 to 160 mg/L				
100	127.8 (127.8)	117.6 (117.6)	120,4 (120.4)	115,6 (115.6)
50	58.5 (117)	53.7 (107.4)	56.7 (113.4)	53,7 (107.4)
25	28.7 (114.8)	26.3 (105.2)	28.4 (113.6)	26,4 (105.6)
10	11.5 (115)	10.15 (101.5)	11.2 (112.)	9,76 (97.6)
5	5.4 (108)	5.33 (106.6)	5.6 (112.)	5,11 (102.2)
2	2.3 (115)	2.2 (110)	2.4 (120)	2,04 (102)
Mean of recoveries %	116.26	108.05	115.04	105.33
Recoveries on range from 0.3 to 2 mg/L				
0.1	0.1 (100)	0,1 (100)	0.1 (100)	0.1 (100)
0.19	0.2 (105.2)	0,18 (94.73)	0.2 (105.2)	0,18 (94.73)
0.27	0.3 (111.1)	0,22 (81.48)	0.3 (111.1)	0,22 (81.48)
0.41	0.4 (97.56)	0,36 (87.80)	0.42 (102.43)	0,36 (87.8)
0.53	0.4 (75.47)	0,32 (60.37)	0.4 (75.47)	0,36 (67.92)
1.05	0.7 (66.66)	0,56 (53.33)	0.7 (66.66)	0,56 (53.33)
1.52	0.9 (59.21)	0,79 (51.97)	0.9 (59.21)	0,81 (53.28)
2	1.7 (85)	1,33 (66.5)	1.7 (85)	1,35 (67.5)
Mean of recoveries %	87.52	74.52	88.13	75.75

^aValues of pure serum or plasma: <0.1 mg/L. **Fig. 2.** **A:** Comparison of the Randox-CRP method with the Olympus-CRP and Olympus-hs CRP methods in 128 serum samples. **B:** Bland-Altman plot analysis of the compared methods. The difference between two values in the y-axis is plotted against the mean value in the x-axis. The solid line represents the mean difference in measured CRP concentration between the methods, and the dashed lines indicate ± 2 SD.

Fig. 3. Comparison of serum vs. heparin samples with Randox-CRP.

(A) correlation between Olympus hs-CRP vs Randox-CRP for serum samples with CRP<10mg/l (n=46)

Fig. 4. **A:** Correlation between Olympus hs-CRP and Randox-CRP for 46 serum samples with CRP <10mg/L. **B:** Bland-Altman plot analysis of the compared methods. The difference between two values in the y-axis is plotted against the mean value in the x-axis. The solid line represents the mean difference in measured CRP concentration between the methods, and the dashed lines indicate ± 2 SD.

CRP and hs-CRP for serum samples (Fig. 2). The slope was close to 1.00 with a positive intercept (0.51). In the lower range (0.5–20 mg/L) a high correlation coefficient ($r^2 > 0.996$) was obtained, and the two assays gave identical results (data not shown).

CRP was diagnosed simultaneously in serum and heparin plasma samples from all 128 patients. The Olympus assay was previously validated on serum and heparin plasma (7). The values of Randox CRP measured on serum correlated strongly with those

determined on heparin plasma, ranging from 0.5 to 160 mg/L (Fig. 3). The regression analysis between serum (x) and heparin plasma (y) values led to correlation coefficients >0.99 (Fig. 3). Moreover, the regression almost passed through true zero, showing the comparability of the two types of samples. No significant difference was found when the median value for serum (22.9) was compared with the median value of heparinized plasma (22.8). According to the Bland-Altman analysis, the serum and heparinized samples showed an excellent agreement in CRP concentrations throughout the entire range (mean difference = -0.035 ± 1.806 mg/L).

Considering the values <10 mg/L ($N=46$), the median value for the Olympus hs-CRP assay (3.79) did not significantly differ from that for the Randox-CRP (3.75). We assessed the degree of agreement between the two assays using the Bland-Altman analysis (Fig. 4). The mean difference was -0.04 ± 0.14 mg/L (Fig. 4). No statistical difference between heparin plasma and serum means was found. When the study population was divided into tertiles of 0.5–10 mg/L, 95.7% of the subjects were classified as “concordant,” and 4.3% of the subjects varied by one tertile. No subject varied by two tertiles.

Our data indicate that Randox full-range CRP measurements using an immunoturbidimetry assay on Olympus systems perform as well for routine diagnostics as other high-sensitivity applications using serum or heparin plasma.

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