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Effect of sample tube type and time to separation on *in vitro* levels of C-reactive protein

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Highly sensitive C-reactive protein (hs-CRP) is assuming increased importance in the evaluation of patients with coronary artery disease (CAD). It is a prognostic indicator in acute coronary syndromes[1-3] and a predictor of future coronary events in those with and without overt CAD.[4,5] As small changes in hs-CRP concentration potentially have considerable clinical impact, all aspects of the analytical process need to be evaluated.

Highly sensitive CRP has undergone numerous evaluations on pre-analytical and analytical variability. In healthy individuals, hs-CRP exhibits no diurnal variation,[6] and hs-CRP shows little variation over a 12-month period, with a similar stability of measurement to total cholesterol.[7] Analytical variability of hs-CRP assays has been assessed recently by Roberts and colleagues, who reported coefficients of variation (CV) <10% at 0.15 mg/L.[8]

It is widely recognised that sample collection tube type may affect analyte concentrations. In particular, 'gel' tube effects have been described for anticonvulsant drugs[9] and intact parathyroid hormone.[10] However, the effect of sample collection tube type on hs-CRP concentration has not been studied.

This study aims to assess the stability of serum and plasma hs-CRP in different collection tubes over a 24-hour period, using the DPC Immulite highly sensitive CRP assay.

Blood samples were collected from seven patients with chronic renal failure (on dialysis) into a 10 mL plain glass tube (tube A; Lip Z10/GN), a 2.7 mL EDTA tube (tube B [2.7 ml KE]; Sarstedt Monovet, Germany) and a 4.2 mL gel tube (tube C [4.2 ml,Z GEL]; Sarstedt Monovet).

Samples were transported to the laboratory on ice and remained unseparated at room temperature (17–23°C) until centrifuged. At 20 min (baseline), 1, 2, 4, 8 and 24 h, a 1 mL sample was taken from each tube and centrifuged. The resultant supernatant was frozen at –20°C until analysis, which was carried out in one batch using a DPC Immulite high sensitivity CRP assay (LKCR, Diagnostic Products, Los Angeles; intra-assay CV = 4.1%), immediately after thawing. Statistical analysis was by repeat measures ANOVA and, where significant, was followed by Tukey-Kramer post-test comparison.

Results of tube-type effect and time on hs-CRP values are shown in Table 1. Significant differences were observed in samples separated at 0.3 h (ANOVA P=0.0192), and hs-CRP values were significantly higher (P<0.0.5) in gel tubes than in plain and EDTA tubes. Otherwise, there were no significant between-tube differences in hs-CRP concentration.

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Table 1. Mean CRP concentration (mg/L) for each sample tube against time

	Time					
	0.3 h	1 h	2 h	4 h	8 h	24 h
Tube A (Plain)	17.0(17.3)	16.6(16.8)	17.2(17.9)	17.4(17.7)	17.7(18.1)	17.8(17.6)
	A/C+					
Tube B (EDTA)	16.8(17.1)	17.2(17.8)	16.7(16.4)	16.0(16.1)	15.9(16.6)	17.2(18.2)
	B/C+					
Tube C (Gel)	18.5(18.3)	17.6(17.7)	17.4(17.3)	17.5(17.9)	16.6(16.3)*	16.7(16.9)*

Results are mean (Standard Deviation)
Within sample tube C (Gel), compared to 0.3 h * = P<0.05
Between sample tubes. A/C + = P<0.05, B/C + =P<0.05

Within the tube type, there were no significant differences in hs-CRP concentration in samples taken into plain tubes and EDTA tubes over the 24-h period. In gel tubes, however, there were significant differences over time (*ANOVA* $P=0.03$), with hs-CRP concentration significantly lower ($P<0.05$) in the samples separated at 8 and 24 h, compared with that in the basal sample separated at 0.3 h. In gel tubes, hs-CRP concentration decreased by 9.7% over 24 h.

These results demonstrate a significant decline in hs-CRP concentration over time, when blood is collected into a gel tube, and support previous studies reporting the effect of gel tubes on other analytes such as therapeutic drugs[9] and intact parathyroid hormone.[10]

Several studies have proposed that hs-CRP be used as a prognostic indicator in acute coronary syndromes[1-3] or a predictor of future coronary events.[4,5] However, the significant decline in hs-CRP in gel collection tubes observed over 24 h in this study could lead to the misclassification of patients in samples left unseparated for eight or more hours. This may be particularly important for samples collected in the community for evaluation of CAD risk.

In conclusion, results from this study suggest that caution should be exercised in interpreting hs-CRP results when the sample is collected into a gel tube, as we report a significant decline in hs-CRP concentration over time. □

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Enzyme-linked immunosorbent assay for β_2-glycoprotein I quantitation: the importance of variability in the plastic support

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β_2-glycoprotein I (β2GPI) is a poorly understood plasma protein that is thought to be an important autoantigen in the antiphospholipid syndrome. In addition, β2GPI may be a key player in the phospholipid-dependent coagulation pathway, and in the clearance of liposomes, phosphatidylserine-expressing cells and foreign particles.[1-9] It shows a high propensity to bind negatively charged surfaces, including phospholipids and irradiated plastic plates.[10] Binding to phospholipids is accompanied *in vitro* by the inhibition of intrinsic coagulation pathway activation,[4] ADP-induced platelet aggregation,[5] prothrombinase activity of activated platelets,[6] as well as the anticoagulant activity of activated protein C.[11]

As relatively little is known about the pathophysiological role of β2GPI, a reliable assay to determine plasma levels would further our understanding of its functions in health and disease. However, non-specific binding of β2GPI to plastic surfaces could significantly decrease the accuracy and reliability of an enzyme-linked immunosorbent assay (ELISA) designed to measure it.

In order to study the effect of non-specific binding of β2GPI to plastic and to determine a normal range for β2GPI concentration in sera from female and male subjects, this study establishes a capture ELISA based on the protocol designed by McNally *et al*.[12] Nunc A/S (Kamstrup, Roskilde, Denmark) provides two commonly used sets of γ-irradiated plates; one certified for consistency in adsorption of protein, the other uncertified. Here, we test six batches of γ-irradiated Nunc Maxisorp 96-well flat-bottomed polystyrene ELISA plates for reproducibility; one of which was certified by the manufacturer for homogeneity in adsorption of IgG.

Briefly, the ELISA plates were incubated overnight at 4°C with 100 μL mouse monoclonal anti-human β2GPI (Chemicon International Inc., Temecula, CA, USA) at a

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