Table 1. Stability of Hcy in EDTA- and citrate-anticoagulated whole blood.

Hours post collection[a]	Fold increase in Hcy, mean ± SD	
	EDTA[b]	Citrate[c]
0	1.0	1.0
2	1.1 ± 0.1	1.0 ± 0.2
4	1.3 ± 0.2	1.2 ± 0.2
8	1.5 ± 0.2	1.6 ± 0.5
24	1.9 ± 0.2	1.8 ± 0.4

[a] For each time point, n = 7 healthy volunteers.
[b] Relative to EDTA result from immediate analysis.
[c] Relative to citrate result from immediate analysis.

agree well with EDTA results if the citrate value is multiplied by 1.163 for females and 1.18 for males. No difference in specimen stability over time was evident in citrate tubes compared with EDTA tubes.

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Serum C-Reactive Protein in Canadian Inuit and Its Association with Genetic Variation on Chromosome 1q21, *Robert A. Hegele,[1*] Matthew R. Ban,[1] and T. Kue Young[2]* ([1] John P. Robarts Research Institute, London, Ontario, N6A 5K8 Canada; [2] Department of Community Health Sciences, University of Manitoba, Winnipeg, Manitoba, R3E 0W3 Canada; * address correspondence to this author at: Blackburn Cardiovascular Genetics Laboratory, John P. Robarts Research Institute, 406-100 Perth Dr., London, Ontario, N6A 5K8 Canada; fax 519-663-3789, e-mail robert.hegele@rri.on.ca)

Mortality from cardiovascular disease among Inuit living in the far north of Canada is ~40% lower than in the rest of the country *(1)*. This might be attributable to the protection resulting from environmental factors such as dietary ω-3 fatty acids in Arctic fish *(1)*. Genetic factors may also be important. For example, the thermolabile variant of methylenetetrahydrofolate reductase is much less prevalent among Inuit than among subjects of European origin *(2)*. However, there are also paradoxical genetic findings in these people. For example, some common genetic variants associated with a higher risk of cardiovascular disease, such as the *APOE E4* and *AGT* T235 alleles, are more prevalent among Inuit than among subjects of European origin *(3)*. These apparent inconsistencies may be related to the fact that there are numerous determinants of susceptibility to cardiovascular disease and that these determinants may differ among ethnic groups *(4)*. As newer determinants of cardiovascular disease risk are identified, these can be evaluated in the Inuit.

C-Reactive protein (CRP), an acute-phase reactant originally detected through its interaction with pneumococcal C polysaccharide *(5)*, has been proposed to be a risk factor for cardiovascular disease *(6)*. When detected with a high-sensitivity assay *(6)*, increased serum CRP could be related to increased vascular disease risk either directly through its association with inflammation *(7)* or indirectly through its association with obesity and insulin resistance *(8)*. The serum CRP concentration in the Inuit has not been reported. Furthermore, the role of possible genetic determinants of serum CRP concentration has not been explored in depth. Our recent discovery of a silent single-nucleotide polymorphism (SNP) in the *CRP* gene, namely 1059G→C within exon 2 *(9)*, and of other chromosome 1q21 SNPs near *CRP (10)* has allowed analysis of the association between the *CRP* locus and serum CRP concentration in Inuit.

The Northwest Territories are located above the 60th parallel of latitude and comprise one-third of the landmass of Canada. In 1986, the population of Northwest Territories was 52 000. Of these, 35% were Inuit (or Eskimos), 15% were Dene (or Athapaskan Indians), and 50% were predominantly migrants of European origin from other parts of Canada. The present study involved residents of eight communities from the Nunavut region, mainly from the western shore of Hudson Bay *(2, 3, 11)*.

Randomly selected individuals (n = 516; age range,

18–80 years) participated; of these, 281 reported themselves as being Inuit, 112 reported themselves as being of mixed ethnic background, 92 reported themselves as being of European background (white), and 31 reported themselves as being of an ethnic background other than Inuit, mixed, or white. At the time of the study, these communities adhered to a traditional lifestyle, which included the consumption of Arctic fish at least three times per week. Of note was the very high prevalence of cigarette smoking *(11)*. The white subjects were included as a contrast sample, which provided reference serum CRP concentration and SNP allele frequency from a regional control white population. The project was approved by the Institutional Review Boards of the Universities of Manitoba and Toronto.

Blood samples were obtained with informed consent. The first exclusion criterion was a self-reported ethnic background that was neither Inuit nor white. This left 373 subjects. The second exclusion criterion was an inadequate blood sample for biochemical and genetic determinations. This left 237 subjects, of whom 180 were Inuit and 57 were white. Serum CRP was measured using a high-sensitivity automated nephelometric method (Beckman Coulter) with a reported lower limit of detection of 0.05 mg/L. Genotypes were determined as described *(9, 10)*, and SAS, Ver. 6.12, was used for all statistical comparisons.

Baseline attributes of the study subjects are shown in Table 1. The Inuit subjects had significantly higher smoking prevalence than the white controls. Serum CRP was not significantly different, but tended to be higher in the Inuit ($P = 0.10$). Sources of variation of serum CRP were then evaluated. Partial regression coefficients from stepwise regression analysis in all subjects indicated that 71%, 16%, and 13% of the attributable variation in serum CRP was related to age, ethnicity, and smoking, respectively (all $P < 0.05$). Univariate analyses performed in Inuit showed serum CRP to be significantly correlated only with age ($r = 0.30$; $P < 0.0001$), the ratio of waist-to-hip circumference ($r = 0.25$; $P < 0.0001$), and body mass index ($r = 0.19$; $P < 0.005$).

The *CRP* 1059C allele frequency was 0.122 in white controls compared with 0.0 in Inuit ($P < 0.0001$). We also genotyped the closely linked *CTSS* gene using genotypes derived from the −25G→A promoter SNP *(10)*. The *CTSS* −25A allele frequency was 0.404 in Inuit and 0.468 in white controls (*P*, not significant). Frequencies did not deviate from those predicted by the Hardy–Weinberg equation in either ethnic group for both genotypes. Chromosome 1q21 haplotypes were created using the *CRP* and *CTSS* genotypes. Multivariate analysis of variance in the Inuit showed that chromosome 1q21 haplotype ($P = 0.0014$) and age ($P = 0.0053$) were each significant sources of variation of serum CRP. Pairwise analyses showed that subjects who were *CTSS* −25A/A homozygotes (11.0 ± 2.1 mg/L) had significantly higher serum CRP than both −25G/A heterozygotes and −25G/G homozygotes (4.1 ± 1.4 and 3.5 ± 1.5 mg/L, respectively; both pairwise $P < 0.005$). Regression analysis in the white control sample showed that no independent variable was a source of variation of log CRP (all $P > 0.05$).

These findings indicate that (*a*) serum CRP in Inuit tended to be higher than in a sample of regional white controls; (*b*) age, cigarette smoking, and obesity were each associated with CRP in the Inuit; and (*c*) chromosome 1q21 haplotype was associated with serum CRP variation. The findings suggest that protection from vascular disease in the Inuit is not associated with serum CRP. The association of serum CRP with obesity indices in the Inuit is consistent with the findings of previous studies *(7)*. The association between serum CRP and chromosome 1q21 haplotype could have been attributable to the impact of an unexamined functional variant within or flanking the *CRP* gene. Alternatively, the association might have resulted from linkage dysequilibrium involving other structural differences at or near the *CRP* gene locus.

Although the basis for the distinctive cardiovascular risk profile of Canadian Inuit remains to be determined, these people do not have lower serum CRP compared with white subjects. There is increasing interest in inflammatory etiologies for atherosclerosis and cardiovascular end points *(8)*. Thus, it is reasonable to consider the possibility that interindividual differences in quantitative inflammatory traits, such as serum CRP, may contribute to differences in disease expression. The results in the Inuit suggest that the low prevalence of vascular disease is unrelated to serum CRP concentration. However, the results also indicate that variation near the *CRP* gene locus on chromosome 1q21 is significantly associated with variation in serum CRP concentration and that this intermediate trait may be subject to genetic control.

Table 1. Baseline clinical and biochemical features (mean ± SE).

	Inuit	European	P
n	180	57	
Age, years	36.6 ± 0.78	37.3 ± 1.16	NS[a]
Female, %	58.9	47.3	NS
BMI, kg/m^2	26.3 ± 0.24	26.9 ± 0.53	NS
Diabetes, %	0.6	1.8	NS
Smoking, %	66.5	23.2	0.0007
CRP, mg/L	5.9 ± 0.89	3.9 ± 0.24	NS

[a] NS, not significant; BMI, body mass index.

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Analytical Performance of Specific-Protein Assays on the Abbott Aeroset System, *Ellen B. Duly,* George Barnes, Sandra Grimason, and Thomas R. Trinick* (Clinical Chemistry Laboratory, Ulster Hospital, Dundonald, BT16 1RH, Northern Ireland; * author for correspondence: fax 44-0-2890487131, e-mail ellie.duly@ucht.n-i.nhs.uk)

The Abbott Aeroset® is an automated, discrete, random-access clinical chemistry analyzer with a stated throughput of up to 2000 tests per hour and is capable of rate and end–point photometry, turbidimetry, and potentiometry. Abbott has recently developed turbidimetric assays for proteins [IgG, IgA, IgM, transferrin, haptoglobin, prealbumin, apolipoproteins (apo) A1 and B, and complements C3, C4] for the Aeroset system. The purpose of this study was to evaluate the analytical performance of these Abbott Aeroset specific-protein assays and, in particular, their ability to correctly handle antigen excess frequently found in immunoglobulin estimations.

Patient samples that had been sent to the laboratory for routine clinical chemistry investigations were collected and frozen over a 3-month period. The study was approved by the local Medical Ethics Committee. Samples were also obtained from the United Kingdom National External Quality Assessment Scheme and the Welsh External Quality Assessment Scheme. Assays were performed according to the manufacturer's recommendations on the Abbott Aeroset analyzer. Sample volume requirements were 2.0–6.5 μL with reagent volumes <200 μL. The Beckman Array Nephelometer and specific-protein reagents were used for comparison studies.

Precision was evaluated according to NCCLS protocol EP5-T2 *(1)* by use of single lots of reagents, calibrators, and controls (normal and high Biorad Immunochemistry controls, LVT1 and LVT2) for the entire study. Within-run imprecision (CV; n = 20) ranged from 0.4% for IgM (mean = 1.17 g/L) to 2.0% for apo A1 (mean, 1.32 g/L). Between-day and total imprecision were measured on two daily analytic runs for 20 days over a 67-day period by use of two control replicates per test (n = 80). The between-day CVs for control samples were <3.0% for all assays with the exception of apo B [LVT2, 6.5% (mean, 0.55 g/L); LVT1, 4.0% (mean, 1.09 g/L)]. The mean total CV across all specific-protein applications was 2.7%. The total CV for 19 of 20 controls was ≤5%, which met the minimum precision criteria on the basis of medical need *(2, 3)*. The exception was apo B (6.9% CV; mean, 0.55 g/L; minimum precision goal of 5.2%). Of the 10 specific-protein applications evaluated, all met the manufacturer's claims for total SD.

Linearity, evaluated by NCCLS protocol EP6-P *(4)*, showed all assays linear across the ranges of their calibration curves: IgG, 1.25–37.36 g/L; IgA, 0.29–7.23 g/L; IgM, 0.14–3.43 g/L; transferrin, 0.54–5.38 g/L; haptoglobin, 0.11–2.77 g/L; prealbumin, 0.12–0.60 g/L; apo A1, 0.33–3.32 g/L; apo B, 0.26–2.56 g/L; complement C3, 0.14–3.22 g/L; and complement C4, 0.03–0.60 g/L.

Detection limits were determined by measuring 20 replicates of saline and 20 replicates of the lowest concentration calibrator and calculating the mean concentration of saline +2 SD of the lowest concentration calibrator. The limit of detection for assays was 0–0.02 g/L, with the exception of IgG at 0.11 g/L.

A within-run precision profile was constructed for IgA by analysis of 20 replicates of 15 patient samples with concentrations near the expected limit of detection (range, 0.01–0.11 g/L). Imprecision (CV) was then calculated for each patient sample and plotted as a function of observed analyte concentration. A 20% CV was seen at 0.02 g/L and a 10% CV at 0.03 g/L. The quoted lowest reportable concentration for the Beckman array IgA is 0.07 g/L.

Method comparison was performed according to NCCLS protocol EP9-T *(5)*. Patient samples were measured on both the Aeroset and Beckman Array within a 2-h time span. A minimum of 52 serum samples were selected for each assay, with analyte concentrations evenly distributed over the assay range. Results of Bland-Altman analysis *(6)* are shown in Table 1. Overall, we found the Abbott Aeroset turbidimetric methods were in agreement with the Beckman Array nephelometric methods. The on-board Aeroset assay protocol for IgA and IgM includes an automated dilution step, so that each sample is analyzed undiluted and diluted, and a ratio is calculated. Results outside a user-defined acceptability criterion for the ratio (e.g., outside 0.5–1.5) suggests antigen excess, and patient results are flagged. Flagged samples are subsequently further diluted (1:10) manually with saline and reassayed. All antigen-excess samples observed for IgA and IgM were appropriately detected by the analyzer. The quoted upper limit for IgG before antigen excess is reached is 95 g/L. The highest concentration of IgG in the current study was 81.4 g/L.

Four replicates of each sample obtained from United Kingdom National External Quality Assessment Scheme and Welsh External Quality Assessment Scheme were measured on both the Abbott Aeroset and Beckman Array within a 2-h time span. The mean bias from the overall mean method value for each sample was calculated.