Stability of Soluble Adhesion Molecules, Selectins, and C-Reactive Protein at Various Temperatures: Implications for Epidemiological and Large-Scale Clinical Studies, Janine Hartweg, Michael Gunter, Rafael Perera, Andrew Farmer, Carole Cull, Casper Schalkwijk, Astrid Kok, Harry Twaalfhoven, Rury Holman, and Andrew Neild. Diabetes Trials Unit, Oxford Centre for Diabetes, Endocrinology and Medicine, University of Oxford, United Kingdom; Division of Public Health and Primary Health Care, University of Oxford, United Kingdom; Clinical Chemistry, Institute for Cardiovascular Research VU University Medical Centre, Amsterdam, The Netherlands; † Dr. Carole Cull died in June 2007; ‡ current address: Department of Internal Medicine, University Hospital Maastricht, Maastricht, The Netherlands; * address correspondence to this author at: Diabetes Trials Unit, Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, Oxford, OX3 7LJ, United Kingdom; fax 44 (0)1865 857240, e-mail janine.hartweg@ndm.ox.ac.uk

Background: We assessed the impact of sample storage conditions on soluble vascular adhesion molecules (sVCAM), soluble intracellular adhesion molecules (sICAM-1), soluble (s)E-selectin, C-reactive protein (CRP), and sP-selectin.

Methods: Markers were measured by ELISA in venous blood from 10 healthy volunteers on aliquots stored as plasma or whole blood at 4, 21, or 30 °C for 1–5 days and after 1–5 freeze-thaw cycles. We compared results on these samples to results for samples processed immediately and stored at −80 °C. Statistical models assessed time-related effects and effects of postprocessing conditions.

Results: Using an upper limit of 10% variation from baseline with \( P > 0.05 \), we found that stability duration in plasma was 5 days for sVCAM-1 and sICAM-1 and at least 2 days for sE-selectin at 4, 21, and 30 °C and 5 days for CRP at 4 and 21 °C and 1 day at 30 °C. Stability duration in whole blood was 5 days for sVCAM-1 and sICAM-1 and at least 2 days for sE-selectin at 4, 21, and 30 °C and 5 days for CRP at 4 and 21 °C and 2 days at 30 °C. sP-selectin was not stable in plasma or whole blood. sICAM-1, sVCAM-1, CRP, and sE-selectin were stable after 5 freeze-thaw cycles.

Conclusions: sVCAM-1, sICAM-1, and CRP are stable in plasma or whole blood at 4 and 21 °C for at least 3 days and sE-selectin for 2 days. sP-selectin is not stable and therefore requires immediate assay.

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Multicenter studies that measure the cardiovascular risk markers soluble intracellular adhesion molecules-1 (sICAM-1), soluble vascular cell adhesion molecules-1 (sVCAM-1), soluble (s)E-selectin, sP-selectin, and C-reactive protein (CRP) in serum or plasma require validated procedures for processing, storage, and transport of samples. Previous studies have not systematically examined effects on these markers of different storage temperatures, duration of storage, freeze-thaw cycles, or delays in separating plasma from whole blood (1–5). Venous blood samples were obtained from 10 nonsmoking healthy staff members (7 male, 3 female, age 24–58 years) who had given informed consent. Clinical research nurses collected the samples into 3–7-mL K3 EDTA glass tubes and 12–2-mL K3 EDTA glass tubes (Becton Dickinson) per person. Samples were randomized into 3 groups. Group 1 samples [six 0.5-mL samples from 5 volunteers (4 males) (see Fig. 1 in the Data Supplement that accompanies this article at http://www.clinchem.org/content/vol53/issue10)] were centrifuged immediately, stored at −80 °C, and then subjected to 0–5 room temperature freeze-thaw cycles. Group 2 samples [1 2.5-mL samples from each volunteer (Supplementary Data Fig. 2)] were centrifuged immediately, and the plasma was then divided into 13 aliquots. One aliquot was frozen immediately, and the others were maintained at 4, 21, or 30 °C for 1, 2, 3, or 5 days and then frozen at −80 °C until assayed. Group 3 samples [1 3-mL sample from each volunteer (Supplementary Data Fig. 2)] were split into 13 aliquots, 1 aliquot was centrifuged immediately and the plasma stored at −80 °C, and the remaining 12 aliquots were stored as whole blood under the same conditions as group 2 before centrifugation and freezing of the plasma at −80 °C. Frozen aliquots were packed in dry ice and sent to the VU University Medical Centre, The Netherlands, where samples were thawed, inverted, and vortex mixed before analysis.

All aliquots were assayed in duplicate at room temperature in batches of 39 samples in up to 2 runs per sampling method for each analyte, including the freeze-thaw aliquots, using semiautomated ELISA methods for sVCAM-1, sICAM-1, and high-sensitivity CRP (all obtained from Diacalone) on the CODA automated EIA reader (Bio-Rad) and manual ELISA methods for sE-selectin (Diacalone) and sP-selectin (R&D Systems) (6).

Baseline values used for each analyte were those obtained from assay of plasma from samples that had been separated immediately. The mean percentage change from baseline was calculated for each time point, temperature, and freeze-thaw cycle using the Statistical Package for the Social Sciences (v12.2, SPSS). Analyte values that differed by >10% from baseline and were statistically significant (\( P < 0.05 \)) were considered to indicate unacceptable stability for given storage/handling conditions (7). We used generalized estimating equations to assess correlations in the data and compared the different storage time and temperature values to baseline values for each sample (8). \( P \) values reported are those from generalized estimating equation models obtained using the statistical package STATA (Intercooled STATA 8.2 for Windows).
Mean (range) baseline values for sVCAM-1 were 873 (715–1283) μg/L, sICAM-1 491 (350–864) μg/L, sE-selectin 41 (31–75) μg/L, high-sensitivity CRP 4 (0.17–12.15) mg/L, and sP-selectin 29 (11.5–40.1) μg/L. These values were all within published reference intervals (9–12) after removal of probable outliers (values < 4 SD from the mean) (13). Interassay CVs ranged from 1.3% to 2.4% at 660 to 703 μg/L for sVCAM-1, 3.6% to 5.6% at 348 to 485 μg/L for sICAM-1, 9.9% to 10.0% at 14.2 to 18.3 μg/L for sE-selectin, 3.0% to 6.1% at 0.78 to 1.59 mg/L for CRP, and 9.0% to 9.4% at 30.2 and 38.2 μg/L for sP-selectin. Intraassay CVs were 4.4% for sVCAM-1, 4.0% for sICAM-1, 4.0% for sE-selectin, 3.9% for CRP, and 2.7% for sP-selectin. VCAM-1 and sICAM-1 were stable up to 5 days under all storage conditions in plasma and whole blood, and after 5 freeze-thaw cycles (Table 1), although whole blood gave more reproducible results than plasma for sICAM-1. sE-selectin was stable for up to 2 days at 4 °C in plasma and whole blood and for 5 freeze-thaw cycles. sP-selectin was unstable under all storage conditions and thus requires immediate processing (Fig. 1 and Table 1) (also see Supplemental Data Table 1 and Supplemental Data Table 2 for all results).

This study is the 1st to assess the effects of sample storage for up to 5 days at different temperatures and exposure to repeat freeze-thaw cycles on stability of soluble adhesion molecule and selectin measurements in whole blood and plasma. Previous studies of CRP stability (1, 2, 5, 8, 14–16) did not consider repeated freeze-thaw cycles or higher storage temperatures beyond 3 days in plasma vs whole blood, and investigated only the effects of storage beyond 5 days at 4 or 21 °C. Our sample size was too small to assess concentration-dependent effects, which should be assessed in a similar manner in future studies.

Assays were conducted at an ambient temperature of 21 °C in single batches by 2 laboratory technicians. To improve precision and run-to-run variability, 1 technician used semiautomated analysis methods on 3 of the markers and whole blood and for 5 freeze-thaw cycles. sP-selectin was unstable under all storage conditions and thus requires immediate processing (Fig. 1 and Table 1) (also see Supplemental Data Table 1 and Supplemental Data Table 2 for all results).

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| Table 1. Number days for which analyte values did not differ significantly (P ≥ 0.05) from baseline, with <10% variation after storage as plasma or whole blood at 4 °C, 21 °C, or 30 °C or after a number of freeze-thaw cycles. |
|---|---|---|---|---|---|---|---|
| Temperature | sVCAM-1 | sICAM-1 | sE-selectin | CRP | sP-selectin |
| Plasma (days) | | | | | | |
| 4 °C | 5 | 5 | 2 | 5 | 0 |
| 21 °C | 5 | 5 | 2 | 5 | 0 |
| 30 °C | 5 | 5 | 2 | 1 | 0 |
| Whole blood (days) | | | | | | |
| 4 °C | 5 | 5 | 2 | 5 | 0 |
| 21 °C | 5 | 5 | 2 | 5 | 0 |
| 30 °C | 5 | 5 | 2 | 2 | 0 |
| Freeze-thaw cycles (n) | | | | | | |
| −80 °C | 5 | 5 | 5 | 5 | 0 |
(sVCAM, sICAM, CRP), and manual methods were used on the other assay selectins. A few measurements do not fit any discernable pattern, notably plasma sVCAM-1 on day 3 at 4 and 21 °C; plasma sE-selectin on day 1 at 4 °C and day 3 at all temperatures, whole blood sE-selectin at 4 °C on day 1 and after freeze-thaw cycle 4; and plasma CRP on day 1 at 4 °C and after freeze-thaw cycle 3. These aberrant values were likely analytic artifacts, because removal of values >4 SDs from the mean or adjustment for run-to-run variability did not change the results qualitatively. The large CRP difference observed after freeze-thaw cycle 3 but not after cycles 4 or 5 suggests assay error or possibly CRP release from LDL and complement factors. It has been demonstrated that a portion of systemic CRP is bound to cholesterol in modified LDL particles (17) and to different complement factors (18). Interaction of CRP with cholesterol and complement factors might hamper the detection of CRP in the assay used. No data were available for sE-selectin, but it is possible that some sE-selectin is bound to different circulating leukocyte types or microvesicles derived from endothelial cells. Freeze-thaw cycles might release these bound forms of sE-selectin, leading to an increase in detectable sE-selectin.

Possible effects of residual platelets in the plasma on marker values obtained are not addressed in our study. sVCAM-1, sICAM-1, and sE-selectin, however, are derived mainly from endothelial cells and not from platelets. Although the presence of platelets in EDTA plasma might contribute to the absolute amounts of these markers, it does not influence their stability. In contrast, sP-selectin is mainly derived from platelets, a characteristic that may explain the variance observed in sP-selectin at different time and temperature points.

Our results show that sP-selectin is unstable under all storage conditions and requires immediate assay. The other cardiovascular risk markers evaluated were stable when stored in whole blood samples for several days at room temperature. sVCAM-1, sICAM-1, and CRP were stable at 4 and 21 °C in plasma or whole blood for 5 days and sE-selectin for 2 days. These findings have important implications for clinical studies measuring these markers, reducing the need for immediate transfer of samples to a laboratory for processing and analysis.

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References


