Homocysteine Stability in Heparinized Plasma Stored in a Gel Separator Tube, Roger R. Calam, Ibrahim Mansoor, and James Blaga (St. John Hospital and Medical Center, Detroit, MI; * address correspondence to this author at: Department of Pathology, St. John Hospital and Medical Center, 22101 Moross Rd., Detroit, MI 48236; fax 313-881-4727)

Recent studies have shown that increased blood concentrations of homocysteine (Hcy) have been linked to increased risk of premature coronary artery disease, stroke, and thromboembolism (venous blood clots), even among people with cholesterol values within the appropriate reference intervals (1–3). As testing for Hcy has become more widespread, it is apparent there are preanalytical issues with respect to specimen type and analyte stability. It is well documented that erythrocytes continue to produce and export Hcy into the plasma after venipuncture (4, 5). This can lead to an artifactual increase in Hcy the longer the plasma is allowed to remain in contact with the cells. Several studies have addressed this issue, with EDTA plasma established as the recommended sample, the collected specimens placed on ice and/or centrifuged within 1 h, and the plasma physically removed from cells (6). Because the specimen is anticoagulated, it can be centrifuged immediately, contributing to rapid handling.

It has been documented that expeditious transport and handling of Hcy specimens for epidemiologic studies is difficult because of preanalytical stability requirements (7, 8). We present the analogy that collecting blood for a test from a predominately outpatient population is the same challenge, the same concern, because these samples are usually collected at sites remote from the testing laboratory. Hence, the requirement for prompt removal and freezing of the plasma until testing is difficult to achieve.

The preanalytical variables for Hcy can be controlled either by inhibiting cellular production and release of Hcy or by prompt removal of plasma from the cells. Studies have looked at the possibility of stabilizing Hcy in the collected blood before plasma separation. Citrate has been evaluated (9, 10), as has fluoride, the latter alone or in combination with EDTA. The combination of sodium fluoride and EDTA has been shown to be effective at preserving Hcy in unseparated blood for up to 7 days at 2–8 °C (7), whereas sodium fluoride alone stabilized Hcy at room temperature for only 3 h (11). Serum separation in a gel tube has been studied, with Hcy stable on the gel for 48 h (12). However, serum preparation requires additional time for the blood to clot before centrifugation. We studied the application of a heparin gel tube [plasma separator tube (PST); BD Preanalytical Solutions] to determine Hcy stability when the plasma was stored on the gel barrier at 2–8 °C. Anticoagulated, the collected blood could be centrifuged immediately, contributing to rapid processing. One study was planned, but an additional study was necessary.

For the first study, 21 paired blood specimens, one EDTA and one PST (tubes from BD Preanalytical Solutions), were drawn from apparently healthy laboratory staff (11 females and 10 males). All collected specimens were centrifuged at room temperature at 1200 g within 30 min from time of collection. EDTA plasma was removed from the cells and frozen (−20 °C) in an aliquot tube; heparinized plasma was frozen on the gel. All samples were thawed at room temperature for assay on day 1. Samples were refrigerated until being reassayed on days 3 and 6.

For the second study, 15 paired blood specimens were collected, one EDTA and one PST. In this study, after centrifugation the EDTA-plasma aliquot was not frozen but was refrigerated at 2–8 °C along with the heparinized plasma, which remained on the separator gel. Day 1 testing was the day of collection with plasma refrigerated after the initial assay until being reassayed on days 3 and 4.

All testing was performed with the Carolina Liquid Chemistries Hcy assay, which uses a recombinant enzy-
motic cycling method. The assay was performed on a Hitachi 917 chemistry analyzer.

During the first study, we observed (Table 1, study I) that the EDTA and heparinized plasma were both stable refrigerated for 48 h after thawing. By day 6, Hcy had decreased in both plasma types. We did not expect to observe the significantly increased values for Hcy in the frozen heparinized plasma compared with the EDTA plasma. We speculated that the gel barrier and/or freezing of the plasma on the gel was the cause of the observed difference.

In the second study (Table 1, study II), the significant difference observed in the first study between heparinized and EDTA plasma was not observed when heparinized plasma was stored at 2–8 °C on the gel rather than frozen on the gel. Thus, freezing the plasma on the gel appears to be the contributing factor to the difference and is not recommended.

Repeated-measures ANOVA was used to compare Hcy stability for the 2 plasma types. EDTA plasma Hcy was stable for 48 h, whereas Hcy in heparinized plasma (PST) was stable on the gel for 72 h at 2–8 °C (P = 0.466). Although PST results compared with EDTA results were statistically different (P = 0.0005), the observed differences were only slightly higher and considered to be negligible.

In conclusion, these studies indicate that Hcy cannot be stored frozen on the PST separator gel. However, Hcy is stable for 72 h in heparinized plasma when stored on the gel at 2–8 °C. This stability and ease of handling has considerable practical application for assuring accurate patient results for Hcy assays when there is a time delay between blood collection and testing.

### Table 1. Comparison of EDTA and heparinized plasma at different times in studies I and II.

<table>
<thead>
<tr>
<th></th>
<th>EDTA plasma</th>
<th>Heparinized plasma</th>
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<tbody>
<tr>
<td>Study I (n = 21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>9.8 (2.6)</td>
<td>11.3 (2.8)</td>
</tr>
<tr>
<td>Day 3</td>
<td>9.9 (2.6)</td>
<td>11.4 (2.7)</td>
</tr>
<tr>
<td>Day 6</td>
<td>8.6 (2.5)</td>
<td>9.6 (2.6)</td>
</tr>
<tr>
<td>Day 6 vs day 1, % difference</td>
<td>−12.20</td>
<td>−15.00</td>
</tr>
<tr>
<td>Study II (n = 15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 h</td>
<td>9.4 (2.4)</td>
<td>9.8 (2.6)</td>
</tr>
<tr>
<td>48 h</td>
<td>9.5 (2.6)</td>
<td>9.9 (2.6)</td>
</tr>
<tr>
<td>72 h</td>
<td>8.5 (2.4)</td>
<td>9.7 (2.5)</td>
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Sensitiv Allele-Specific PCR Assay Able to Detect FGFR3 Mutations in Tumors and Urine from Patients with Urothelial Cell Carcinoma of the Bladder, Ashraf A. Bakkar,1 Viviane Quach,2 Anaïg Le Borgne,2 Marianne Toublanc,3 Dominique Henin,3 Hervé Wallerand,4 François Raudamy,4 Hugues Bittard,4 Vincent Ravory,4 Laurent Boccon Gibod,6 Sixtina Gil Diez de Medina,1 Dominique K. Chopin,1 and Bernard Grandchamp5 (1 EMI INSERM U37 and Service d’Urologie, Université Paris 12, AP-HP, Hôpital Henri Mondor, Créteil, France; 2 Service de Biochimie et Génétique, 3 Service d’Anatomie et Cytologie Pathologiques, and 4 Service d’Urologie, Université Paris 7, AP-HP, IFR02, Hôpital Bichat-Claude Bernard, Paris, France; 4 UMR 144, CNRS Institut Curie, Paris, France; 5 Service d’Urologie CHRU Besançon, France; 6 address correspondence to this author at: Service de Biochimie et Génétique, Hôpital Bichat, 46 rue Henri Huchard 75018 Paris, France)

Activating somatic point mutations in exons 7, 10, and 15 of the FGFR3 gene are frequently observed in urothelial cell carcinoma (UCC) (1, 2). These mutations have been found primarily in superficial papillary pTa tumors and were absent in carcinoma in situ (3).

Given the high frequency of FGFR3 mutations and the possible implication of this receptor in the development of UCC, it was important to develop a simple, fast, and reliable method to identify these mutations in greater detail as a potential tool for the diagnosis and follow-up of UCC patients.

Ten different FGFR3 mutations have been described in UCC, but 4 of them (R248C, S249C, G372C, and Y375C) account for >95% of cases (2). These mutations therefore represent an excellent target for assays, such as allele-specific PCR (AS-PCR), which depend on the specific detection of point mutations.

### References


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