heparinized plasma samples stored at −20 °C for an extended time resulted in a variable loss of KIC. To characterize this loss and to define better conditions for sample storage, we tested the effects of EDTA and iodoacetic acid (IAA) on the stability of KIC in serum and plasma. EDTA inhibits ferric ion-catalyzed oxidative decarboxylation of alpha-keto acids (2), and IAA scavenges thiol compounds that could condense with the α-keto group of KIC (3).

KIC concentrations were measured by using the N-methylquinazoline derivative for GC/MS analysis as described previously (4). Methyleute (Fierce Chemical Co.) was used as solvent and as an on-column methylating agent. With α-ketocaproic acid as an internal standard, samples were analyzed in a HP5985A (Hewlett-Packard) mass spectrometer at m/e 174.

In a preliminary study, heparinized plasma samples were prepared with no additions, with EDTA (disodium salt, 10 mM/L), or with IAA (sodium salt, 2 mM/L). They were stored at room temperature (RT) or −70 °C, and assayed for KIC at various intervals. After eight weeks, all samples stored at −70 °C showed no significant change in KIC concentrations. The controls and the EDTA- and IAA-treated samples stored at RT showed KIC concentrations that were 2%, 45%, and 101% of their −70 °C counterparts, respectively.

The effect of heparin concentration and its interaction with EDTA were further examined (Table 1). At RT and 0 or 1 USP unit of heparin added per milliliter, KIC was unstable, with recoveries of less than 10%; high heparin concentrations in plasma resulted in significant KIC loss. In this and other experiments, IAA showed excellent stabilizing effects, regardless of heparin concentration.

While we have not defined the mechanisms of KIC degradation or of its protection by various agents, we believe the above results would be of use to researchers involved in protein metabolism studies. We recommend that plasma samples used for KIC studies be stored in the presence of IAA and (or) at −70 °C, and that the combination of EDTA and commercial heparin preparations be avoided.

**References**


**Table 1. Stability of α-Ketocapric Acid in Serum and in Plasma Treated with Heparin, EDTA, and IAA**

<table>
<thead>
<tr>
<th>Heparin, USP</th>
<th>Additions,</th>
<th>KIC, μM/L</th>
<th>% recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>units/mL *</td>
<td>mmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>0</td>
<td>1.1 (34.3)</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>14.3 (30.0)</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>28.7 (32.4)</td>
<td>89</td>
</tr>
<tr>
<td>Plasma</td>
<td>0</td>
<td>0.2 (32.8)</td>
<td>1</td>
</tr>
<tr>
<td>Inv., 1</td>
<td>EDTA, 10</td>
<td>3.6 (29.0)</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>IAA, 2</td>
<td>1.8 (32.5)</td>
<td>6</td>
</tr>
<tr>
<td>Inv., 20</td>
<td>EDTA, 10</td>
<td>6.1 (28.4)</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>EDTA, 10</td>
<td>26.4 (31.2)</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>IAA, 1</td>
<td>1.6 (28.5)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>21.1 (29.8)</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>EDTA, 10</td>
<td>4.7 (29.2)</td>
<td>16</td>
</tr>
</tbody>
</table>

*Two heparin preparations were tested, one from Invenex (Inv.), Chagrin Falls, OH, and one from Elkins-Sinn (ES), Cherry Hill, NJ.

aSamples were stored at room temperature (or at −70 °C, results listed in parentheses) for six weeks before assay.

bRatio of KIC concentrations in room temperature samples to those in −70 °C samples.

**Effect of Storage Conditions on Saliva Thiocyanate Concentration**

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After absorption via the pulmonary, nasal, and oral mucosa, cyanide compounds in tobacco smoke are transformed into thiocyanates (SCN) and excreted into urine, saliva, and sweat. Thiocyanate has thus been proposed as a biochemical marker of smoking exposure. Saliva tests, generally considered the most sensitive, have the additional advantage of easy, non-invasive sampling (1, 2).

However, little is known about stability of SCN in saliva samples or optimal storage conditions between the time of sampling and analysis. Consequently, it has been proposed to analyze saliva samples immediately after collection (1). This requires the close proximity and availability of a trained laboratory and limits the widespread use of SCN analysis in epidemiological studies.

We evaluated the influence of storage conditions on the variability of salivary SCN as determined by the method of Densen et al. (3). We analyzed 48 saliva samples (group A) immediately and two months after sampling. Samples collected in sterile containers were stored in airtight tubes at
4 °C. For 12 additional patients (group B) the analysis was performed immediately and repeated 10 and 30 days after collection of the samples, which were stored at room temperature in airtight containers.

The data were examined by Student’s paired t-test and linear-regression analysis.

In neither group did storage conditions significantly influence the SCN measured two months (group A, \( t = 0.569, \text{NS} \)), 10 days (group B, \( t = 0.419, \text{NS} \)), or 30 days (group B, \( t = 0.01, \text{NS} \) after sampling. Results of prompt and postponed analysis correlated well (Figure 1). In group B, culture of the samples confirmed in all cases the presence of polymicrobial flora normally present in oropharynx.

We conclude that SCN is stable in saliva stored in airtight containers for as long as one month at room temperature or two months at 4 °C—an additional advantage for the large-scale application of the test in epidemiological studies.

References


Glycated proteins reduce alkaline nitroblue tetrazolium chloride (NBT) reagent, and this property has been used for their estimation in fructosamine assays (1). Could the same property be used for estimation of globin-bound fructosamine, as an index of hemoglobin glycation?

We measured glycated hemoglobin by affinity chromatography (2) and hemoglobin by the cyanmethemoglobin method (3). To determine globin-bound and serum fructosamine, we modified the NBT reagent, using carbonate buffer (0.2 mol/L, pH 10.35), containing 500 μmol of NBT and 2 mmol of p-chloromercuribenzoic acid per liter.

Hemolysate, free of cell debris, was prepared from saline-washed erythrocytes. Hemolysate (0.5 to 1.0 mL) containing 10 mg of hemoglobin was added dropwise to 10 mL of cold acetone containing 30 μL of hydrochloric acid (2 mol/L), and mixed for 10 min. We then centrifuged (1000 × g, 10 min), decanted the acetone, and dissolved the globin precipitate in 1 mL of 9 g/L sodium chloride within 1 min.

We mixed 0.2 mL of test serum or standardized pooled serum with 1 mL of 9 g/L sodium chloride, incubated these and the globin solutions at 37 °C for 5 to 10 min, then added 1 mL of prewarmed NBT reagent to each sample at 1-min intervals. The globin solution initially precipitates, but redissolves when all of the reagent has been added. We measured the absorbance in a Beckman spectrophotometer (Model 25 UV/Vis) at 530 nm 10 (A1) and 20 min (A2) later, and calculated the concentration of globin-bound and serum fructosamine from ΔA(A2 minus A1), expressing the results as nmol/10 mg of globin and mmol/L of serum, respectively.

It is essential to centrifuge the globin precipitate at slow speed (1000 × g, 10 min) and dissolve it immediately (within 1 min) in 9 g/L sodium chloride, to prevent denaturation of globin from heat or air exposure. The p-chloromercuribenzoic acid in the NBT reagent is necessary for measuring the absorbance change attributable to globin-bound fructosamine in the presence of SH groups; otherwise these groups reduce NBT to a deep purple.

Results of this test for 30 normoglycemics and 41 diabetics were as follows:

<table>
<thead>
<tr>
<th>% Gly-Hb by affinity chromatography</th>
<th>Globin-bound fructosamine, mmol/10 mg</th>
<th>Serum fructosamine, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoglycemics</td>
<td>4.38 (0.56)</td>
<td>124.81 (22.68)</td>
</tr>
<tr>
<td></td>
<td>1.42 (0.20)</td>
<td>296.21 (96.71)</td>
</tr>
<tr>
<td>Diabetics</td>
<td>10.65 (4.10)</td>
<td>2.26 (0.58)</td>
</tr>
</tbody>
</table>

Glycated hemoglobin, serum fructosamine, and globin-bound fructosamine were all significantly higher in diabetics than in normoglycemics (P < 0.001).

Intra-assay CVs for globin-bound fructosamine assay at mean values of 103.7 and 244.3 mmol/10 mg were 6.27% and 2.67%, respectively. For these 71 subjects, globin-bound fructosamine values (y) correlated well with glycated hemoglobin (y = 24.23x + 26.94; S_yx = 27.93; r = 0.96) and serum fructosamine (y = 171.7x – 104.5; S_yx = 56.3; r = 0.92).

Unlike other methods (4, 5) this method avoids the hydrolysis of glucon and eliminates interference from residual hemoglobin without requiring a separate control; the same solution acts as control and test (absorbance measured at 10 and 20 min). Moreover both long- and medium-term control of blood glucose can be monitored simultaneously, as globin-bound and serum fructosamine. The method also offers the possibility of automated analysis.

References


Comparison of Seven Sensitive Thyrotropin (TSH) Assays to Predict a Suppressed Thyrotropin (TRH) Test, J. J. Body, F. Seraj, and V. Keymolen (Service de Médecine et Laboratoire d’Investigation Clinique H. Tagnon, Unité d’Endocrinologie, Institut J. Bordet Centre des Tumeurs de l’Université Libre de Bruxelles, 1 Rue Héger-Bordet, 1000 Bruxelles, Belgium)

Newer, more sensitive TSH assays represent a considerable advance in the evaluation of thyroid disorders (1). Much literature exists on technical performances of numer-