exchange chromatography (Pharmacia Fine Chemicals) by using a 0–0.2 mol/L NaCl gradient in a stepwise elution with 15 mmol/L phosphate buffer, pH 7.2, at a flow rate of 10 mL/h. The dissociated Ig was incubated with the purified AST isoenzymes (s-AST 386 U/L, m-AST 986 U/L) in a ratio of 4:1 by volume at 25°C for 60 min, and the recombined enzyme-Ig complex was detected by immunoturbidimetry [11]. Immunoturbidimetry showed that the absorbances in tubes to which anti-IgM and the patient’s IgM and s-AST had been added were higher than in tubes containing m-AST (Table 1). The results showed that the purified IgM was able to recombine with s-AST. These results show that the patient’s increased serum AST was related to the presence of an s-AST-IgM-λ-type complex.

We found 53 published cases of high serum AST concentrations that were due to the formation of an Ig-AST complex [12]. In 28 cases, the macro form of AST was found to be an IgA-AST complex; in 18 cases, an IgM-AST complex; and in 6 cases, AST was found to be complexed with IgG and IgA [12, 20]. The reported cases with Ig-AST complexes can be divided into three groups, according to the clinical characteristics of the patients: (a) 40 patients with various liver diseases, malignancies, and diseases involving immune disorders; (b) 8 apparently healthy individuals [1–6]; and (c) 5 patients whose disease was not described. However, three of the healthy individuals with IgG-AST complex had received medication (one, an oral contraceptive [1]; one, a griseofulvin [5]; and one, a weight-reducing drug [5]), and one healthy individual with IgG-AST complex had hepatitis B vaccination before the increased serum AST activity was detected. Our patient showed a high antimicrosomal antibody concentration, suggesting an autoimmune disorder. These reports suggest that not only the patients with serious liver disease or immune disorder, but also most of the healthy individuals had experienced possibly hepatotoxic or host immune response-modifying episodes before the increased serum AST activity was detected [15], and these episodes or diseases may be involved in the production of Ig-AST complexes.

Our patient was discharged with no treatment. After 3 years, the patient is still clinically well, and her AST serum concentrations are still high. We are following her enzyme activity to determine the duration of the high AST activity and whether the IgM bound to AST will switch to another class of Ig. When high concentrations of serum AST are observed in the absence of organ-specific disease, the presence of an Ig-enzyme complex should be investigated to avoid unnecessary or invasive examinations.

### Table 1. Recombination of dissociated IgM and AST by immunoprecipitation reaction in free liquid media.

<table>
<thead>
<tr>
<th></th>
<th>Anti-IgG</th>
<th>Anti-IgA</th>
<th>Anti-IgM</th>
<th>Anti-κ</th>
<th>Anti-λ</th>
</tr>
</thead>
<tbody>
<tr>
<td>s-AST</td>
<td>0.1338</td>
<td>0.1333</td>
<td>0.2068</td>
<td>0.1325</td>
<td>0.1467</td>
</tr>
<tr>
<td>m-AST</td>
<td>0.1333</td>
<td>0.1306</td>
<td>0.1299</td>
<td>0.1307</td>
<td>0.1313</td>
</tr>
</tbody>
</table>

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**Novel Reductant for Determination of Total Plasma Homocysteine**, Brian M. Gilfix,1,2* David W. Blank,2 and David S. Rosenblatt1 (1 Div. of Med. Genetics, 2 Div. of Clin. Biochem., Royal Victoria Hosp., 687 Pine Ave. W., Montreal, QC H3A 1A1, Canada; * author for correspondence: fax 514-843-1499, e-mail mcgb@musica.mcgill.ca)

Homocysteine (Hcy), increasingly being recognized as a risk factor for vascular disease [1], is found primarily in plasma in the form of homocystine and mixed disulfides, both protein-bound and unbound; total Hcy (tHcy) is the
sum of all Hcy species obtained after quantitative reduction. Several methods are currently used to measure tHcy in plasma, including GC/MS, ion-exchange chromatography, and HPLC [2]. In one of the most popular HPLC techniques, any homocystine and homocystine-mixed disulfides present are reduced with tri-butyl phosphine (TBP) to Hcy, which is subsequently derivatized with ammonium 7-fluorozeno-2-oxa-1,3-diazole-4-sulfonate (SBD-F) to produce a fluorescent product [3, 4].

TBP has a highly disagreeable odor and is poorly soluble in water so that it must be dissolved in dimethylformamide for use, both of which properties hinder the routine use of TBP in a clinical laboratory. Here, we call attention to a newer phosphine reagent, tris(2-carboxyethyl)phosphine (TCEP), which is nonvolatile, stable, and soluble in aqueous solution and thus is more suitable for routine use.

We have therefore modified the method of Ubbick and Vermaak [4] as follows. First, add 9 \( \mu \)L of 100 g/L TCEP (aq) (Pierce Chemical Co., Rockford, IL) to 90 \( \mu \)L of plasma, mix, and allow to react for 30 min at room temperature. Then, add 90 \( \mu \)L of 100 g/L trichloroacetic acid containing 1 mmol/L EDTA. After centrifuging the sample for 10 min at 13,000 g, add 100 \( \mu \)L of the supernatant to a tube containing 20 \( \mu \)L of 1.55 mol/L NaOH, 250 \( \mu \)L of 0.125 mol/L borate buffer containing 4 mmol/L EDTA, pH 9.5, and 100 \( \mu \)L of SBD-F (Wako Chemicals USA, Richmond, VA), 1 g/L in the borate buffer. After mixing, incubate the solution for 1 h at 60 °C, let cool to room temperature, and inject 20 \( \mu \)L of this onto a C18 column to run at a flow rate of 1.5 mL/min [4].

Figure 1 shows the relation between the Hcy determined after reduction with TCEP as a function of that determined after reduction with TBP in aliquots of the same plasma samples [5]. All plasma samples were obtained in accordance with the guidelines of the research ethics board at our institution.

For the Hcy determinations with TCEP, Hcy calibrators were prepared in isotonic saline (NaCl 9 g/L), the assigned concentration being based on a molar absorptivity for Hcy of 397 L mol\(^{-1}\) cm\(^{-1}\) at 244 nm. These saline calibrators were chromatographed and used to prepare a calibration curve from which the Hcy concentrations in the plasma samples were calculated according to peak heights. When Hcy was added to plasma samples, recovery was 100% (results not shown). Hcy determinations in plasma with TBP were performed at the Institut de Recherches Cliniques de Montreal (by L.-J. Fortin, whom we thank for these determinations). For the Hcy determinations done with TBP, Hcy calibrators were prepared in plasma, and the Hcy concentrations were based on peak areas [5].

The correlation coefficient between the results obtained with the two different reductants is 0.988, although the TCEP method yields values ~21% higher. This may reflect simple differences in methodology—e.g., the calculation of results (peak height vs peak area), the calibration matrix (saline vs plasma), or quantification of the calibrators (molar absorptivity vs dry weight, respectively)—or differences in the efficiency of reduction with TCEP, and suggests the necessity of developing an interlaboratory standardization procedure. The 5th, 50th, and 95th percentiles for plasma tHcy determined with TCEP reduction for a population of 30 individuals (15 men, 15 women; mean ± SD ages, 36.9 ± 10.6 years) were 5.2, 9.8, and 14.9 \( \mu \)mol/L, respectively. Usable values can be produced from as little as 15 \( \mu \)L of plasma. The TCEP solution can be stored at ~20 °C and is stable to freezing and thawing.

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Initial Test Cutoff Selection Based on Regression Analysis of Initial Test Apparent Analyte Result vs GC/MS Test Analyte Result—Evaluation of Two Radioimmunoassay Kits’ Test Data, Jeffrey Brendler and Ray H. Liu* (Doctoral Training Program in Forensic Sci., Univ. of Alabama at Birmingham, Birmingham, AL 35294-2060; *author for correspondence: fax 205-934-2067, e-mail rliu@sbs.sbs.uab.edu)

Current workplace drug-testing programs require reporting positive results only if the gas chromatography/mass