Tenslotte kunnen vraagtekens gezet worden bij de gegevens vermeld in de bijsluiter van de Immulite 2500. In de bijsluiter is de volgende correlatie beschreven: Immulite 2500 = 0,93 x Immulite 2000 + 10,7 pg/ml (5). Vertaald naar pmol/l zou een waarde van 150 pmol/l op de Immulite 2000 een waarde van 147 pmol/l op de Immulite 2500 moeten opleveren. Onze data laten zien dat dit niet klopt en dat eerder een waarde van 123 pmol/l wordt gevonden. Daarbovenop komt dat in sommige monsters een variatie van maximaal 33,4% is waargenomen. Juist in het klinisch belangrijke gebied treden dus grote afwijkingen op met implicaties voor de betrokken patiënten. Bij gedetailleerde bestudering van de door de firma gekozen correlatiemonsters valt op dat in het gebied tussen de 111 en 150 pmol/l slechts enkele monsters zijn geïncludeerd en onder de 136 pmol/l geen enkel monster (5). Hierdoor is de door ons geobserveerde bias tussen de twee methoden onopgemerkt gebleven.

Samenvattend kan gezegd worden dat de problemen van de VB12-bepaling zo complex zijn, dat ze alleen door goede en open communicatie tussen de aanvragers van labdiagnostiek, de laboratoria en de diagnosticafabrikanten goed zijn te tackelen.

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Evaluation of blood collection tubes specific for homocysteine measurement on AxSYM, Immulite and LC-MS/MS systems

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Introduction

Homocysteine is an intermediary aminoacid with a reactive sulfhydryl group that is formed in the metabolism of methionine. Hyperhomocysteinemia is associated with increased risk for atherosclerotic vascular disease and thromboembolic events (1, 2). Furthermore, increased plasma homocysteine concentration is a sensitive marker for folate and vitamine B12 (cobalamin) deficiency (3). Measurement of homocysteine is complicated by sustained metabolism by red blood cells after blood collection, resulting in increase of homocysteine levels in vitro. To prevent this artifact, it is recommended that blood samples are placed and stored on ice immediately after collection (4). Samples should also be centrifuged and plasma or serum should be separated from blood cells as soon as possible. These preanalytical measures are impractical in routine blood collection and may be unsuitable for out-of-hospital facilities. Several studies have investigated the effect of different additives and anticoagulants on the stability of homocysteine levels in blood collection tubes. The use of acidic citrate as an anticoagulant can stabilise homocysteine levels in tubes at room temperature for up to 6h (5-7). Sodium fluoride (NaF) can reduce the artifactual homocysteine increase at ambient temperature for only short periods of time (2-3h). However, the combination of NaF and EDTA may be effective in maintaining homocysteine stability at room temperature for up to several days (8). Recently, a new tube for homocysteine measurement, containing a stabiliser and Z-gel, was brought on the market by Sarstedt (Nümbrecht, Germany). According to the manufacturer's claim this tube is able to stabilise homocysteine levels in vitro at room temperature for up to 8h without centrifugation.

Methods

The stability of homocysteine was investigated in blood samples of healthy volunteers (n=10, laboratory coworkers). Three commercially available blood collection tubes specific for homocysteine measurement were tested; the HCY-Z-gel tube (S-Monovette 2.6 ml HCY/Z-gel with clot activator and unknown homocysteine stabilisator, Sarstedt, Nümbrecht, Germany), HCY-C/acidic citrate tube (S-Monovette 2.9 ml 9NC/HCY with 0.5 mol/l trisodium citrate and citric acid buffer solution at pH 4.3, Sarstedt, Nümbrecht, Ger-

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many) and the EDTA-NaF tube (BD Vacutainer 4.0 ml with 12.0 mg disodium EDTA and 6.0 mg sodiumfluoride, Becton Dickinson, Plymouth, UK). Of each volunteer blood was collected in these three special homocysteine tubes as well as in regular EDTA and serum tubes. Control samples (t=0h) for each tube were put on ice immediately after collection and centrifuged as soon as possible. Other samples were stored at room temperature for 8 or 24h. Subsequently, serum and plasma were separated from the cell fraction and stored at -20 °C. Homocysteine was determined by isotope-dilution liquid chromatography tandem mass spectrometry (LC-MS/MS; Waters Acquity UPLC Quattro Premier XE). For chromatographic separation, a Waters Symmetry C8 column (2.1 x 100 mm, reference WAT 058961, Waters, Etten-Leur, The Netherlands) was used with a precolumn (Waters, reference 205000343). The column was eluted at 0.25 ml/min and no splitter was used. Calibration was performed with aqueous standards because they gave similar results as plasma-based standards.

We compared homocysteine results in special homocysteine tubes to those in regular serum and EDTA tubes. Blood was collected from 60 outpatients requiring homocysteine testing after informed consent was given. Blood samples were collected in HCY-C (acidic citrate), HCY-Z-gel and EDTA-NaF tubes, as well as in standard EDTA and serum tubes. All samples were placed on ice immediately after collection and centrifuged within the hour. Subsequently, serum or plasma was separated from blood cells, aliquotted and stored at -20 °C. Homocysteine was determined using LC-MS/MS, AXSYM and Immulite 2000 systems. Data were compared between special homocysteine tubes and regular serum and EDTA tubes by linear regression using EP Evaluator release 7 (David G. Rhoads Associates, Inc.).

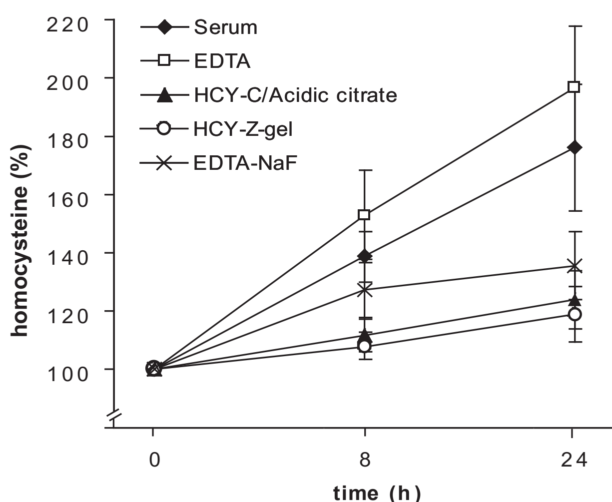

Figure 1. Homocysteine stability in different blood-collection tubes at room temperature. Homocysteine levels at 8 and 24h are given as percentages of the homocysteine concentration in the tubes at t=0h. Mean percentages of homocysteine levels per tube type are shown in the graph. Mean basal levels of homocysteine were 11.2 (EDTA), 11.4 (serum), 10.0 (HCY-C), 11.3 (HCY-Z-gel), 11.0 (EDTA-NaF) µmol/l. Bars represent standard deviation.

Results

Homocysteine levels in the different blood collection tubes stored at room temperature for 8 and 24h were compared to homocysteine levels of control samples. As expected, in vitro homocysteine concentration in regular EDTA and serum tubes increased when tubes were kept at room temperature. In EDTA tubes homocysteine levels increased by 53 and 97 % after 8 and 24h, respectively (figure 1). In serum tubes homocysteine levels increased by 39 and 76 % after 8 and 24h, respectively. Homocysteine concentrations in HCY-C and HCY-Z-gel tubes remained stable for up to 8h (12 and 8 % increase, respectively) and showed a small but significant rise after 24h (24 and 19 % increase, respectively), taking into account an allowable total error of 17.7 % for homocysteine (9). The data of the EDTA-NaF tubes showed that homocysteine levels increased significantly by 27 % after 8h and 36 % after 24h (figure 1).

Table 1 shows the results from the comparison of homocysteine testing in patient samples between the different tubes. Results from linear regression analysis for each of the special homocysteine tubes versus standard EDTA or serum tubes for all three methods are given. Homocysteine results from HCY-C tubes were recalculated using a correction factor of 1.11 to compensate for dilution of the sample caused by the volume of liquid citrate anticoagulant (table 1A). We found differences in homocysteine levels between HCY-C, HCY-Z-gel and EDTA-NaF tubes versus routine EDTA and

Table 1. Comparison of homocysteine in special homocysteine tubes versus regular serum and EDTA tubes measured by different methods. Blood samples from patients were collected in different tubes. Homocysteine was measured on AXSYM, Immulite 2000 and LC-MS/MS systems. Data were compared betrween special homocysteine tubes (HYC-C, HCY-Z-gel and EDTA-NaF) and regular serum and EDTA tubes by linear regression. *indicates that there is a significant slope (≠ 1) or intercept (≠ 0) with 95% confidence.

Method	Tube	Slope	Intercept	R
HCY-C with 1.11 correction factor				
AXSYM	serum	0.930*	0.062	0.981
	EDTA	0.947*	0.445	0.980
Immulite 2000	serum	0.903*	0.592	0.953
	EDTA	0.806*	1.895*	0.949
LC-MS/MS	serum	0.887*	0.428	0.991
	EDTA	0.944*	0.482*	0.991
HCY-Z-gel				
AXSYM	serum	0.957	0.024	0.983
	EDTA	0.995	0.123	0.987
Immulite 2000	serum	0.933	0.086	0.937
	EDTA	0.849*	1.260*	0.951
LC-MS/MS	serum	0.881*	0.671*	0.986
	EDTA	0.942*	0.673*	0.991
EDTA-NaF				
AXSYM	serum	0.888*	0.362	0.978
	EDTA	0.910*	0.661*	0.984
Immulite 200	serum	0.998	- 0.643	0.938
	EDTA	0.905*	0.649	0.959
LC-MS/MS	serum	0.836*	0.751*	0.990
	EDTA	0.944*	0.482*	0.991

serum tubes, revealed by varying slopes and intercepts that were significant in most cases (slopes ≠ 1 and intercepts ≠ 0 with 95 % confidence). We found that correlation factors were similar for the different tubes when homocysteine was measured using the same method. The highest correlation factors were found using the LC-MS/MS method (mean R=0.990, SD 0.002), followed by the AXSYM immunoassay (mean R=0.982, SD 0.003), whereas the Immulite 2000 immunoassay showed the lowest correlation factors (mean R= 0.947, SD 0.008). These correlation factors are in line with the analytical variation of the methods.

Discussion

Artifactual increase of homocysteine due to ongoing metabolism by red blood cells in standard blood collection tubes poses a problem for routine homocysteine testing. Our results indicate that the artifactual rise in homocysteine in serum is lower than in EDTA anticoagulated blood. Potentially clot formation and trapping of red blood cells within the clot could play a role in this effect in serum tubes. The data for acidic citrate (HCY-C tubes) are consistent with previous reports (5-7). HCY-Z-gel tubes were recently introduced by Sarstedt and so far no studies have reported on its performance. Our data for homocysteine stability in HCY-Z-gel tubes corroborate claims from the manufacturer. Therefore, both HCY-C and HCY-Z-gel tubes can be used for blood collection for homocysteine determination without storage on ice or rapid centrifugation, provided that samples are processed within 8h. Although it is not known which homocysteine stabilising agent is used in HCY-Z-gel tubes, these tubes may have an advantage over HCY-C tubes, because there is no dilution of the sample, which reduces pre-analytical error. Contrary to the findings of Clark et al. (8) we did not find that EDTA-NaF could maintain homocysteine levels at room temperature. This inconsistency may be explained by different ratios of EDTA, NaF and blood in the tubes or differences in experimental protocols.

The comparison of homocysteine levels in patient samples revealed differences between HCY-C, HCY-Z-gel and EDTA-NaF tubes versus routine EDTA and serum tubes. These differences were relevant in most cases and should be compensated for when switching from standard tubes to the tubes specific for homocysteine testing. Therefore, new reference values may need to be assessed. Alternatively, correction factors may be calculated to compensate for the differences in results, as correlation factors (R) are high. Specific correction factors should be calculated for each type of tube and method of homocysteine determination. The slopes and intercepts given in this paper may be used as guidelines for evaluation procedures. The differences between the new homocysteine tubes and routine tubes may be explained by interference of homocysteine metabolites or other components in the homocysteine tubes with the analytical methods. Alternatively, different properties of the tubes, like coagulation (serum versus plasma) or dilution may contribute to the bias found in our experiments. The 1.11 correction factor for dilution did not fully compensate the slopes in the HCY-C tubes. Therefore, an additional factor would be required to correct the homocysteine concentration determined in HCY-C tubes. The differences in homocysteine in HCY-C/acidic citrate that we found are in line with earlier studies (5, 6, 10).

Conclusion

In conclusion, our data indicate that both Sarstedt HCY-C and HCY-Z-gel tubes can be used for homocysteine testing without pre-analytical measures, such as placing tubes on ice and rapid centrifugation. These tubes may be stored at room temperature for up to 8h without significant effect on homocysteine concentration. EDTA-NaF tubes are not suitable for routine homocysteine testing without measures to prevent artifactual homocysteine increase. A difference in homocysteine values in HCY-C and HCY-Z tubes should be taken into account when interpreting results; e.g. by assessing new reference values or by introducing tube-specific and method-specific correction factors.

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