Regression analysis incorporating all serum samples measured on both analyzers revealed a proportional difference of 21% for the Immulite analyzer (n = 135; r = 0.97, Spearman rank correlation). Samples measured in EDTA or cooled EDTA plasma on the Immulite showed differences of 15% and 19%, respectively (n = 135; r = 0.97 and 0.96, Spearman rank correlation), compared with the Immulite 2000.

Finally, we tested the in vitro stability of PTH by measuring PTH before and after storage of serum and plasma for 48 h at 4 °C. Serum PTH decreased from a median concentration of 2.6 pmol/L to 2.4 pmol/L within 48 h (P = 0.0003, Wilcoxon test). PTH in EDTA plasma increased marginally, from 3.8 pmol/L to 4.1 pmol/L (P = 0.008, Wilcoxon test), and PTH in ice-cold EDTA plasma remained constant at ~3.2 pmol/L (P = 0.52, Wilcoxon test). The quality-control data indicated that there was no obvious drift. Although for serum and EDTA plasma the changes in PTH concentrations with time are statistically significant, the clinical relevance of the changes is questionable.

The results presented here confirm previous reports describing higher apparent PTH concentrations in EDTA plasma than in serum (1, 2) and changes in PTH concentrations with time (1–6). In agreement with what has been published for the DPC PTH assay (1), we found a slight increase in PTH concentration in EDTA plasma with time. The decrease in serum PTH concentrations was much less profound after 2 days (this study) than after 3 days (1) after venipuncture. This may be attributable to the fact that in the study of Glendenning et al. (1) the samples were stored at room temperature, whereas we stored them, similar to patient material, at 4 °C. The observation that PTH does not decrease in EDTA plasma at room temperature may be attributable to chelation by EDTA of cations essential to (metallo)protease activity.

In conclusion, reference intervals for PTH are dependent on sample type, and reports on the stability of PTH in serum and plasma are inconsistent. Therefore, within one laboratory, PTH should be determined in one type of sample only and as soon as possible after venipuncture. Excess EDTA in an underfilled tube can inhibit signal production in methods that use alkaline phosphatase; for such methods, serum should be the material of choice. Laboratories measuring PTH on both the Immulite and Immulite 2000 analyzers should make sure that the analyzers give similar results for patient material.

DPC Nederland (Breda, The Netherlands) kindly provided the PTH reagents used in this study.

References

Volkker Scharnhorst* Jan Valkenburg Cor Vosters Huib Vader
Clinical Laboratory Máxima Medical Center PO Box 7777 5500 MB Veldhoven, The Netherlands

*Author for correspondence. Fax 31-40-8888929; e-mail V.Scharnhorst@mmc.nl.

Editor’s Note: A representative of the manufacturer declined to send a reply for publication, but indicated in comments to us that the letter is accurate and that the problem has been rectified.
The proteinase K+ sample was then centrifuged at 12 000g for 2 min, and 600 µL of absolute ethanol was added to the supernatant, with mixing during the addition. The sample was then applied to a RNeasy mini spin column. From this point, the same procedure was followed for both the proteinase K− and proteinase K+ samples. The column was washed once with 350 µL of RW1 buffer followed by on-column treatment with 80 µL of DNase solution (10 µL of DNase + 70 µL of RDD buffer from the Qiagen RNase-free DNase reagent set) for 15 min, according to the Qiagen recommendations. The column was then washed once with 350 µL of RW1 and twice with 500 µL of RPE buffer. Finally, the RNA was eluted with 50 µL of RNase-free water. We assessed the quality of the RNA by measuring the 28S:18S ribosomal RNA ratio with the Agilent 2100 Bioanalyzer (Agilent Technologies).

Proteinase K treatment increased the mean yield of RNA more than 10-fold, from 1.54 µg (range, 0–64.3 µg) to 20.3 µg (range, 0–125.6 µg). The proteinase K treatment increased the number of samples that could be used for microarray analyses from 10 of 209 to 152 of 209 samples (P <0.0001, Fisher exact two-tailed test) with 2 µg as the minimum amount of RNA per tumor required for the microarray studies.

To determine whether the addition of proteinase K has an effect on the gene expression profile, we performed microarray analyses according to the Affymetrix protocol, using Affymetrix HG-U133 (45 000 probe sets) chips because Affymetrix arraying to the Affymetrix protocol, using formed microarray analyses according to the Qiagen RNeasy protocol and markedly increases the amount of RNA purified from primary breast tumors by this method.

In summary, proteinase K treatment dramatically improves the yield of RNA without causing any significant changes in the expression profile when included in the Qiagen RNeasy protocol and markedly increases the amount of RNA purified from primary breast tumors by this method.

Our research groups are supported by grants from the Swedish Cancer Society, the Stockholm Cancer Society, King Gustav V’s Jubilee Fund, and Bristol-Myers Squibb.

References

Suzanne Egyházi1*
Judith Bjöhle1
Lambert Skoog1
Fei Huang2
Anna-Lena Borg1
Marianne Frostvik Stolt1
Torsten Hägerström1
Ulrik Ringborg1
Jonas Bergh1

1 Department of Oncology-Pathology Cancer Center Karolinska Karolinska University Hospital-Solna S-171 76 Stockholm, Sweden
2 Bristol-Myers Squibb Princeton, NJ

*Author for correspondence. Fax 46-8-5177-4245; e-mail Suzanne.Egyhazi@onkpat.ki.se.

Effects of Hemolysis on the Roche Ammonia Method for Hitachi Analyzers

To the Editor:
The manufacturer’s product literature for the Roche ammonia reagent (cat. no. 11877984) states that “there is no interference from hemolysis up to a hemolysis index of 50 (approximate hemoglobin concentration: 50 mg/dL).” This statement can and has been interpreted to mean there are artifactual changes in the ammonia results above this hemolysis index limit. The likely result is that laboratories could use this low limit as the definitive guide and not report any results above it. This would lead to rejection of up to 10% of samples.

The effect of hemolysis on analytes is often studied [e.g., Ref. (1)] by adding a hemolysate (2) to a pool of serum/plasma.

To investigate the effects of hemolysis on ammonia, we used a slightly different method. We split an ~50-mL freshly collected EDTA-blood sample (recommended sample type) from a volunteer into three pools. Pool 1 was immediately aliquoted into equal sample volumes (2 mL). The first sample was centrifuged, and the EDTA plasma that was separated contained minimal hemolysis. The remaining samples were passed through a blood collection needle to mimic a typical collection process. The number of times a sample was passed through a needle increased with each subsequent sample to produce a range of hemolysis. Ammonia was added to pools 2 and 3, which were then processed similarly to pool 1. The samples were centrifuged, and the EDTA-plasma was separated and analyzed on an Hitachi Modular P analyzer (Roche Diagnostics Australia).

A separate EDTA-whole-blood sample was used to prepare hemolysate (2). The hemolysate was then added to portions of EDTA plasma to produce a range of hemoglobin concentrations.

When hemolysis was induced by mimicking a typical collection process, hemoglobin up to 11 g/L had

DOI: 10.1373/clinchem.2003.027102