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Immulite 2000 parathyroid hormone assay: stability of parathyroid hormone in EDTA blood kept at room temperature for 48 h

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SUMMARY. Parathyroid hormone (PTH) concentrations were compared in serum and EDTA plasma from 36 patients attending a renal stone clinic. Serum PTH concentrations ranged from 0·9 to 10·9 pmol/L, with a mean of 4·6 pmol/L. When serum and EDTA plasma results were compared, in samples frozen within 30 min of collection, EDTA plasma results were found to be significantly higher than those in serum ($P < 0·0001$; Wilcoxon test), with an average increase of 19·5% over the serum result. Results from EDTA-preserved blood left to stand at room temperature for 48 h were on average 14·8% lower than results from the corresponding EDTA plasma samples frozen within 30 min, with a highly significant difference ($P < 0·0001$). Freshly frozen serum and 48 h EDTA plasma PTH results were not significantly different.

Parathyroid hormone in EDTA-preserved blood is not completely stable, and this could lead to misclassification of results for samples which are not frozen quickly.

Parathyroid hormone (PTH) has a half-life of approximately 5 min in the circulation and is unstable in whole blood or serum.[1–3] The need to centrifuge clotted blood samples and to freeze serum rapidly limits the use of the service by general practices and clinics at a distance from the main laboratory.

Recently there has been much interest in the use of EDTA as an anticoagulant for the collection of blood samples for PTH analysis. The manufacturers of the Immulite and Immulite 2000 PTH assays have reported that PTH is stable in whole blood for up to 72 h when it is collected into EDTA tubes. However, the evidence on the stability of PTH in EDTA whole blood or plasma is not entirely consistent, and it is possible that sample stability may be influenced by the assay method used.

Levin and Nisbet[4] reported that PTH concentrations were stable in EDTA plasma for up to 72 h at room temperature in a study of 28 patients using the Nichols Allegro assay. Using the Immulite assay, PTH in EDTA plasma was found to be stable for up to 48 h at room temperature in a study of seven renal patients reported by Fern and Holder.[5] Collins *et al.*,[6] in a study of 16 renal patients using the Immulite assay, showed that although PTH was stable in EDTA-preserved whole blood kept at room temperature for 24 h, zero-time PTH results in EDTA plasma were 11·4% higher than the corresponding zero-time serum result. Recently Walker and Seth,[7] also using the Immulite assay, found PTH to be stable for up to 12 h at room temperature in EDTA-preserved whole blood from eight renal patients.

From a practical point of view we felt that it was important to determine, first, whether there was any significant difference in PTH measured in serum and EDTA plasma frozen immediately; and second, whether PTH remained stable in EDTA-preserved whole blood for 48 h at room temperature. The period of 48 h was chosen because if specimens were found to be stable for this time, local hospital transport and first-class post could be used for specimen delivery.

METHODS

Blood samples from 36 patients attending the renal stone clinic at Southampton General Hospital were collected into both 10 mL plain and EDTA vacutainers (Becton-Dickinson Ltd, Plymouth, UK). Serum was separated from the

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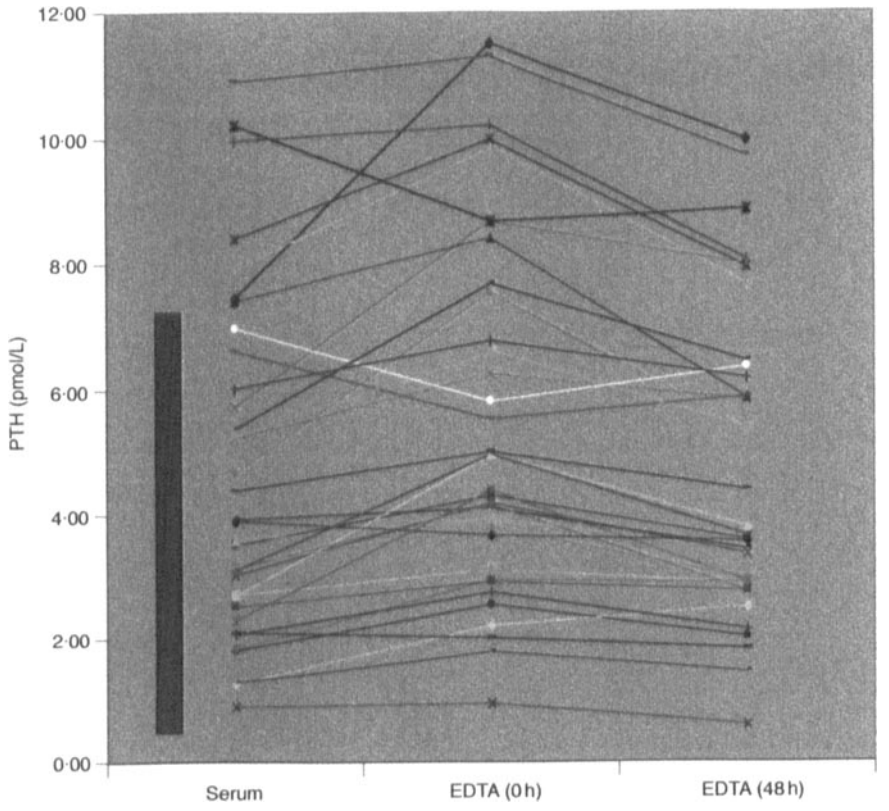

FIGURE 1. *PTH concentrations in freshly frozen serum, freshly frozen EDTA plasma and plasma separated from EDTA blood left at room temperature for 48 h. Lines connect results in individual patients. The laboratory reference range is indicated by the shaded block.*

clotted blood sample and frozen within 30 min. A 5 mL aliquot of EDTA blood was centrifuged and EDTA plasma was frozen within 30 min. The remainder of the EDTA blood was left to stand at room temperature for 48 h, after which plasma was separated and stored frozen.

For maximum precision all specimens were thawed and analysed in the same run on the Immulite 2000 analyser (DPC-UK, Glyn Rhonwy, Gwynedd, Wales, UK) and for each patient, samples were analysed in the sequence zero-time serum, zero-time EDTA plasma and 48 h EDTA plasma.

Inter-assay precision was determined at three levels from the analysis of in-house freeze-dried control samples in 27 batches. Percentage coefficients of variation (CV) were: low, mean 0·9 pmol/L, %CV 9·5%; medium, mean 13·4 pmol/L, %CV 5·5%; high, mean 125·6 pmol/L, %CV 8·2%.

Results were compared using Deming regression analysis and bias was determined using Bland–Altman bias plots ('Analyse-It' Software Ltd, Leeds, UK). The significance of differences in results for patients was assessed using Wilcoxon paired signed ranks test.

RESULTS AND DISCUSSION

Serum PTH values measured on the 36 patients ranged from 0·9 to 10·9 pmol/L, with a mean of 4·6 pmol/L for serum samples.

Results from zero-time serum and EDTA plasma and from plasma separated from blood kept at room temperature for 48 h are presented in Fig. 1. When zero-time serum and EDTA plasma results were compared, EDTA plasma results were on average 19·5% higher than those of serum. These differences were highly significant ($P < 0·0001$).

A comparison of zero-time and 48 h EDTA plasma showed that the latter results were on average 14·8% lower than the former. These

results were significant ($P < 0.0001$). The percentage biases between zero-time and 48 h EDTA results showed no correlation with the PTH concentrations.

Zero-time serum PTH and 48 h EDTA plasma PTH concentrations were not significantly different.

It is clear from the results of this study that, following analysis by the Immulite 2000, freshly frozen EDTA plasma gives PTH results which are higher than those from freshly frozen serum from the same patient. Our findings on freshly frozen serum/EDTA plasma differences are similar to those of Collins *et al.*,[6] who found an 11·6% difference with the same PTH assay method. Fern and Holder[5] and Walker and Seth[7] did not see this difference. However, all three previous studies using the Immulite assay[5–7] have used samples taken from renal patients with much higher PTH results. For example, in the study of Walker and Seth,[7] the mean serum PTH concentration was 25·7 pmol/L. The PTH concentrations found in this study of renal stone patients are closer to those found in normal subjects. A possible explanation for the higher zero-time EDTA plasma concentrations is that PTH is relatively more stable in EDTA blood, and that PTH in serum deteriorates slightly during the clotting process before separating and freezing.

When freshly frozen EDTA plasma PTH results were compared with results from EDTA blood left at room temperature for 48 h a highly significant fall was observed, such that levels at 48 h were an average of 14·8% less than those at zero time. Therefore, although PTH in EDTA-preserved blood is considerably more stable than in serum, it is not completely stable. The finding of a fall in PTH levels in EDTA blood left at room temperature for 48 h has not previously been published. However, the two earlier studies looking at PTH stability at room temperature in EDTA-preserved whole blood have used a smaller number of patients and have only tested stability up to 12 h[6] or 24 h.[7] Other confounding factors may be the type of EDTA blood tubes (Collins *et al.*[6] used Sarstedt tubes), or even the batch of tubes from a particular manufacturer.

It is possible that small changes in PTH results could be 'masked' by the higher PTH concentrations in samples from renal patients used in other studies, but in this study we found no dose-related changes in bias for PTH concentrations up to 10·9 pmol/L.

In practice, a change to EDTA blood tubes could be considered if all samples were frozen quickly and if the reference range was changed appropriately. However, because it is often unclear how long samples have been in transit to the laboratory, if they have not been separated and frozen quickly then the zero-time EDTA reference range may be not appropriate. Freshly frozen EDTA plasma would be an alternative to freshly frozen serum, but reference ranges would have to be re-established.

In conclusion, we have found that freshly frozen EDTA plasma samples give higher PTH results than serum samples separated in the same way. Over 48 h plasma from EDTA-preserved whole blood yields PTH results which are comparable to those of freshly frozen serum. It would be difficult to define an appropriate reference range for EDTA plasma samples unless they were frozen quickly, and this would increase the possibility of misclassification of borderline results for samples sent in to the laboratory.

In view of this we feel that a change to EDTA blood samples for the Immulite 2000 PTH assay would result in difficulties with interpretation of results for samples sent to the laboratory, and that this outweighs any advantages arising from specimen collection.

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