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Preanalytical Influences on DPC IMMULITE 2000 Intact PTH Assays of Plasma and Serum from Dialysis Patients, *Daniel T. Holmes,[1*] Adeera Levin,[2] Barry Forer,[3] and Frances Rosenberg[1]* ([1] Department of Pathology and Laboratory Medicine and [2] Department of Medicine, Division of Nephrology, University of British Columbia, St. Paul's Hospital, Vancouver, Canada; [3] Measurement, Evaluation, and Research Methodology Program, University of British Columbia, Vancouver, Canada; * address correspondence to this author at: Department of Pathology and Laboratory Medicine, St. Paul's Hospital, 1081 Burrard St., Vancouver, BC, V6Z 1Y6, Canada; fax 604-806-8815, e-mail dtholmes@interchange.ubc.ca)

Measurement of intact parathyroid hormone (PTH) is essential to the diagnosis and management of metabolic bone disease *(1)*, hypercalcemia, hypocalcemia *(2)*, and renal osteodystrophy *(3)*. The effects of specimen type, collection temperature, and storage temperature on the in vitro stability of PTH differ by method and platform *(4–10)*. Characterization of preanalytical effects unique to each method, platform, and patient population is important to prevent potential clinical misclassification.

The Diagnostics Product Corporation (DPC) IMMULITE 2000 intact PTH assay is a solid-phase two-site chemiluminescent immunoassay with a monoclonal mouse capture antibody and a polyclonal goat signal antibody conjugated to alkaline phosphatase. The effects of preanalytical factors on this assay have been partially investigated. Underfilling of EDTA-plasma tubes decreased measured PTH in samples from both healthy persons and those with chronic kidney disease (CKD) *(11)*. Storage of SST serum samples for 3 days at room temperature decreased measured PTH in SST serum samples, an effect not seen with EDTA plasma *(6)*. Results from EDTA plasma paradoxically increased after storage at 4 °C *(12)*. Despite evidence that PTH is more stable in EDTA-anticoagulated specimens *(5–7, 9, 10)*, the manufacturer describes an instability associated with EDTA-plasma samples and therefore recommends cold (2–8 °C) collection, centrifugation, and storage until analysis *(13)*.

At our institution, PTH is most frequently measured for management of CKD and helps direct therapies, including calcium, vitamin D, bisphosphonates, and parathyroidectomy. Preanalytical influences on PTH assays may be atypical in CKD because samples contain increased concentrations of N-terminal truncated PTH fragments that cross-react with intact PTH assays *(14)*. Accordingly, we have investigated preanalytical variables in a dialysis-dependent CKD population. The study was approved by the St. Paul's Hospital ethics committee.

Five predialysis samples were obtained from 31 CKD patients between 0800 and 0930 h. Two specimens were drawn into Becton Dickinson (BD) 6-mL dipotassium EDTA Vacutainer™ plastic tubes: the first was immediately placed on ice and then centrifuged at 4 °C ($EDTA_{cold}$), whereas the second was collected and centrifuged at room temperature ($EDTA_{RT}$). The three remaining samples were drawn into 5-mL BD Vacutainer™ SST® plastic tubes containing gel and clot activator. The first (SST_{cold}) and second (SST_{RT}) serum specimens were collected in the same manner as their EDTA counterparts. A third specimen ($SST_{spuncold}$) was collected in accordance with the manufacturer's indication that SST specimens can be collected at room temperature but require cold centrifugation and subsequent refrigeration *(15)*. All tubes were filled completely. Each of the five tubes was separated into four aliquots. Aliquots from the $EDTA_{cold}$, SST_{cold}, and $SST_{spuncold}$ tubes were maintained at 4 °C until analysis, whereas aliquots from the $EDTA_{RT}$ and SST_{RT} tubes remained at room temperature. Each aliquot was analyzed at baseline (within 3 h) and after 24, 48, and 72 h, according to the manufacturer's protocols (reagent lot 121).

Using SPSS (Ver. 13.0), we performed two separate $2 \times 2 \times 4$ within-subject repeated-measures ANOVA analyses: the first was (EDTA/SST) × (room temperature/cold) × (time), and the second was (EDTA/SST) × (room temperature/spuncold) × (time). We explored the interactions between sample type and temperature and sample type and time and found them to be significant; we therefore considered the effects of temperature and time separately. Bonferroni corrections for multiple comparisons were applied.

The mean PTH concentrations for each sample type are displayed in Table 1 and Fig. 1. Results for specimens collected in EDTA were higher than those for specimens collected in SST tubes irrespective of temperature ($P < 0.001$). Furthermore, mean PTH values were significantly higher in $EDTA_{RT}$ specimens than $EDTA_{cold}$ ($P < 0.001$). Time had no significant effect on PTH measurements in $EDTA_{cold}$ specimens ($P = 0.172$), whereas for

$EDTA_{RT}$, there was a slight increase and decrease over time. Post hoc analysis confirmed significance for the 8.3% ($P = 0.006$) and 8.8% ($P = 0.004$) increases seen at 24 and 48 h, respectively. At baseline, PTH values for the SST_{cold}, $SST_{spuncold}$, and SST_{RT} specimens did not differ significantly ($P = 0.193$), and the results for the SST_{cold} and $SST_{spuncold}$ specimens did not change significantly over time (both $P > 0.05$). However, for SST_{RT}, there was a strong downward linear trend. By 48 and 72 h, significant changes from baseline [−17.3% ($P = 0.040$) and −24.9% ($P = 0.004$)] had occurred. Additionally, the 48 and 72 h values for SST_{RT} specimens were significantly lower than the corresponding values for SST_{cold} and $SST_{spuncold}$ specimens (all $P < 0.05$).

The data demonstrate variation in results within and between samples collected and stored differently, with certain discrepancies large enough to alter clinical decisions. The discrepancies are not unique to reagent lot 121. We have seen similar differences with lots 114 and 115 (data not shown), as have others with lots 109 and 112 *(12)*. In addition, the negative difference for SST serum compared with EDTA plasma was not a matrix effect attributable to the SST tube, as reported for other analytes on the Immulite 2000 *(16)*. We have seen similar results in sera from plain tubes (data not shown). We have seen no such effects for intact PTH measured on our Elecsys 1010 (data not shown). This study clarifies an apparent discrepancy between the DPC technical bulletin *(13)* and the published literature. The bulletin describes an instability associated with EDTA-plasma specimens *(13)*, whereas other reports indicate that PTH is more stable with EDTA *(5–7, 9, 10)*. As we now describe, both the technical bulletin and the literature are correct, depending on the delay before analysis.

At baseline, mean PTH in $EDTA_{RT}$ specimens was 9.8% higher than in $EDTA_{cold}$ specimens. This implies that even 3 h at room temperature will produce a shift in results for EDTA specimens to higher values; this may be the "instability" that the DPC labeling cites *(13)*. Interestingly, there was no significant difference in baseline PTH results for SST_{RT}, SST_{cold}, and $SST_{spuncold}$ specimens. Thus, in the short term, SST serum specimens demonstrate greater stability, irrespective of temperature. However, after 48 and 72 h, the mean measured PTH in SST_{RT} specimens showed a greater percentage decrease from baseline than the percentage increases seen in $EDTA_{RT}$ specimens. In this sense, PTH shows greater apparent stability in EDTA over longer times.

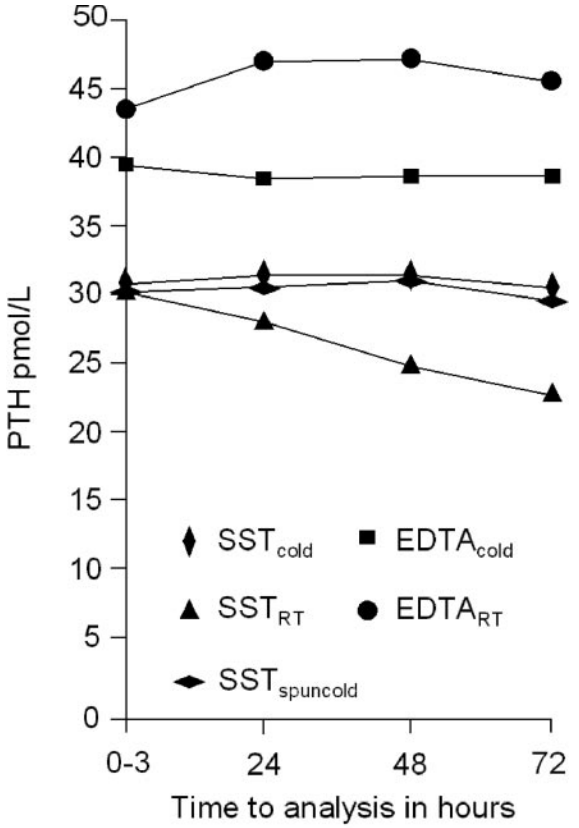

Fig. 1. Mean PTH concentrations measured at baseline (0–3 h) and 24, 48, and 72 h for respective protocols.
95% confidence intervals are provided in Table 1.

The consistently higher baseline results in EDTA plasma over SST have been noted previously in more heterogeneous populations *(6, 12)*. Appropriately, DPC has issued EDTA-specific reference intervals *(13)*. A statistically nonsignificant increase in measured PTH in EDTA specimens has been reported after storage at room temperature *(6)*. A similar but statistically significant effect has been seen in EDTA plasma collected and centrifuged at room temperature and then refrigerated *(12)*. The mechanism of the effect is unclear. Because "intact PTH" assays show cross-reactivity with PTH frag-

Table 1. Mean PTH results in pmol/L (with 95% confidence intervals) obtained on the IMMULITE 2000.[a]

	Time to analysis			
Sample	**0–3 h**	**24 h**	**48 h**	**72 h**
$EDTA_{cold}$	39.5 (26.4–52.6)	38.4 (25.8–50.9)	38.7 (26.1–51.2)	38.7 (25.9–51.5)
$EDTA_{RT}$	43.4 (29.1–57.8)	47.0 (31.3–62.8)[b]	47.2 (31.5–63.0)[c]	45.4 (30.3–60.4)
SST_{cold}	30.7 (9.2–36.5)	31.4 (8.5–32.8)	31.3 (8.0–31.0)	30.5 (6.8–27.5)
SST_{RT}	30.1 (20.3–39.9)	28.0 (19.2–36.7)	24.9 (17.3–32.5)[d]	22.6 (15.6–30.0)[c]
$SST_{spuncold}$	30.1 (20.3–39.9)	30.4 (20.6–40.2)	31.0 (20.8–41.2)	29.6 (20.0–39.1)

[a] Manufacturer's reference intervals are 1.3–6.8 pmol/L for serum and 1.7–9.2 pmol/L for plasma.
[b–d] Significantly different from baseline: [b]$P = 0.006$; [c]$P = 0.004$; [d]$P = 0.04$. All differences not indicated are not significant.

ments *(14)*, it is possible that apparent increases in PTH are attributable to fragment detection, i.e., fragments unique to the degradation process in EDTA plasma; no such increase is observed in serum samples. Reassuringly, the increase is modest and of questionable clinical consequence.

The ideal specimen type remains unclear, but standardization of preanalytical handling is critical in clinical and research settings. If analysis occurs within 3 h, SST serum affords the most consistent results, regardless of acquisition and storage temperature. However, if a specimen remains at room temperature for several days before analysis, then EDTA-plasma results are less discrepant from their baseline. DPC does not make any recommendation to immediately cool SST specimens *(15)*. Our data support this recommendation and further suggest that a 3-h delay at room temperature will not affect results. When analysis must be delayed beyond 3 h, it is reassuring that even after 24 h, the mean decrease in SSR_{RT} results was only 7.3% (95% confidence interval, 3.2–11.2%), a change that failed to reach significance. DPC recommends that EDTA specimens be collected and maintained at 2–8 °C *(13)*. Our data suggest that this practice will prevent a paradoxical increase in PTH results, although we cannot comment on the necessity to precool EDTA tubes. In all cases, refrigeration minimizes effects of storage. We use room temperature SST serum for in-house tests and have verified and adopted the manufacturer's reference interval of 1.3–6.8 pmol/L. Referred-in samples are cooled or frozen as appropriate.

In summary, we examined samples from 31 hemodialysis patients, using two different matrices while varying acquisition temperature, storage temperature, and time to analysis. In the dialysis-dependent CKD population, EDTA-plasma specimens gave higher mean PTH results than SST serum. Mean PTH values from EDTA-plasma specimens collected and stored at room temperature increased modestly over time for the first 48 h. However, larger absolute percentage changes from baseline were seen in SST specimens similarly handled. Different reference intervals are needed for EDTA plasma and SST serum. The reference interval for room temperature plasma specimens should not be assumed to be identical to that for refrigerated plasma. If analyzed within 3 h of collection, SST specimens do not require cooling. As each PTH method is affected differently by preanalytical factors, conclusions should not be drawn about other PTH assays based on these results. Peripheral laboratories and clinicians conducting trials should be informed of the need to collect and store specimens according to protocols established at their laboratory lest results be affected and patient care be compromised.

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Anti-Actin Antibodies in Celiac Disease: Correlation with Intestinal Mucosa Damage and Comparison of ELISA with the Immunofluorescence Assay, *Antonio Carroccio,[1*] Ignazio Brusca,[2] Giuseppe Iacono,[3] Lidia Di Prima,[1] Saverio Teresi,[3] Giuseppe Pirrone,[1] Ada Maria Florena,[4] Stella Maria La Chiusa,[2] and Maurizio Rocco Averna[1]* ([1] Internal Medicine and [4] Pathology Department, University Hospital of Palermo, Palermo, Italy; [2] "Buccheri La Ferla" Hospital of Palermo, Palermo, Italy; [3] Pediatric Gastroenterology, "Di Cristina" Hospital of Palermo, Palermo, Italy; * address correspondence to this author at: Internal Medicine, University Hospital of Palermo, via del Vespro 141, 90127 Palermo, Italy; fax 39-091-6552936, e-mail acarroccio@hotmail.com)

The presence in the sera of celiac disease (CD) patients of anti-actin autoantibodies (AAAs) has been suggested as a