Long-Term Stability of Osteocalcin at -70° Determined by the Elecsys 2010 Analyzer


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ABSTRACT

Serum Osteocalcin (OC) is used for assessing bone turnover and monitoring therapy. OC assays have suffered from analyte instability. OC measurement on the Elecsys 2010 (N-MID Osteocalcin, Roche Diagnostics) is specific for both the intact OC and the main N-terminal fragment. Short-term and long term OC stability was determined using 1) a set of serum, lithium heparin plasma and K3EDTA plasma obtained from three subjects, and 2) two human serum pools. All samples were separated from cells within 2 hours of collection. Aliquot storage at -70°C was within 8 hours. Osteocalcin was analyzed at baseline and after 1, 3, 6, 12, 18 and 24 months. In addition, 171 samples from post-menopausal osteoporosis patients undergoing antiresorptive therapy was used to assess long-term OC assay reproducibility. For the latter samples, analyses were batched, and separate serum aliquotes were assayed within 6 months of collection and after a 34-month storage. The 24-month stability of the individual serum and plasma samples is shown in the figure. The serum pools gave values over the same period of 25.9 ± 1.0 ng/mL (mean ± SD; range 24.4 - 26.3) and 90.2 ± 3.6 ng/mL (mean ± SD; range 84.8 - 92.0), without a trend over time. The comparison of 171 serum sample from post-menopausal women yielded a regression of:

Final OC Concentration = 1.022 x Initial OC Concentration - 1.658; r² = 0.941 over a concentration range of 13 - 85 ng/mL. In summary, the Elecsys OC assay is consistent over time. Lithium heparin and K3EDTA plasma samples do not degrade significantly at -70°C for at least 2 years, and for serum, OC is stable for 3 years. The latter were analyzed using different reagent lots without compromising the results. The Elecsys 2010 OC assay is suitable for use in clinical research where samples have been in long-term storage.

BACKGROUND AND OBJECTIVE

Osteocalcin (OC), a 49 amino acid peptide is the major noncollagen protein of bone. It contains gamma-carboxyglutamic acid (GLA) residues at positions 17, 21 and 24 and is therefore, also known as bone gla-protein or BGP. The three gamma-carboxyglutamic acid residues in OC confer a very strong ability to bind hydroxyapatite and calcium. Vitamin K is essential for the biosynthesis of OC, which is stimulated by 1,25-di-OH vitamin D. OC is synthesized by osteoblasts during the process of bone formation and mostly incorporated into bone matrix with some escaping into the blood. The circulating level of total OC is primarily composed of the intact molecule (aa 1-49) and a large N-terminal midregion fragment (aa 1-43) resulting from the cleavage of the intact molecule. Since the half-life in blood is relatively short (about 5 min.), the OC level in blood reflects new protein synthesis and therefore its measurement provides a valuable tool for assessing bone formation and bone turnover.

The use of serum OC for assessing bone turnover and monitoring therapy has been complicated by analyte instability and assay non-specificity. We therefore assessed the robustness of the OC assay on the Elecsys 2010 (N-MID Osteocalcin, Roche Diagnostics, IN) for its suitability of different sample types as well as the reproducibility of results from clinical samples analyzed three years apart.
ASSAY PRINCIPLE
The N-MID OC is a double monoclonal antibody sandwich (Mab) assay that recognizes both the intact (aa 1-49) and the large N-Mid fragment (aa 1-43). Step 1: Sample and a biotinylated OC Mab are incubated together. Step 2: Following addition of streptavidin-coated paramagnetic microparticles and a ruthenium labelled partner Mab for another OC epitope, a sandwich complex is formed which binds to the solid phase via a biotin-streptavidin interaction. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured on the surface of the electrode. Unbound substances are then removed by a wash. Application of a voltage to the electrode then induces chemiluminescent emission, which is measured by a photomultiplier. Results, which are derived from a 2-point calibration and a master curve provided via the reagent bar code, are available in 18 minutes.

STUDY DESIGN
1. Serum, lithium heparin and K3EDTA samples from three subjects and two serum pools were prepared from freshly collected blood. All samples were separated from cells within 2 hours of collection and aliquots frozen at -70°C within 8 hours in cryogenic vials. N-MID OC was analyzed fresh (within 8 hr) and after 1, 3, 6, 12, 18 and 24 months of frozen storage. OC recoveries of the three sample matrices were compared.

2. 171 Frozen serum samples from osteoporotic postmenopausal women undergoing anti-resorptive therapy were used to assess the long-term reproducibility of N-MID OC. The samples were analyzed in one batch immediately after the conclusion of a three-month treatment period. Another aliquot of the same samples was analyzed in a batch, after 34 months storage at -70°C.

RESULTS

- The subject means represent duplicate analyses at each time point (fresh, 1, 3, 6, 12, 18 and 24 mo) of each sample type. The order of the analysis was randomized.
- Means of the frozen in-house serum pools represent duplicate analyses measured at the same time points as samples from the three subjects.
- A total of four lots of reagents and calibrators were used during the 24 mo period.
- Serum, lithium heparin plasma and K3EDTA plasma stored at -70°C were stable for 24 months. Except for the EDTA plasma baseline value of Subject A (which appeared to be an outlier compared to all other time points and sample types), recoveries were within 10% of baseline values (EDTA plasma values were not corrected for the dilution by the anticoagulant).
- Total imprecision (CV) of all sample types over 24 months was 5.7%.

FIGURE 3: Comparison of N-MID OC in Sera Stored at -70°C Analyzed 3 Years Apart

- 171 Sera from osteoporotic postmenopausal women collected at baseline, 1 month and 3 months after anti-resorptive treatment were analyzed 34 months apart using different reagent and calibrator lots.
- For comparison, published reference values in postmenopausal women indicates a mean of 27 ng/mL and 5-95 percentile range of 5-46 ng/mL.
CONCLUSION

- Serum, lithium heparin, and K3EDTA samples are acceptable and comparable sample types for N-MID OC measurements on the Elecsys.
- Serum is stable at -70°C for at least 3 years. Lithium heparin, and K3EDTA samples are stable at -70°C for at least 2 years.
- The Elecsys 2010 N-MID OC platform is stable and robust across multiple reagent and calibrator lots. The assay is suitable for short-term and long term assessment of bone metabolism.