

1.1. Serum PINP

Serum N-propeptide of Type I Procollagen (PINP)

Serum intact PINP was measured with a two site immunoassay based on monoclonal antibodies raised against purified intact human PINP and detecting both intact mono and trimeric forms, but not fragments using an automated analyzer (Elecsys, Roche Diagnostics). Intra-assay variation is lower than 2 % and inter-assay variation lower than 4%. The mean (SD) value for pre-menopausal women is **46 (14)** ng/ml. The mean (SD) value for postmenopausal women is **63.4 (17)** ng/ml.

Ref: *Garnero P., Delmas P.D. Evaluation of a Fully Automated Assay for Serum Procollagen Type I-N Propeptide in Osteoporosis. J Bone and Miner Res, 17, supp. 1:S316.*

1.2. Urinary N-terminal crosslinking telopeptide of type I collagen (U-NTX-I)

Urinary NTx was measured by ELISA using the OSTEOMARK* assay (Ostex Inc, Seattle, USA) on an automated machine (Vitros ECi) from ortho-clinical diagnostics.

Intra assay range from 1.1 to 6.7%.

Inter assay variation range from 3.8 to 6.1%.

The lower limit of detection, also referred as sensitivity is 10 nM BCE.

Results were provided separately for U-NTX (nM BCE) and corrected for urine creatinine (nM BCE/mM Creat).

1.3. Pentosidine

Urinary Pentosidine was measured by an HPLC technique using purified bone Pentosidine as a standard.

Pentosidine was measured in acid hydrolysate by high performance liquid chromatography. After extraction by SPE partition column chromatography, Pen was eluted from a reversed phase HPLC on a Beckman ultrasphere ODS (5 m, 25 cm X 4.6 mm) at a flow rate of 1 ml/ min with an isocratic elution of 17 % acetonitrile in 0.15% of HBFA and quantified by fluorimetry. Effluent was monitored for fluorescence at an emission of 385 nm and an excitation of 335 nm. The amount of pentosidine was quantified using as synthetic standard. Intra- and interassay variations are lower than 8 and 15 % respectively. The sensitivity of the assay is 0.7 pmol/L.

Reported with correction for urine creatinine as nmol/mmol U-Cr

Reference: Garnero P., Borel O., Gineyts E., Duboeuf F., Solberg H., Bouxsein M.L., Christiansen C., Delmas P.D. Extracellular post-translational modifications of collagen are major determinants of biomechanical properties of fetal bovine cortical bone. *Bone* 2005 Nov 2; [Epub ahead of print]