



Osteomark NTx

Accurate measurement of bone resorption

Urine **ELISA** Serum

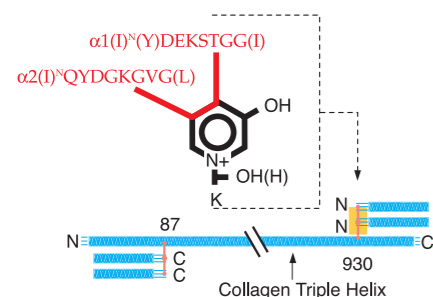
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Introduction

Osteomark NTx assays provide a quantitative measurement of the cross-linked N-telopeptides of bone type I collagen (NTx). NTx is a specific biochemical indicator of bone resorption that is generated as result of osteoclast activity on bone. The NTx molecule is specific to bone due to the unique amino acid sequence and orientation of the cross-linked alpha-2 (I) N telopeptide.¹

Intermolecular cross-linking of bone type 1 collagen

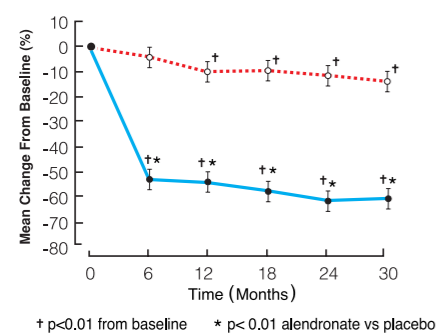


Osteomark NTx assays are sensitive and specific assays that can be used to indicate subtle changes in levels of bone resorption.²⁻⁹

Clinical utility

- To monitor response to anti-resorptive therapy in Osteoporosis.¹⁰

NTx/Cr response in alendronate-treated (†) vs placebo (†*) groups¹⁰



- To determine probability for accelerated decrease in bone mass in postmenopausal women
- To help assess the need to initiate anti-resorptive therapy by predicting skeletal response (BMD)
- To counsel patients about continuation of therapy.

Osteomark NTx vs Bone mineral density (BMD)

Bone mineral density is currently the best way to evaluate bone mass. Densitometry measurements taken 12 to 24 months apart, post therapy can be used to detect changes in bone mass from baseline. However, they are not sufficiently sensitive to accurately measure changes in bone mass that are less than 3%. Osteomark NTx provides a dynamic indicator of the current level of bone resorption, shows significantly earlier response to therapy (within 3-6 months) and when used in conjunction with BMD gives a more complete picture of bone status than either technology alone.¹¹

Assay principles

The Osteomark NTx assays are competitive-inhibition enzyme linked immunosorbent assays (ELISAs) that use microwells in strips as the solid phase onto which NTx has been absorbed. NTx in the patient sample competes with the NTx absorbed on the solid phase for binding sites on a horseradish peroxidase labelled monoclonal antibody. The amount of antibody bound is inversely proportional to the amount of NTx in the sample. The NTx concentration in the patient sample is determined spectrophotometrically and calculated from a standard calibration curve. In urine, ELISA results are expressed in nM of bone collagen equivalents (nMBCE) per mM of creatinine. In serum, ELISA results are expressed in nMBCE, i.e. no correction for creatinine is necessary.

Urine

Sample specifications

- Second void of morning urine
- No dietary modification required
- No specimen pre-treatment required
- Specimens with visible blood should be discarded as assay interference may occur
- Specimens can be stored for up to 72 hrs in refrigeration or at room temperature for 24 hrs.

Procedure summary

- Take required number of microwells
- Add 25 µl urine sample, calibrator or control
- Add 200 µl of monoclonal antibody conjugate
- Seal and incubate for 90 minutes at room temperature
- Wash wells
- Add Chromogen
- Incubate for 15 mins at room temperature
- Add stop reagent and read on Spectrophotometer
- Calculate results using 4-parameter curve fitting equation

Features and benefits

- Standard ELISA format
- Simple and convenient to perform
- Requires no specialised equipment
- Fast assay time, results available in less than 2 hours

Performance characteristics

- Intra-assay variability 7.6%CV
- Inter-assay variability 4.0% CV
- Antigen recovery 105%
- Dilutional linearity r=0.999
- Assay range 20-3000 nMBCE

Reference ranges for urine assay (nM BCE/mM Creatinine)

	Mean	Range
Pre-menopausal women	35	5-65
Men	27	3-51



Serum

Sample specifications

- Serum sample collected by standard venipuncture technique
- No dietary modification required
- Samples can be stored refrigerated up to 24 hrs or frozen for longer term storage.
- Samples may undergo three freeze/thaw cycles
- For monitoring purposes collect sequential samples at approximately the same time of day.

Procedure summary

- Take required number of microwells
- Prepare 1:5 dilutions of calibrators, controls and specimens
- Add 100µl of diluted calibrator, control or sample to appropriate microwells
- Add 100µl of monoclonal antibody conjugate
- Seal and incubate at room temperature for 90 minutes
- Wash wells
- Add 200µl of Chromogen
- Seal and incubate at room temperature for 15 minutes
- Add stop reagent and read in Spectrophotometer (Plate reader)
- Calculate results using 4-parameter logistic curve fitting equation

Features and benefits

- Standard ELISA procedure
- No creatinine testing or correction required
- Fast assay time, results available in less than 2 hrs
- Reproducible results with good precision
- Low within patient variability

Performance characteristics

- Intra-assay variability 4.6%CV
- Inter-assay variability 6.9% CV
- Antigen recovery 94-105%
- Dilutional linearity mean 98% across range
- Assay range 3.2-40 nM BCE

Reference ranges for serum assay (nM BCE)

	Mean	Range
Pre-menopausal women	12.6	6.2-19.0
Men	14.8	5.4-24.2