N-MID® Osteocalcin ELISA

For the Quantitative Determination of
Osteocalcin in human serum and plasma

CE REF AC-11F1 Σ 96

Immunodiagnostic Systems Limited is not responsible for any other use of the kit or consequence hereof than the one specified above. Neither for misuse, e.g. use deviating from the procedure described in this manual. Furthermore, Immunodiagnostic Systems Limited is not to be made responsible for any diagnoses or conclusions made by the user or third party based on the results obtained with the kit nor for any consequences such interpretations may cause.

INTRODUCTION

Intended use

The N-MID® Osteocalcin ELISA is an enzyme immunological test for the quantitative measurement of osteocalcin, an indicator of osteoblastic activity in human serum and plasma and is ***intended to be used as an aid in the prevention of osteoporosis.***

Limitations

Osteocalcin values may vary depending upon the person's age (years post menopause), "circadian rhythm", rate of glomerular filtration and duration of treatment.
Results should be used in conjunction with information available from the clinical evaluation of the patient and other diagnostic procedures. Therefore, osteocalcin values are not recommended for use as a screening procedure to detect the presence of osteoporosis in the general population. Also, medication dosage should not be changed or stopped based solely on the osteocalcin values.
When evaluating subsequent samples, collect at the same time of day as baseline and use the same specimen type, serum or anticoagulated plasma.

Summary and explanation of the test

Osteocalcin, or bone Gla protein (BGP), is the major non-collagenous protein of bone matrix. It has a molecular weight of approximately 5800 Dalton and consists of 49 amino acids, including three residues of gamma-carboxyglutamic acid.
Osteocalcin is synthesized in bone by osteoblasts. After production, it is partly incorporated into the bone matrix and partly delivered to the circulatory system. The precise physiological function of osteocalcin is still unclear. A large number of studies have shown that the circulating level of osteocalcin reflects the rate of bone formation (1-14).
Determination of serum osteocalcin has proved to be valuable as an aid in identifying women at risk of developing osteoporosis, for monitoring bone metabolism during the perimenopause and postmenopause and during antiresorptive therapy.

Principle of the procedure

The N-MID® Osteocalcin ELISA is based upon the application of two highly specific monoclonal antibodies (Mabs) against human osteocalcin. An antibody recognizing the midregion (amino acids 20-29) is used as the capture antibody and for detection a peroxidase conjugated antibody recognizing the N-terminal region (amino acids 10-16) is used. In addition to intact osteocalcin (amino acid 1-49) the N-terminal-Mid fragment (amino acids 1-43) is also detected.
Standards, control and unknown samples are pipetted into the appropriate microtitre wells coated with streptavidin. Then a mixture of a biotinylated antibody and a peroxidase conjugated antibody is added. Following incubation for 2 hours at room temperature the wells are washed and a chromogenic substrate is added and the colour reaction is stopped with sulfuric acid. Finally, the absorbance is measured.

PRECAUTIONS

The following precautions should be observed in the laboratory:

- *Do not eat, drink, or smoke where immunodiagnostic materials are being handled*
- *Do not pipette by mouth*
- *Wear gloves when handling immunodiagnostic materials and wash hands thoroughly afterwards*
- *Cover working area with disposable absorbent paper*

Storage

Store the N-MID® Osteocalcin ELISA kit upon receipt at 2-8°C. Under these conditions the kit is stable up to the expiry date stated on the box.
Following reconstitution the ***Standards*** *and the* ***Controls*** *should be stored below -18°C for up to 3 months, and should only be frozen and thawed twice. When the components of the* ***Antibody Solution*** *are mixed, the remaining solution should be stored at 2-8°C for no longer than 1 month or frozen below -18°C. The remaining reagents and immunostrips should be stored at 2-8°C.*

Note:
The specimens' storage and stability information stated above are general recommendations for use in a variety of settings of laboratories. Each laboratory should follow the guidelines or requirements of local, state, and/or federal regulations or accrediting organizations to establish its own specimens handling and storage stability. For guidance on appropriate practices, please refer to the CLSI GP44-A4, Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guideline - Fourth Edition

Warnings

For in vitro use only.

- *All reagents and laboratory equipment should be handled and disposed of as if they were infectious.*
- *Do not use kit components beyond the expiry date and do not mix reagents from different lots.*

MATERIALS

Specimen collection

Collect blood by venipuncture taking care to avoid haemolysis. Separate the serum from the cells within 3 hours after collection of blood. It is recommended to freeze (<-18°C) samples immediately.
When analysing plasma, both heparin and EDTA plasma may be used.

Materials supplied

Before opening the kit, read the section on ***Precautions****. The kit contains reagents sufficient for 96 determinations. For reconstitution of lyophilized material, add appropriate volume of distilled water and leave for 10 minutes before mixing. Make sure to avoid foam.*

Streptavidin coated microtitre plate **MICROPLAT**

Microwell strips (12 x 8 wells) pre coated with streptavidin. Supplied in a plastic frame.

Osteocalcin Standard 0 **CAL 0**

One vial (lyophilized) containing a PBS-buffered solution with protein stabilizer and preservative. Reconstitute with 5.0 mL of distilled water. The standard must be stored below -18°C after use.

Osteocalcin Standards **- CAL 1 - 5**

Five vials (lyophilized) containing synthetic human osteocalcin in a PBS-buffered solution with protein stabilizer and preservative. Reconstitute with 0.5 mL of distilled water. The exact value of each Standard is printed on the QC Report. The standards must be stored below -18°C after use, and should only be frozen and thawed twice.

Controls **- CTRL 1-2**

Two vials (lyophilized) containing synthetic human osteocalcin in a PBS-buffered solution with protein stabilizer and preservative. Reconstitute with 0.5 mL of distilled water. Controls must be stored below -18°C, and should only be frozen and thawed twice. Please refer to enclosed QC Report for control range.

Peroxidase Conjugated Antibody **ENZYMCONJ**

One vial (min. 0.25 mL) of a concentrated solution of a peroxidase conjugated murine monoclonal antibody specific against the N-terminal region of osteocalcin in a TRIS-buffered solution with protein stabilizer, detergent and preservative. Prior to use, add 10 mL ***Conjugate Diluent Solution****.*

Biotinylated Antibody **Ab BIOTIN**

One vial (min. 0.25 mL) of a concentrated conjugate solution of biotinylated murine monoclonal antibody against the mid-region of osteocalcin in a TRIS-buffered solution with protein stabilizer, detergent and preservative. Prior to use, add 10 mL ***Conjugate Diluent Solution****.*

Conjugate Diluent Solution **BUF**

One vial (min. 22 mL) of a PBS-buffered solution with protein stabilizer, detergent and preservative.

Substrate Solution **SUBS TMB**

One vial (min. 12 mL) of a ready for use tetramethylbenzidine (TMB) substrate in an acidic buffer.
Please note that the chromogenic substrate might appear slightly bluish.

Stopping Solution **H2SO4**
One vial (min. 12 mL) of ready for use 0.18 mol/L sulfuric acid.

Washing Solution **WASHBUF 50x**
One vial (min. 20 mL) of a concentrated washing buffer with detergent and preservative. Dilute 1+50 times in distilled water before use.

Sealing tape
Adhesive film for covering wells during incubation.

Materials required — not supplied
- *Containers for preparing the Antibody Solution and the Washing Solution.*
- *Precision micropipettes to deliver 20 µL*
- *Distilled water*
- *Precision 8- or 12-channel multipipette to deliver 100 µL and 150 µL.*
- *Microtiter plate reader with both 450 nm and 650 nm filters*

ASSAY PROCEDURE

For optimal perfomance of the assay it is important to comply with the instructions given below

Assay Procedure
Prior to use, prepare and equilibrate all solutions to room temperature. ***Perform the assay at 18-22°C.***

Determine the number of strips needed for the assay. It is recommended to test all samples in duplicate. In addition, for each run a total of 16 wells are needed for the standards and controls. Place the appropriate number of strips in the plastic frame. Store unused immunostrips in the tightly closed foil bag with desiccant capsules.

1 Preparation of the Antibody Solution:

The **Antibody Solution** is prepared by 1) adding 10 mL of **Conjugate Diluent Solution** **BUF** to both the **Peroxidase Conjugated Antibody Solution** **ENZYMCONJ** and the **Biotinylated Antibody Solution** **Ab biotin** , and 2) mixing the two conjugate solutions in equal volumes.

2 Incubation in Immuno Strips

Pipette 20 µL of either **Standards** **CAL 0 - 5** **-**, **Controls** **CTRL 1 - 2** **-** or unknown samples into appropriate wells followed by 150 µL of the **Antibody Solution**. Cover the immunostrips with sealing tape and incubate for 120±5 minutes at 18-22°C (without any mixing).

3 Washing

Wash the immunostrips 5 times manually with 300 µL diluted **Washing Buffer** (**WASHBUF 50x** diluted 1+50 in distilled water). Using an automated plate washer, follow the instructions of the manufacturer or the guidelines of the laboratory. Usually 5 washing cycles are adequate. Make sure that the wells are **completely emptied** after each manual or automatic washing cycle.

4 Incubation with chromogenic substrate solution

Pipette 100 µL of the **Substrate Solution** **SUBS TMB** into each well and incubate for 15±2 minutes at 18-22°C in the dark (without any mixing). Use sealing tape. Do not pipette directly from the vial containing TMB substrate but transfer the needed volume to a clean reservoir. Remaining substrate in the reservoir should be discarded and not returned to vial TMB.

5 Stopping of colour reaction

Pipette 100 µL of the **Stopping Solution** **H2SO4** into each well.

6 Measurement of absorbance

Measure the absorbance at 450 nm with 650 nm as reference within two hours.

Limitations of the procedure

If the absorbance of a sample exceeds that of **Standard 5**, the sample should be diluted in **Standard 0** and re-analysed.

QUALITY CONTROL

Good Laboratory Practice (GLP) requires the use of quality control specimens in each series of assays in order to check the performance of the assay. Controls should be treated as unknown samples, and the results analysed with appropriate statistical methods.

RESULTS

Calculation of results

A four-parametric logistic curve fit can be used.

Alternatively, calculate the mean of the duplicate absorbance determinations. Construct a standard curve on graph paper by plotting the mean absorbances of the six standards (ordinate) against the corresponding osteocalcin concentrations (abscissa). Determine the osteocalcin concentration of the controls and each patient sample by interpolation.

Example of results obtained

Standards/ Controls/ Samples	Osteocalcin (ng/mL)	$A_{450nm-650nm}$ Obs 1/ Obs 2	Mean $A_{450nm-650nm}$	Interpolated Osteocalcin (ng/mL)
Standard 0	0.0	0.019 / 0.020	0.020	
Standard 1	4.2	0.108 / 0.096	0.102	
Standard 2	7.6	0.183 / 0.177	0.180	
Standard 3	19.4	0.627 / 0.656	0.642	
Standard 4	35.4	1.271 / 1.278	1.275	
Standard 5	56.1	1.988 / 1.873	1.931	
Control 1		0.474 / 0.459	0.467	15.1
Control 2		1.147 / 1.132	1.140	31.8
Sample I		0.099 / 0.105	0.102	4.6
Sample II		0.899 / 0.850	0.875	25.1
Sample III		1.412 / 1.375	1.394	38.7

Please note:

The data above are for illustration only and should not be used to calculate the results of another assay.

Performance characteristics

All performance data have been established using second morning void urine samples unless otherwise indicated.

Detection limit: 0.5 ng/mL Osteocalcin

This is the concentration corresponding to three standard deviations above the mean of 21 determinations of the blank ("Osteocalcin Standard 0").

Precision

The precision of the N-MID® Osteocalcin ELISA was evaluated for three serum samples. The results are summarised in the table below:

InterAssay Variation (n=10)

Mean (ng/mL)	SD (ng/mL)	CV (%)
6.7	0.2	5.1
26.2	0.7	2.7
53.9	2.3	4.2

IntraAssay Variation (n=10)

Mean (ng/mL)	SD (ng/mL)	CV (%)
6.7	0.1	1.3
26.2	0.4	1.8
53.9	1.2	2.2

Dilution/Linearity

It was investigated if the N-MID® Osteocalcin ELISA assay was sensitive to any effect of the serum matrix. Four serum samples were diluted in Standard 0, and the concentrations were determined in the N-MID® Osteocalcin ELISA

Sample	Dilution	Observed (ng/mL)	Expected (ng/mL)	Recovery (% of expected value)
1	1 / 1	12.0	12.0	100
	1 / 2	5.1	6.0	84
	1 / 4	2.7	3.0	88
	1 / 8			
2	1 / 1	36.1	36.1	100
	1 / 2	18.1	18.1	106
	1 / 4	9.0	9.0	105
	1 / 8	4.7	4.5	104
3	1 / 1	38.2	38.2	100
	1 / 2	20.5	19.1	107
	1 / 4	9.9	9.6	104
	1 / 8	4.3	4.8	90
4	1 / 1	48.1	48.1	100
	1 / 2	23.8	24.1	99
	1 / 4	12.2	12.0	101
	1 / 8	6.3	6.0	105

Recovery

The accuracy of the N-MID® Osteocalcin ELISA was determined by spiking human serum with different amounts of synthetic osteocalcin. Three serum samples were mixed in equal volumes with 4 standard solutions and assayed in the N-MID® Osteocalcin ELISA

Sample	Standards synthetic osteocalcin (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (% of expected value)
1	50	26.9	30.5	88
	25	16.4	18.0	91
	12.5	10.7	11.7	92
	6.3	8.4	8.6	98
2	50	30.8	35.6	87
	25	19.9	20.6	97
	12.5	14.6	14.3	102
	6.3	11.0	11.2	98
3	50	37.5	40.5	93
	25	26.6	28.0	95
	12.5	21.2	21.7	98
	6.3	18.6	18.6	100

Measuring Range
The measuring range for N-MID® Osteoclacin ELISA is between 0.5 ng/mL and 100 ng/mL osteocalcin.

Interference:
In the concentration listed below no interference was detected:
Lipid (IntraLipid): 15 g/L
Bilirubin: 200 mg/L
Hemoglobin: 5 g/L

Expected values
It is advisable for a laboratory to establish its own range of normal and pathological values. As an example, the mean values and standard deviations for various populations are given below. For further reading, please refer to the reference list.

Populations	Number of subjects	Mean Values (ng/mL)	95% Confidence Interval
Premenopausal women	77	17.4	8.4 – 33.9
Postmenopausal women [1)]	131	26.5	12.8 – 55.0
Males	85	19.8	9.6 – 40.8

[1)] The average years after menopause is 10.3 years.

Day to Day Intra-individual Variation
The Day to Day Intra-individual Variation was assessed by analyzing serum samples (morning fasting) from 11 healthy postmenopausal women at five time points over 2 weeks

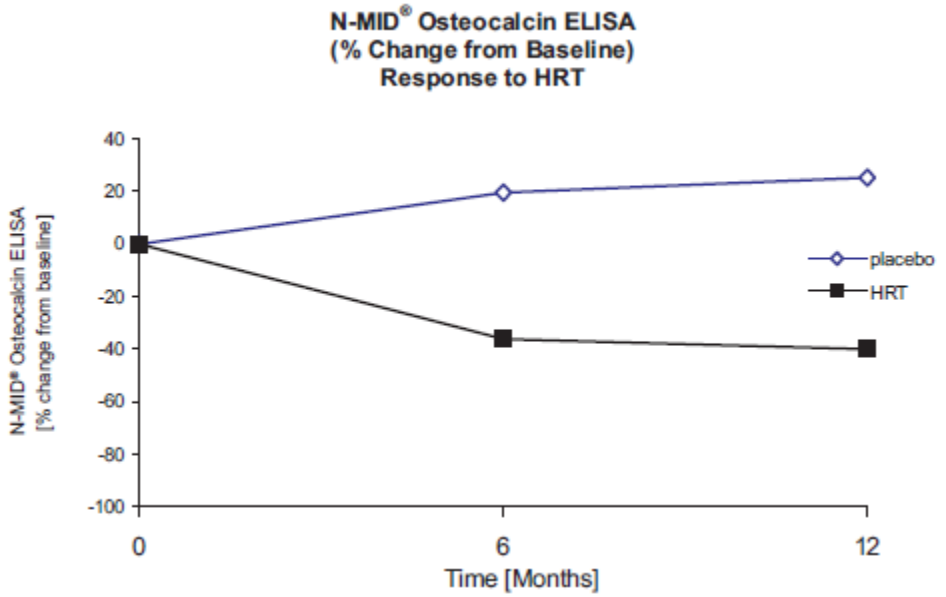
Subject	Mean (ng/mL)	SD (ng/mL)	CV (%)
1	22.0	3.5	16
2	13.4	1.0	7
3	19.6	1.3	7
4	18.0	3.1	17
5	12.9	1.1	9
6	9.9	0.9	9
7	14.4	2.2	15
8	7.5	0.4	5
9	15.3	2.2	15
10	14.5	1.6	11
11	14.8	0.6	4

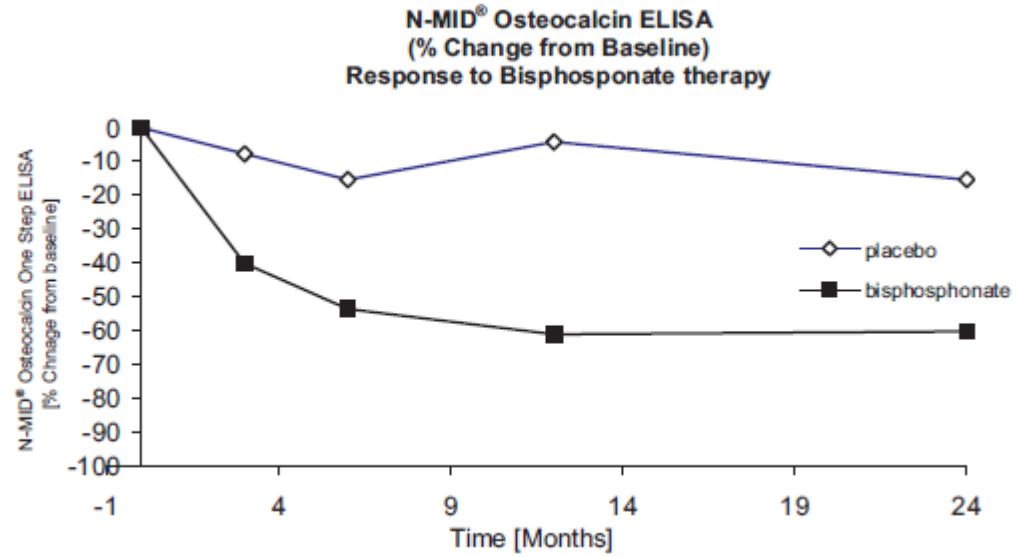
CLINICAL DATA

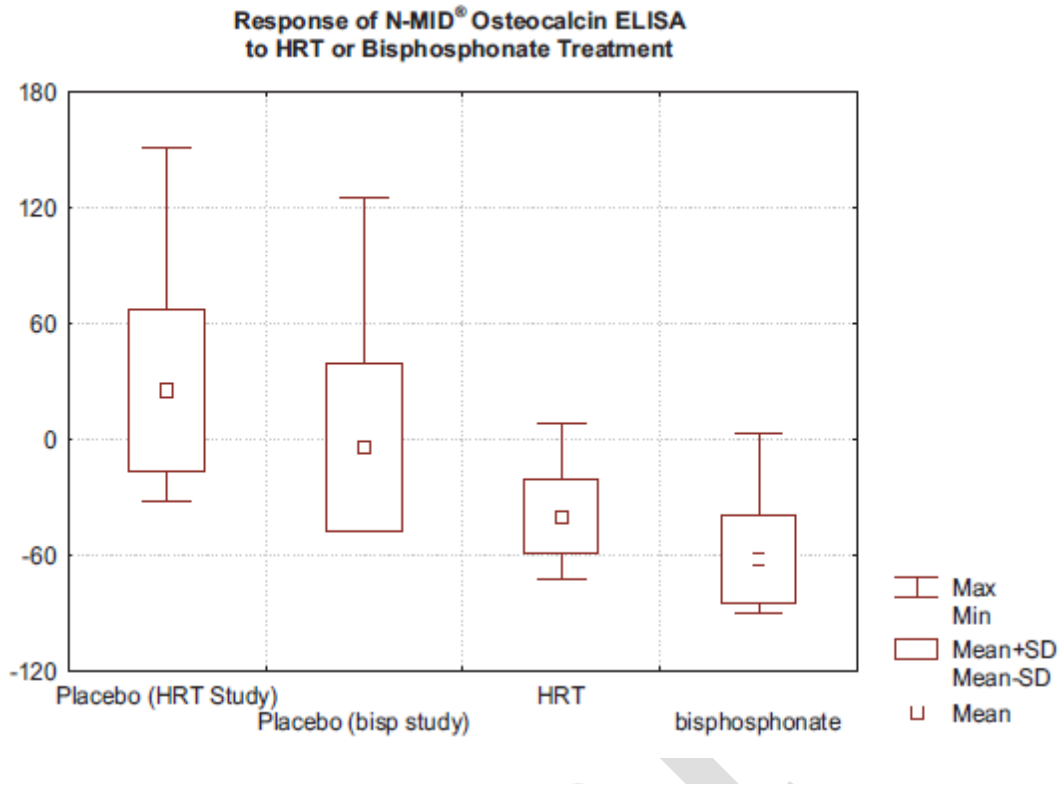
The N-MID® Osteocalcin ELISA has been used to monitor treatment in several clinical studies and the osteocalcin values have been compared to Bone Mineral Density (BMD_{spine}) measurements.

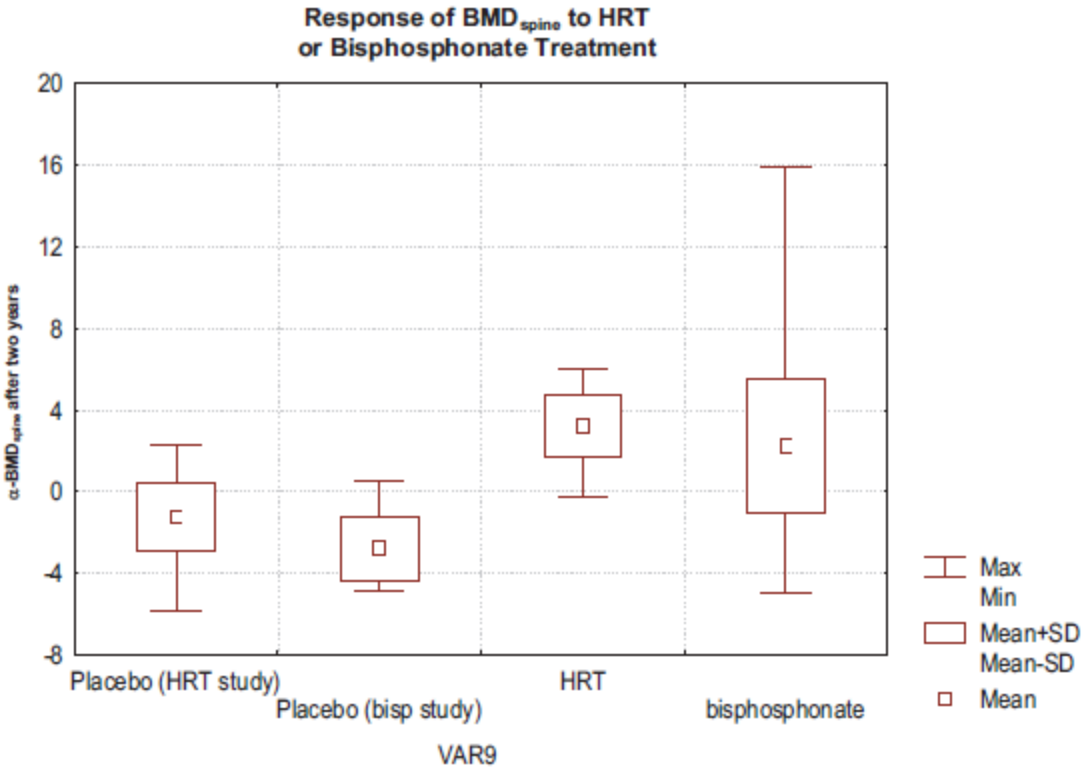
All the clinical studies presented below were performed according to the European Standard for good clinical practice (GCP and GLP).
The clinical studies presented here were conducted on white Danish women. However, several studies have been published showing that other demographic groups display similar osteocalcin decrease in response to anti-resorptive therapies (7, 8, 11, 12, 14).
The Bone Mineral Density (BMD) was measured at the Lumbar spine (L1 – L4). The change in the bone mineral density presented below is α–BMD, which is defined as the slope of the linear regression line for BMD_{spine} versus time (years) for the period of treatment, i.e. α–BMD represents the % change in BMD_{spine} per year.

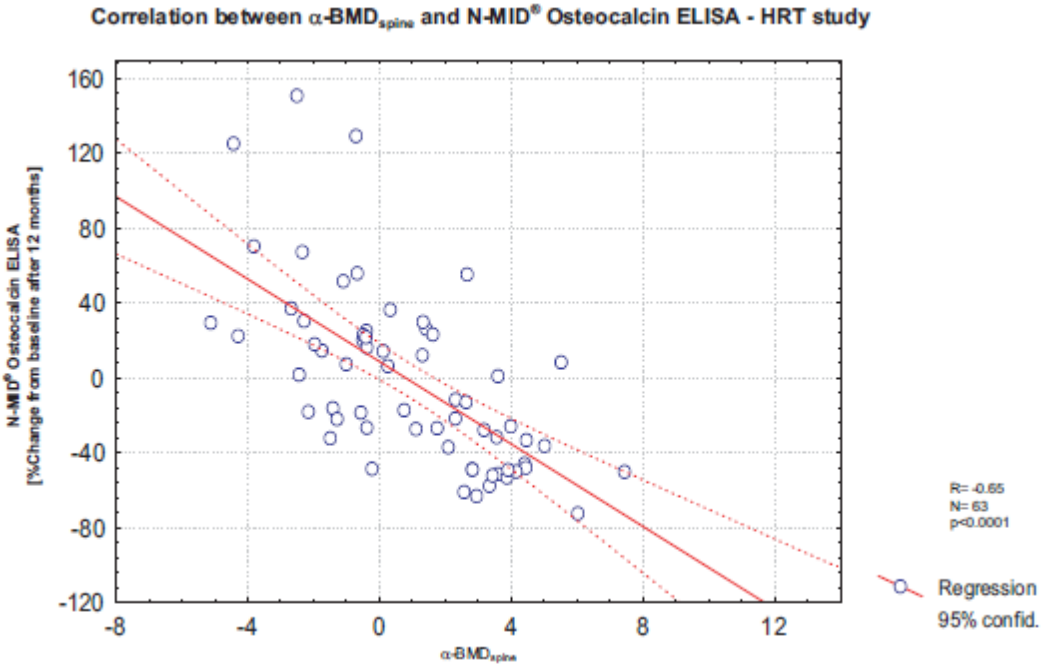
HRT study: • Women more than 45 year and 1-6 years since menopause • 35 participants on placebo • 26 participants on active treatment	**Bisphosphonate study:** • Women between age 40 and 59 years, and 6 months to 3 years since menopause. • 12 participants on placebo • 31 participants on active treatment

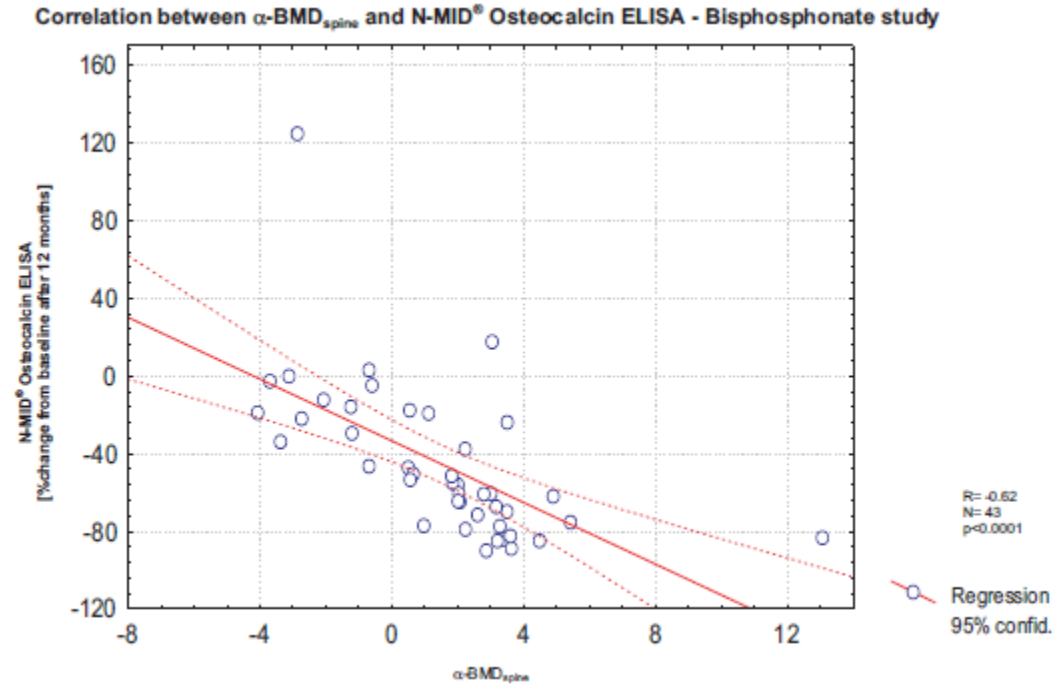

N-MID® Osteocalcin ELISA
(% Change from Baseline)
Response to HRT
N-MID® Osteocalcin ELISA
[% change from baseline]
40
20
0
-20
-40
-60
-80
-100
0
6
12
Time [Months]
placebo
HRT


N-MID® Osteocalcin ELISA
(% Change from Baseline)
Response to Bisphosphonate therapy
N-MID® Osteocalcin One Step ELISA
[% Chnage from baseline]
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-1
4
9
14
19
24
Time [Months]
placebo
bisphosphonate


Response of N-MID® Osteocalcin ELISA
to HRT or Bisphosphonate Treatment
180
120
60
0
-60
-120
Placebo (HRT Study)
Placebo (bisp study)
HRT
bisphosphonate
Max
Min
Mean+SD
Mean-SD
Mean


Response of BMDspine to HRT
or Bisphosphonate Treatment
20
16
12
8
4
0
-4
-8
α-BMDspine after two years
Placebo (HRT study)
Placebo (bisp study)
HRT
bisphosphonate
VAR9
Max
Min
Mean+SD
Mean-SD
Mean


Correlation between α-BMD$_{spine}$ and N-MID® Osteocalcin ELISA - HRT study
N-MID® Osteocalcin ELISA [%Change from baseline after 12 months]
160
120
80
40
0
-40
-80
-120
-8
-4
0
4
8
12
α-BMD$_{spine}$
R= -0.65
N= 63
p<0.0001
Regression
95% confid.


Correlation between α-BMD$_{spine}$ and N-MID® Osteocalcin ELISA - Bisphosphonate study
N-MID® Osteocalcin ELISA [%change from baseline after 12 months]
160
120
80
40
0
-40
-80
-120
-8
-4
0
4
8
12
α-BMD$_{spine}$
R= -0.62
N= 43
p<0.0001
Regression
95% confid.

Below is summarized the specificity and sensitivity using different cut-off values of N-MID® Osteocalcin ELISA, change from baseline after 12 months HRT or Bisphosphonate treatment for α-BMD cut-off of 0.5%.
95% confidence intervals are indicated.

HRT study

Cut-off of change in osteocalcin	Specificity	Sensitivity
-10 [% change from baseline]	83 (64 – 94)	74 (55 – 87)
-20 [% change from baseline]	76 (56 – 90)	85 (69 – 95)
-30 [% change from baseline]	62 (42 – 79)	94 (80 – 99)
-40 [% change from baseline]	48 (29 – 67)	97 (85 – 100)

Bisphosphonate study

Cut-off of change in osteocalcin	Specificity	Sensitivity
-10 [% change from baseline]	100 (89 – 100)	35 (14 – 62)
-20 [% change from baseline]	96 (80 – 100)	59 (33 – 82)
-30 [% change from baseline]	96 (80 – 100)	76 (50 – 93)
-40 [% change from baseline]	92 (75 –99)	82 (57 – 96)

REFERENCES

1. Bjarnason NH. et al., Tibolone: prevention of bone loss in late postmenopausal women. *J Clin Endocrinol Metab (1996) 81: 2419-2422.*
2. Christiansen C. et al., Dose dependent effects on bone resorption and formation of intermittently administered intravenous ibandronate. *Osteoporos Int (2003) 14: 609-613.*
3. Junqueira PA. et al., Comparison of bone remodeling indicators in climacteric women. *Int J Fertil Womens Med (2002) 47: 174-181.*
4. Marcus R. et al., The relationship of biochemical markers of bone turnover to bone density changes in postmenopausal women: results from the Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. *J Bone Mineral Res (1999) 14: 1583-1595.*
5. McClung M. et al., Alendronate prevents postmenopausal bone loss in women without osteoporosis. A double-blind, randomized, controlled trial. Alendronate Osteoporosis Prevention Study Group. *Ann Intern Med (1998) 128: 253-261.*
6. Meunier P. et al., Prevention of early postmenopausal bone loss with cyclical etidronate therapy (a double-blind, placebo-controlled study and 1-year follow-up). *J Clin Endocrinol Metab (1997) 82: 2784-2791.*
7. Ohishi T. et al., Changes of biochemical markers during fracture healing. *Arch Orthop Trauma Surg (1998) 118: 126-130.*
8. Ravn P. et al., Changes in biochemical markers and bone mass after withdrawal of ibandronate treatment: prediction of bone mass changes during treatment. *Bone (1998) 22: 559-564.*
9. Ravn P. et al., Biochemical markers can predict the response in bone mass during alendronate treatment in early postmenopausal women. Alendronate Osteoporosis Prevention Study Group. *Bone (1999) 24: 237-244.*
10. Ravn P. et al., High bone turnover is associated with low bone mass and spinal fracture in postmenopausal women. *Calcif Tissue Int (1997) 60: 255-260.*
11. Ravn P. et al., The effect on bone mass and bone markers of different doses of ibandronate: a new bisphosphonate for prevention and treatment of postmenopausal osteoporosis: a 1-year, randomized, double-blind, placebo-controlled dose-finding Study. *Bone (1996) 19: 527-533.*
12. Rosenquist C. et al., Measurement of a more stable region of osteocalcin in serum by ELISA with two monoclonal antibodies. *Clin Chem (1995) 41:1439-1445.*
13. Takahashi M. et al., Comparison of the analytical and clinical performance characteristics of an N-MID versus an intact osteocalcin immunoradiometric assay. *Clin Chim Acta (2000) 294:67-76.*
14. Tanko LB. et al., Oral ibandronate: changes in markers of bone turnover during adequately dosed continuous and weekly therapy and during different suboptimally dosed treatment regimens. *Bone (2003) 32: 687-693.*

	GB DE ES IT FR NL DK CZ SK GR PT HU SE PL	*Use By* *Verwendbar bis* *Fecha de caducidad* *Utilizzare entro* *Utiliser jusque* *Houdbaar tot* *Holdbar til* *Použitelné do* *Použiteľné do* *Ημερομηνία λήξης* *Prazo de validade* *Felhasználható* *Använd före* *Użyć przed*	**LOT**	GB DE ES IT FR NL DK CZ SK GR PT HU SE PL	*Batch code* *Chargenbezeichnung* *Código de lote* *Codice del lotto* *Code du lot* *Lot nummer* *Lotnummer* *Číslo šarže* *Číslo šarže* *Αριθμός Παρτίδας* *Código do lote* *Sarzsszám* *Lot nummer* *Kod partii*
REF	GB DE ES IT FR NL DK CZ SK GR PT HU SE PL	*Catalogue number* *Bestellnummer* *Número de catálogo* *Numero di catalogo* *Référence du catalogue* *Catalogus nummer* *Katalognummer* *Katalogové číslo* *Katalógové číslo* *Αριθμός καταλόγου* *Referência de catálogo* *Katalógusszám* *Katalognummer* *Numer katalogowy*		GB DE ES IT FR NL DK CZ SK GR PT HU SE PL	*Manufacturer* *Hersteller* *Fabricante* *Fabbricante* *Fabricant* *Fabrikant* *Producent* *Výrobce* *Výrobca* *Κατασκευαστής* *Fabricante* *Gyártó* *Tillverkare* *Producent*
	GB DE ES IT FR NL DK CZ SK GR PT HU SE PL	*Contains sufficient for <n> tests* *Inhalt ausreichend für <n> Prüfungen* *Contenido suficiente para <n> ensayos* *Contenuto sufficiente per "n" saggi* *Contenu suffisant pour "n"tests* *Inhoud voldoende voor "n" testen* *Indeholder tilsttrækkeligt til "n" test* *Lze použít pro <n> testů* *Obsah postačuje na <n> stanovení* *Περιεχόμενο επαρκές για «ν» εξετάσεις* *Conteúdo suficiente para "n" ensaios* *A doboz tartalma <n> vizsgálat elvégzéséhez elegendő* *Räcker till "n" antal tester* *Wystarczy na wykonanie <n> testów*	**IVD**	GB DE ES IT FR NL DK CZ SK GR PT HU SE PL	*In Vitro Diagnostic Medical Device* *In-Vitro-Diagnostikum* *Producto sanitario para diagnóstico in vitro* *Dispositivo medico-diagnostico in vitro* *Dispositif médical de diagnostic in vitro* *Medisch hulpmiddel voor in-vitro diagnostiek* *Medicinsk udstyr til in vitro-diagnostik* *In Vitro diagnostický zdravotnický prostředek* *Zdravotnícka pomocka in vitro* *In Vitro Διαγνωστικό Ιατροτεχνολογικό προϊόν* *Dispositivo médico para diagnóstico in vitro* *In vitro diagnosztikum* *Medicintekniska produkter för in vitro diagnostik* *Wyrób do diagnistyki In Vitro*
	GB DE ES IT FR NL DK CZ SK GR PT HU SE PL	*Temperature limitation* *Temperaturbegrenzung* *Límite de temperatura* *Limiti di temperatura* *Limites de température* *Temperatuurlimiet* *Temperaturbegrænsning* *Teplotní rozmezí od do* *Teplotné rozmedzie od do* *Περιορισμοί θερμοκρασίας* *Limites de temperatura* *Hőmérséklettartomány* *Temperaturbegränsning* *Przestrzegać zakresu temperatury*		GB DE ES IT FR NL DK CZ SK GR PT HU SE PL	*Consult Instructions for Use* *Gebrauchsanweisung beachten* *Consulte las instrucciones de uso* *Consultare le istruzioni per l'uso* *Consulter les instructions d'utilisation* *Raadpleeg de gebruiksaanwijzing* *Se brugsanvisning* *Viz návod k použití* *Viď návod na pužitie* *Συμβουλευτείτε τις οδηγίες χρήσης* *Consulte as instruções de utilização* *Nézze meg a Használati utasítást* *Se handhavandebeskrivningen* *Sprawdź w instrukcji obsługi*

Immunodiagnostic Systems Limited.

UK Immunodiagnostic Systems Limited, 10 Didcot Way, Boldon Business Park, Boldon, Tyne & Wear, NE35 9PD, UK

Tel: +44 (0) 191 519 0660 • Fax: +44 (0) 191 519 0760 • e-mail: info.uk@idsplc.com • www.idsplc.com
USA Immunodiagnostic Systems (IDS) Inc, 948 Clopper Road, Gaithersburg, MD 20878, USA

Tel: +1(877)852-6210 • Fax: +1 (301)990-4236 • e-mail: info.us@idsplc.com • www.idsplc.com

Germany Immunodiagnostic Systems GmbH (IDS GmbH), Mainzer Landstrasse 49, 60329 Frankfurt am Main

Tel: +49 (0) 69 3085-5025 • Fax: +49 (0) 69 3085-5125 • e-mail: info.de@idsplc.com • www.idsplc.com

France Immunodiagnostic Systems France SA (IDS France SA) 153 Avenue D'Italie, 75013 PARIS

Tel: +33 (0)1 40 77 04 50 • Fax: +33 (0)1 40 77 04 55 • e-mail: info.fr@idsplc.com • www.idsplc.com
Scandinavia Immunodiagnostic Systems Nordic a/s (IDS Nordic a/s), International House, Center Boulevard 5, 2300 København S, Denmark
Tel:+45 44 84 0091 e-mail: info.nordic@idsplc.com • www.idsplc.com