Instructions for Use

Crosslaps Serum ELISA

IVD

CE

REF **EIA-2124**

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Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
Si prega di usare la versione valida delle istruzioni per l'uso a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.
Utilisez seulement la version valide des Instructions d'utilisation fournies avec le kit.

Table of Contents

1 INTENDED USE

For *In Vitro* Diagnostic Use.

The Crosslaps Serum ELISA is an enzyme immunological test for the quantification of degradation products of C-terminal telopeptides of Type I collagen in human serum and plasma.

The Crosslaps Serum ELISA assay is intended for *in vitro* diagnostic use as an indication of human bone resorption and may be used as an aid in:

- Monitoring bone resorption changes of anti-resorptive therapies (such as hormone replacement therapies (HRT) with hormones or hormone like drugs and bisphosphonate therapies) in postmenopausal women and individuals diagnosed with osteopenia
- Predicting skeletal response (Bone Mineral Density) in postmenopausal women undergoing anti resorptive therapies, such as HRT with hormones or hormone like drugs and bisphosphonate therapies

2 SUMMARY AND EXPLANATION

Type I collagen accounts for more than 90% of the organic matrix of bone and is synthesized primarily in bone (1). During renewal of the skeleton, Type I collagen is degraded, and small peptide fragments are excreted into the bloodstream. These fragments can be measured by Crosslaps Serum ELISA. The measurements of the specific degradation products of Type I collagen in both urine (2) and serum (3) by a competitive CrossLaps have been reported.

The sandwich assay has been reported as useful for follow up of anti resorptive treatment of patients with metabolic bone diseases (3-17).

3 METHOD DESCRIPTION

The Crosslaps Serum ELISA is an enzyme immunological assay which is based on two highly specific monoclonal antibodies against the amino acid sequence of EKAHD-β-GGR, where the aspartic acid residue (D) is β-isomerised. To obtain a specific signal in the Crosslaps Serum ELISA, two chains of EKAHD-β-GGR must be cross linked.

50 µL of each standards, controls or unknown serum samples are pipetted into microtiter wells coated with streptavidin, followed by application of a mixture of a biotinylated antibody and an enzyme (horseradish peroxidase – HRP) conjugated antibody. Then, a complex between CrossLaps® antigens, biotinylated antibody and HRP conjugated antibody is generated; this complex binds to the streptavidin surface via the biotinylated antibody. Following the one step incubation at room temperature (18 °C - 22 °C), the wells are emptied and washed. The colour is developed using a chromogenic substrate (TMB). The colour reaction is stopped and the absorbance of the stopped reaction mixture is read in a microtiter plate reader. The colour intensity of the reaction mixture is proportional to the concentration of the cross-linked CTX-I in the original sample.

4 WARNINGS AND PRECAUTIONS

The Crosslaps Serum ELISA is for *in vitro* diagnostic use only and is not for internal use in humans or animals. This product must be used strictly in accordance with the instructions set out in these Instructions for Use (IFU). DRG will not be held responsible for any loss or damage (except as required by statute), howsoever caused, arising out of non-compliance with the instructions provided.

CAUTION: This kit contains material of animal origin. Handle kit reagents as if capable of transmitting an infectious agent. Appropriate precautions and good laboratory practice must be used in the storage, handling and disposal of the kit reagents. Disposal of kit reagents should be in accordance with local regulations.

Human materials

Human material used in the preparation of this product has been tested by FDA recommended assays for the presence of antibody to Human Immunodeficiency Virus (HIV I and II), Hepatitis B surface antigen, antibody to Hepatitis C, and found negative. As no test can offer complete assurance that infectious agents are absent, the reagents should be handled according to Biosafety Level 2.

Reagents containing Sodium Azide

Some reagents in this kit contain sodium azide (NaN_3) <0.1 % (w/w) which may react with lead, copper or brass plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent azide build up.

Classification according to Regulation (EC) CLP:

Skin sensitivity, Category 1

Warning

Contains ProClin 300

Hazard statements:

H317 - May cause an allergic skin reaction.

Precautionary statements:

P261 - Avoid breathing dust/fume/gas/mist/vapour/spray.

P280 - Wear protective gloves/protective clothing/eye protection/face protection/hearing protection.

P321 - Specific treatment /see supplemental first aid instructions on this label).

P333 + P313 - If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 - Takeoff contaminated clothing and wash it before use.

5 SHELF LIFE AND STORAGE OF REAGENTS

This kit is stable until the expiry date printed on the box if stored as specified.

Upon receipt, store all reagents at 2 °C - 8 °C.

Do not use any kit component beyond their expiry date.

Indications of possible deterioration of kit reagents include:

- The presence of abnormal particulate matter in any of the reagents.
- A decrease in the maximum binding.
- A high non-specific binding.
- A shift in the slope of the curve from its normal position.

6 SAMPLE COLLECTION AND STORAGE

For optimal results it is recommended to draw blood as fasting morning samples (18). For monitoring individual patients, it is recommended that follow up samples should be collected under same conditions as the baseline sample.

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.

The assay should be performed using serum or plasma (heparin or potassium EDTA) samples.

Note:

- Some commercially available sample collection tubes may affect the results of testing in particular cases.
- Follow the blood collection tube manufacturer's recommendations for handling and processing the samples.
- Samples containing particulate matter must be centrifuged before performing the assay. Centrifuged samples with a lipid layer on the top must be transferred to a sample cup or secondary tube. Care must be taken to transfer only the clarified samples without the lipemic material
- Samples displaying microbial contamination, highly lipemic or grossly haemolysed should not be assayed with the kit.
- Before performing assays, make sure that samples, calibrators and controls are at room temperature (18 °C - 22 °C).
- Do not use heat-inactivated samples.
- Avoid repeat freeze/thaw cycles for samples.
- 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”.

7 MATERIALS

7.1 Materials Provided

The kit contains reagents sufficient for 96 determinations.

Streptavidin coated plate **MICROPLAT**
Microwell strips (12 × 8 wells) pre-coated with streptavidin, supplied in a plastic frame.

Biotinylated Antibody **Ab BIOTIN**
Concentrated solution of biotinylated monoclonal anti-CTX-I, in buffered solution with protein stabiliser and sodium azide as preservative (<0.1 %); **1 vial, 0.25 mL**

Peroxidase Conjugated Antibody **ENZYMCONJ**
Concentrated peroxidase conjugated monoclonal antibody specific for CTX-I; provided in a buffered solution with protein stabiliser; **1 vial, 0.25 mL**

Incubation Buffer **BUF**
Ready to use buffered solution containing protein stabiliser and detergents, with Proclin 300 as preservative (<0.1 %); **1 vial, 19.0 mL**

Substrate Solution **SUBS TMB**
Ready to use tetramethylbenzidine (TMB) substrate in an acidic buffer; **1 vial, 12.0 mL**
Please note that the chromogenic substrate might appear slightly blueish.

Stopping Solution **H2SO4**
Ready to use solution of 0.18 mol/L Sulfuric acid; **1 vial, 12.0 mL**

Washing Buffer **WASHBUF 50x**
Concentrated washing buffer with detergent; **1 vial, 20.0 mL**

Standard **CAL 0**
Ready for use Phosphate buffered solution containing protein stabiliser and ProClin 300 as preservative (<0.1 %); **1 vial, 5.0 mL**

Standards **CAL 1 - 5**
Ready for use Phosphate buffered solution containing CTX-I antigen, protein stabiliser ProClin 300 as preservative (<0.1 %); **1 each of 5 concentration levels, 0.4 mL per vial.**
The exact value of each standard is printed on the QC report.

Controls **CTRL 1 – 2**
Ready for use Phosphate buffered solution containing desalted urinary antigen of human origin with protein stabilisers and ProClin 300 as preservative (<0.1 %); **1 each of 2 concentration levels, 0.4 mL per vial.**
The established ranges for the controls are printed on the QC report.

Adhesive Plate Sealer 2 per kit.

Documentation Instructions for Use and QC report.

7.2 Materials Required But Not Provided

- Containers for preparing the Antibody Solution and the Washing Solution
- Precision pipetting devices to deliver 50 – 200 µL
- Distilled water
- Precision 8 or 12 channel multipipette to deliver 100 µL to 300 µL
- Vortex mixer
- Microwell mixing apparatus
- Automatic microplate washer (optional)
- Photometric microplate reader and data analysis equipment

8 PREPARATION OF REAGENTS

Allow all reagents to come to room temperature for a minimum of 60 minutes before use. Do not interchange kit components from different lots.

Antibody Solution

Prepare the Antibody Solution a maximum of 30 minutes before starting the assay.
Mix the Biotinylated Antibody **Ab BIOTIN**, Peroxidase Conjugated Antibody **ENZYMCONJ** and Incubation Buffer **BUF** in the volumetric ratio 1 + 1 + 100 in an empty container. Mix carefully and avoid formation of foam.

Prepare a fresh solution before each run of the assay.

Wash buffer preparation

Prepare by adding 1-part Wash Concentrate **WASHBUF 50x** to 50-parts distilled water.

All other reagents are supplied ready for use.

N.B. To avoid potential microbial and / or chemical contamination, unused reagents should never be returned into the original vials.

9 ASSAY PROCEDURE

Prepare reagents as described in § 8. Preparation of Reagents. Mix all reagents and samples before use (avoid formation of foam).

NOTE: To ensure consistent results between runs, between operators, and to minimise any drift effect; strictly adhere to the following procedure:

a. Bring all reagents to room temperature (18 °C – 22 °C) prior to use – this will take approximately 60 minutes.

b. Set up the assay within 30 minutes of preparing the Antibody Solution.

c. Seal the plate during incubations using the plate sealers which are supplied with the assay kit.

d. Do not stack plates during incubation in order to ensure a consistent temperature for all plates.

e. Do not under or over-fill the assay wells during the washing steps.

f. Add reagents in the same sequence each time to reduce time deviation between reactions

Do not pipette directly from the vial containing TMB substrate. The required volume should first be transferred to a clean container. Solution remaining in the container should be discarded following use and NOT returned to the stock vial **SUBS TMB** **.**

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 any unused strips in the tightly closed foil bag with desiccant capsules.

1. Pipette 50 µL of each standard **CAL 0 - 5**, control **CTRL 1 – 2** or sample SPE to the appropriate wells on the Streptavidin coated plate **MICROPLAT**
   followed by 150 µL of the Antibody Solution **AB SOLN**.
2. Cover the plate with an adhesive plate seal.
3. Incubate at room temperature (18 °C – 22 °C) for 120 ±5 minutes on a microtiter plate mixer (300 rpm).
4. Wash all wells 5 times with diluted wash solution **WASHBUF SOLN**.
   Automatic plate wash — Set plate washer to dispense 300 µL of wash solution per well. Fill and aspirate for 5 cycles.
   Manual wash — Decant the contents of the wells by inverting sharply. Pipette 300 µL of wash solution into each well, decant and repeat 5 times. Remove excess wash buffer by tapping firmly on absorbent tissue before proceeding.
5. Pipette 100 µL of Substrate Solution **SUBS TMB** into each well.
6. Cover the plate with an adhesive plate seal.
7. Incubate at room temperature (18 - 22°C) for 15 minutes in the dark on a microtiter plate mixer (300 rpm).
   NOTE: do not pipette directly from the vial containing TMB substrate. The required volume should first be transferred to a clean container. Solution remaining in the container should be discarded following use and NOT returned to the stock vial **SUBS TMB**.

8. Pipette 100 µL of Stopping Solution **H2SO4** into each well.
9. Measure absorbance at 450 nm with reference at 650 nm using a microplate reader within 2 hours of stopping the reaction.

N.B. Microplate readers measure vertically; when pipetting, do not touch the bottom of the wells

Automated Platforms

The Crosslaps Serum ELISA kit was designed and developed to be performed manually using the protocol described above. The protocol is not necessarily applicable to automated platforms.

If automated platforms are used it is the responsibility of the user to ensure the kit has been appropriately tested. To improve the performance of the kit on automated platforms, it is recommended to increase the number of wash cycles at each wash step.

10 CALCULATION OF RESULTS

A variety of data reduction software packages are available, which may be employed to generate the mean calibration curve and to calculate the mean concentrations of unknown samples and controls. A quadratic curve fit, **including Calibrator 0 is required.**

Alternatively, a calibration curve may be prepared on semi-log graph paper by plotting mean absorbance on the Y-axis against concentration of CTX-I on the X-axis. Calibrator 0 should be included in the calibration curve. Read the mean absorbance value of each unknown sample off the curve.

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

11 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.

The two kit controls provided in the kit should be tested as unknowns and are intended to assist in assessing the validity of results obtained with each assay plate.

DRG recommends the users to maintain graphic records of the control values generated with each assay run, including the running means, SDs and %CVs. This information will facilitate the controls trending analysis relating to the performance of current and historical control lots relative to the supplied Quality Control data. The trending will assist in the identification of assays which give control values significantly different from their average range.

When interpreting control data, users should note that this product was designed and developed as a manual product. The range stated on the QC report should be appropriate for assays that are performed manually and with strict adherence to the Assay Procedure described above. It is recognised by Quality Control professionals, that as a result of differences in conditions and practices, there will always be variability in the mean values and precision of control measurements between different laboratories (27).

12 MEASUREMENT RANGE (REPORTABLE RANGE)

The reportable range of the assay is from 0.020 ng/mL.

Any value that reads below 0.020 ng/mL should be reported as “< 0.02 ng/mL”.

Samples with CTX-I concentrations above the absorbance of calibrator 5 should be diluted with calibrator 0. The results for diluted samples must be multiplied by the appropriate dilution factor.

13 TRACEABILITY

The Crosslaps Serum ELISA has been standardised against in-house reference standards (CTX-I in analyte-free buffered protein matrix).

14 LIMITATIONS OF USE

- As in the case of any diagnostic procedure, results must be interpreted in conjunction with the patient’s clinical presentation and other information available to the physician.
- The performance characteristics of this assay have not been established in a paediatric population.

- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays (28). Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed.
- The recommended daily allowance of Biotin (defined as 0.03 mg) does not typically cause interference with immunoassays that use biotin-streptavidin technology. Higher biotin levels may cause interference and affect corresponding test results, false highs or false lows (29,30). Biotin has a very rapid elimination half-life of about 2 hours; theoretically, most of it should clear from the body within 4–5 hours (31). Concentrations of biotin up to 1200 ng/mL may be present in specimens collected from patients taking up to 300 mg per day (30).
- For results that are not consistent with the patient's clinical presentation, biotin interference may be considered. Physicians and other practitioners should inquire and advise patients to abstain from biotin intake before blood draw31. The lowest Biotin level that does not significantly raise the results (<10% bias) within the Crosslaps Serum ELISA kit is 10 ng/mL.
- The following substances do not interfere in the Crosslaps Serum ELISA when the concentrations presented in the following table are below the stated threshold.

Potentially Interfering Agent	Threshold Concentration
Ditaurobilirubin	600 mg/L
Haemoglobin	10 g/L
Intralipid	10 g/L

15 EXPECTED VALUES

The following ranges were determined using the Crosslaps Serum ELISA and are provided for information only. For further reading, please refer to the reference list.

All samples were morning fasting samples from healthy individuals.

Populations	Number of subjects	Mean Values* (ng/mL)	95% Confidence Interval (ng/mL)
Post-menopausal women	193	0.439	0.142 – 1.351
Pre-menopausal women	226	0.287	0.112 – 0.738
Males	125	0.294	0.115 – 0.748

The above ranges should be considered as guidelines only; it is recommended that each laboratory establish its own expected range based upon its own patient population.

16 CLINICAL STUDIES

The Crosslaps Serum ELISA has been used to monitor treatment in several clinical studies and the CrossLaps 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).
Most of the clinical studies presented here were conducted on white Danish women. However, several studies have been published showing that other demographic groups display similar CrossLaps decrease in response to anti resorptive therapies (9-13).

For all the data presented below fasting morning samples have been used.

The Bone mineral density was measured at the Lumbar spine (L1, L4).

The change in the bone mineral density is presented below α-BMD. α-BMD is defined as the slope of the linear regression line for BMD-spine versus time (years) for the period of treatment. In most cases the calculation of α BMD involves a minimum of 5 BMD-spine measurements. The α-BMD thus represents the % change in BMD-spine per year.

Because there to this date is no universal agreement as to what constitutes positive BMD response we have calculated the sensitivity and specificity using two different cut off values for α-BMD; α-BMD > 0 and α-BMD > 1.

The sensitivity is defined as the percent of the study population with a positive BMD response and who have a % change from baseline of Crosslaps Serum ELISA which is 40% or greater.

The specificity is defined as the percent of the study population without a positive BMD response and who have a % change from baseline of Crosslaps Serum ELISA that is less than 40%.

16.1 Bisphosphonate studies

Below is shown the Crosslaps Serum ELISA data from two different bisphosphonate studies.

Alendronate

- Women between age 40 and 59 years, 6 months to 3 years since menopause
- 12 participants on placebo (500 mg calcium)
- 42 participants on active treatment (5 mg (n=16), 10 mg (n=14), 20 mg (n=12)) Alendronate and 500 mg calcium)
- Treatment period: 2 - 3 years

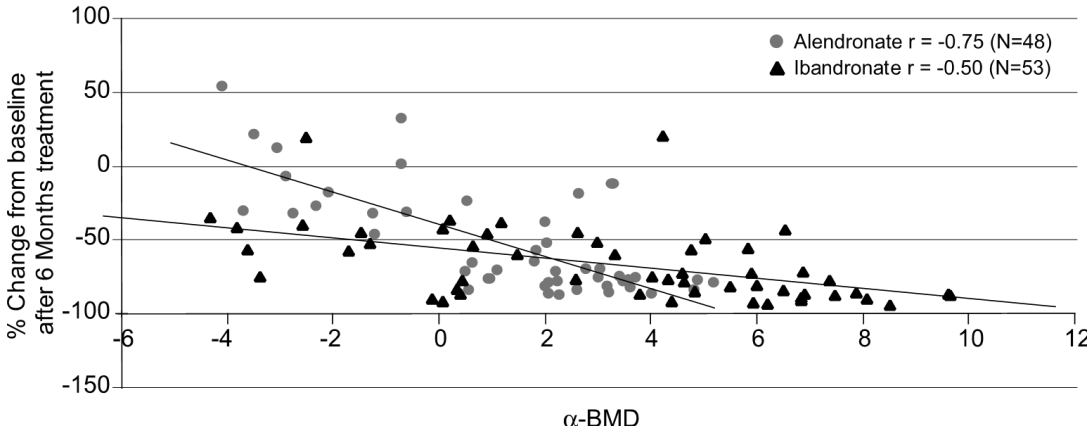
	Placebo Group			Alendronate Group		
Crosslaps Serum ELISA	**Mean (ng/mL) (CI)**	**SD**	**SEM**	**Mean (ng/mL) (CI)**	**SD**	**SEM**
Baseline	0.676 (0.543 - 0.809)	0.235	0.068	0.603 (0.540 - 0.666)	0.208	0.032
After 6 months treatment	0.623 (0.498 - 0.748)	0.221	0.064	0.191 (0.144 - 0.238)	0.153	0.024

Ibandronate

- Women less than 75 years, more than ten years after menopause and have a BMD forearm 1.5 SD or more below the standard for healthy pre-menopausal women
- 17 participants on placebo (1000 mg calcium)
- 36 participants on active treatment: (2.5 mg (n=20), 5 mg (n=16)) ibandronate and 1000 mg calcium
- Treatment period: 1 year

	Placebo Group			Ibandronate Group		
Crosslaps Serum ELISA	**Mean (ng/mL) (CI)**	**SD**	**SEM**	**Mean (ng/mL) (CI)**	**SD**	**SEM**
Baseline	0.590 (0.502 - 0.678)	0.185	0.045	0.614 (0.536 - 0.692)	0.240	0.040
After 6 months treatment	0.325 (0.241 - 0.409)	0.178	0.043	0.136 (0.093 - 0.179)	0.131	0.022

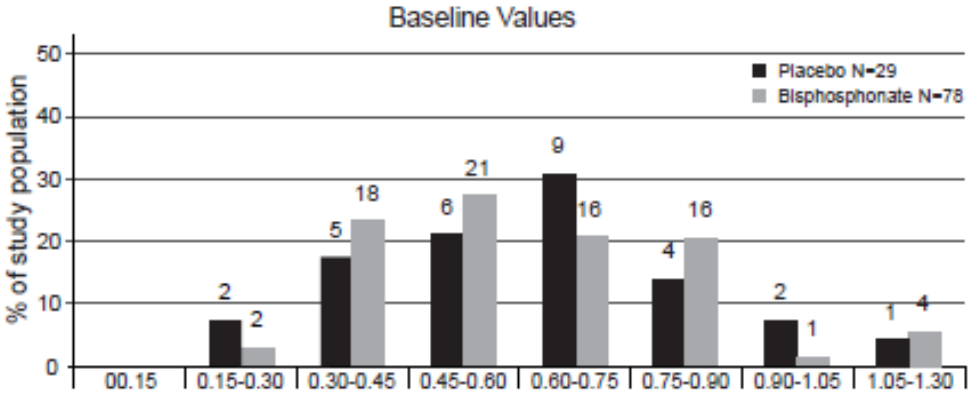
Crosslaps Serum ELISA versus α-BMD for patients treated with bisphosphonates

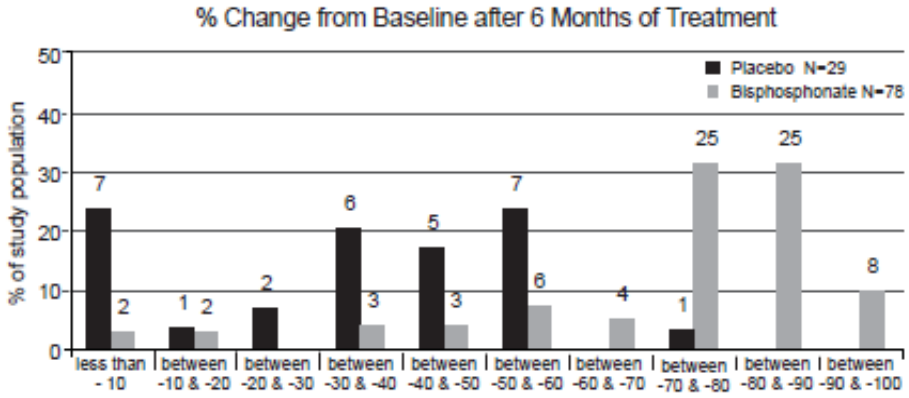


Using a cut-off for Crosslaps Serum ELISA of 40% change from baseline the following sensitivities, specificities and 95% confidence intervals are obtained. The corresponding data is reported below:

	Ibandronate		Alendronate	
	Sensitivity	**Specificity**	**Sensitivity**	**Specificity**
α-BMD > 0	93% (81 - 99) 40/43	30% (7 - 65) 3/10	89% (73 - 97) 31/35	92% (64 - 100) 12/13
α-BMD > 1	94% (81 - 99) 33/35	22% (6 - 48) 4/18	90% (73-98) 26/29	68% (43 - 87) 13/19

Below are shown distribution plots for the combined bisphosphonate studies. The number over each bar indicates the number of participants in each class.





16.2 HRT Studies

Below is shown the Crosslaps Serum ELISA data from three different HRT studies.

Tibolone

- Women less than 75 years and more than ten years after meno pause
- 13 participants on placebo (400 mg calcium/day)
- 49 participants on active treatment (1.25 mg (n=25) or 2.5 mg (n=24) Tibolone and 400 mg calcium/day
- Treatment period 2 years

	Placebo Group			Tibolone Group		
Crosslaps Serum ELISA	**Mean (ng/mL) (CI)**	**SD**	**SEM**	**Mean (ng/mL) (CI)**	**SD**	**SEM**
Baseline	0.264 (0.217 - 0.311)	0.085	0.024	0.339 (0.302 - 0.376)	0.130	0.019
After 6 months treatment	0.287 (0.232 - 0.342)	0.099	0.028	0.192 (0.161 - 0.223)	0.113	0.016

HRT I

- Women more than 45 years, 1 to 6 years since menopause
- 42 participants on placebo (400 mg calcium/day)
- 120 participants on active treatment:

Days 1 - 16	Days 17 - 28
E 1 mg	E 1 mg + G 25 µg
E 2 mg	E 2 mg + G 25 µg
E 2 mg	E 2 mg + G 50 µg
E 1 mg + G 25 µg	E 1 mg + G 25 µg continuously

E = estradiol 17β, G = gestodene, active treatment also receive 400 mg calcium/day

- Treatment period: 2 years.

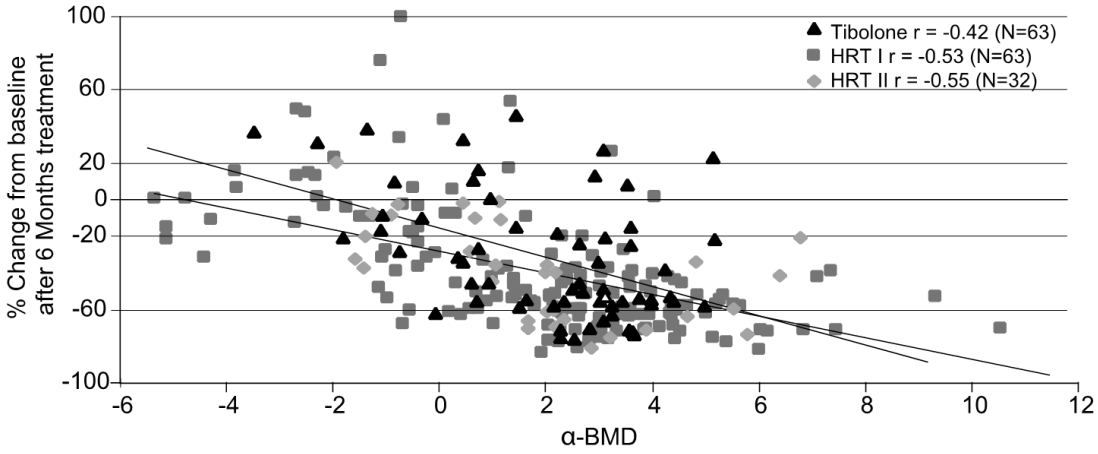
Crosslaps Serum ELISA	Placebo Group			HRT I Group		
	Mean (ng/mL) (CI)	SD	SEM	Mean (ng/mL) (CI)	SD	SEM
Baseline	0.389 (0.348 - 0.430)	0.136	0.021	0.411 (0.386 - 0.437)	0.140	0.013
After 6 months treatment	0.396 (0.349 - 0.443)	0.153	0.024	0.182 (0.164 - 0.200)	0.098	0.009

HRT II

- Women between 65 and 70 years and BMC forearm below 1 SD of healthy pre-menopausal women
- 17 participants on placebo (1000 mg calcium/day)
- 15 participants on active treatment: 50 µg estradiol, 1 mg norethisterone and 1000 mg calcium/day

Crosslaps Serum ELISA	Placebo Group			HRT II Group		
	Mean (ng/mL) (CI)	SD	SEM	Mean (ng/mL) (CI)	SD	SEM
Baseline	0.350 (0.301 - 0.399)	0.099	0.025	0.371 (0.306 - 0.436)	0.135	0.033
After 6 months treatment	0.293 (0.240 - 0.346)	0.106	0.027	0.159 (0.108 - 0.210)	0.106	0.026

Crosslaps Serum ELISA versus α-BMD for patients treated with HRT

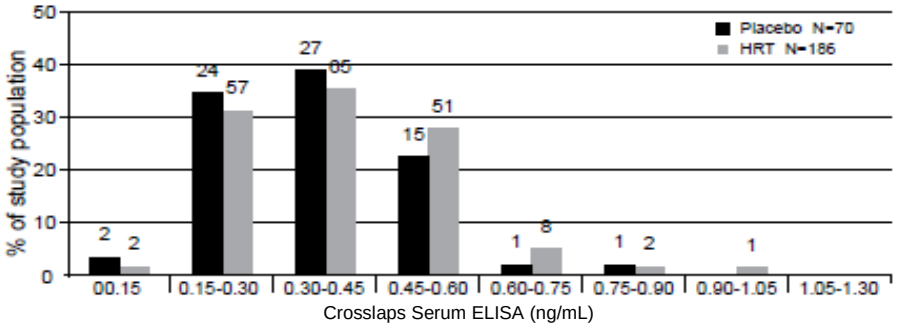


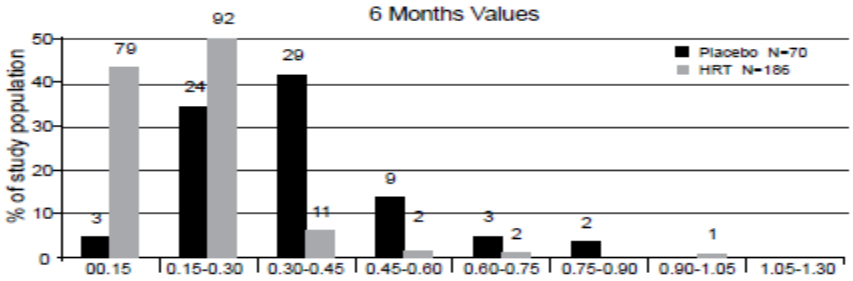
Using a cut off for Crosslaps Serum ELISA of 40% change from baseline the following sensitivities, specificities and 95% confidence intervals are obtained. The corresponding data is reported below:

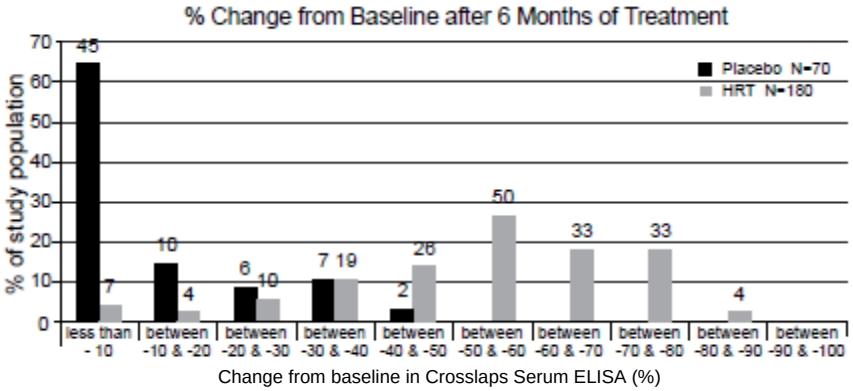
	Tibolone		HRT I		HRT II	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
α-BMD > 0	58% (44 - 72) 31/53	90% (56 - 100) 9/10	80% (72 - 87) 16/20	90% (77 - 97) 37/41	60% (39 - 79) 15/25	100% (59 - 100) 7/7
α-BMD > I	65% (49 - 79) 28/43	80% (56 - 94) 16/20	83% (74 - 94) 90/109	78% (64 - 88) 42/54	62% (38 - 82) 13/21	91% (59 - 100) 10/11

Below is shown distribution plots for the combined HRT studies. The number over each bar indicates the number of participants in each class

Change from baseline in Crosslaps Serum ELISA (%)







17 PERFORMANCE DATA

Representative performance data are shown. Results obtained at individual laboratories may vary.

17.1 Sensitivity

The sensitivity, defined as the concentration corresponding to the mean minus 3 standard deviations of 21 replicates of the zero calibrator, is 0.020 ng/mL.

17.2 Precision

The imprecision of the Crosslaps Serum ELISA was evaluated for three serum samples. The results are summarised in the table below:

	Inter Assay Variation (n=10)		Intra Assay Variation (n=10)	
Mean (ng/mL)	**SD (ng/mL)**	**CV (%)**	**SD (ng/mL)**	**CV (%)**
0.121	0.013	10.9	0.004	3.0
0.444	0.043	9.7	0.007	1.7
1.967	0.050	2.5	0.035	1.8

Intra-individual variation was assessed by evaluating patient sera, collected as morning fasted sample from 11 healthy postmenopausal women on 5 occasions within a 2-week period:

	Visit Number					Mean	SD	CV
ID	**1**	**2**	**3**	**4**	**5**	**(ng/mL)**	**(ng/mL)**	
1	0.423	0.428	0.396	0.46	0.445	0.430	0.024	6%
2	0.461	0.523	0.500	0.535	0.539	0.512	0.032	6%
3	0.850	0.731	0.761	0.782	0.764	0.778	0.044	6%
4	0.377	0.468	0.455	0.499	0.440	0.448	0.045	10%
5	0.918	0.834	0.791	0.781	0.714	0.808	0.075	9%
6	0.268	0.249	0.246	0.257	0.258	0.256	0.009	3%
7	0.431	0.457	0.468	0.494	0.506	0.471	0.030	6%
8	0.666	0.587	0.670	0.595	0.728	0.655	0.063	10%
9	0.323	0.357	0.341	0.409	0.345	0.355	0.033	9%
10	0.419	0.520	0.541	0.491	0.470	0.488	0.047	10%
11	0.353	0.472	0.429	0.464	0.400	0.424	0.049	12%

17.3 Dilution/Linearity

The Crosslaps Serum ELISA is linear in the range 0.020 ng/mL to 3.380 ng/mL of CTX-I.

Serum samples with the concentration of 0.460 - 0.668 ng/mL CTX-I were diluted with standard 0 and the concentration of CTX-I were determined with Crosslaps Serum ELISA. The serum neat sample is set to 100%.

The data below is calculated from 3 different runs:

Dilution Procedure		
Serum [%]	**Standard 0 [%]**	**Recovery [% of expected value]**
100	0	100
90	10	103
80	20	103
70	30	101
60	40	102
50	50	105
40	60	109
30	70	107
20	80	97
10	90	100
Mean		103

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SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français	Português
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes	Conformidade Europeia
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation	Consultar as instruções de uso
IVD	*In vitro* diagnostic medical device *	*In-vitro*-Diagnostikum *	Diagnostica in vitro	Diagnóstico in vitro	Diagnostic in vitro	Dispositivo médico para diagnóstico in vitro
REF	Catalogue number *	Katalognummer *	No. di Cat.	No de catálogo	Référence	Número de catálogo
LOT	Batch code *	Chargen-bezeichnung *	Lotto no	Número de lote	No. de lot	Código do lote
	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests	Suficiente para <n> determinações
	Temperature limit *	Temperaturgrenzwerte *	Temperatura di conservazione	Temperatura de conservacion	Temperature de conservation	Limites de temperatura
	Use-by date *	Verwendbar bis *	Data di scadenza	Fecha de caducidad	Date limite d'utilisation	Prazo de validade
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant	Fabricante
	Distributor *	Vertriebspartner *	Distributore	Distribuidor	Distributeur	Distribuidor
	Date of manufacture *	Herstellungsdatum *	Data di produzione	Fecha de fabricación	Date de production	Data de fabricação
	Biological risks *	Biologische Risiken *	Rischi biologici	Riesgos biológicos	Risques biologiques	Riscos biológicos
	Caution *	Achtung *	Attenzione	Precaución	Attention	Cuidado
UDI	Unique device Identifier *	eindeutige Produktidentifizierung *	Identificativo unico del dispositivo*	Identificación exclusiva del dispositivo *	Identifiant de dispositif unique*	Identificador único do dispositivo *
RUO	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches	
Content	Content	Inhalt	Contenuto	Contenido	Conditionnement	Conteúdo
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité	Volume / Quantidade