

Serum CrossLaps[®] (CTX-I) ELISA

For the quantification of degradation products of C-terminal telopeptides of Type I collagen in human serum and plasma

Quantification des produits de dégradation des télopeptides C-terminaux du collagène de type I dans le sérum et le plasma humains

Zur quantitativen Bestimmung von CrossLaps aus Serum und Plasma

Kit per il dosaggio quantitativo dei prodotti di degradazione del C-terminale del collagene umano di tipo I in siero e plasma.

Para la determinación cuantitativa de CrossLaps en suero y plasma human.



AC-02F1



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INTRODUCTION

Intended use

The Serum CrossLaps® (CTX-I) 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 Serum CrossLaps® (CTX-I) ELISA assay is intended for in vitro diagnostic use as an indication of human bone resorption and may be used as an aid in

A. Monitoring bone resorption changes of

- 1) Anti resorptive therapies in postmenopausal women:
 - a) Hormone Replacement Therapies (HRT) with hormones and hormone like drugs
 - b) Bisphosphonate therapies
- 2) Anti resorptive therapies in individuals diagnosed with osteopenia;
 - a) Hormone Replacement Therapies (HRT) with hormones and hormone like drugs
 - b) Bisphosphonate therapies

B. Predicting skeletal Response (Bone Mineral Density) in postmenopausal women undergoing anti resorptive therapies

- a) Hormone Replacement Therapies (HRT) with hormones and hormone like drugs
- b) Bisphosphonate therapies

Limitations

The use of the test has not been established to predict the development of osteoporosis or future fracture risk. The use of the test has not been established in hyperparathyroidism or hyperthyroidism.

When using the test to monitor therapy, results may be confounded in patients afflicted with clinical conditions known to affect bone resorption e.g. bone metastases, hyperparathyroidism or hyperthyroidism.

Serum CrossLaps® (CTX-I) ELISA results should be interpreted in conjunction with clinical findings and other diagnostic results and should not be used as a sole determinant in initiating or changing therapy

Do not interchange Serum CrossLaps® (CTX-I) ELISA values with Urine CrossLaps® (CTX-I) ELISA values.

Summary and explanation of the test

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 Serum CrossLaps® (CTX-I) 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).

Principle of the procedure

The Serum CrossLaps® (CTX-I) ELISA 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-isomerized. In order to obtain a specific signal in the Serum CrossLaps® (CTX-I) ELISA, two chains of EKAHD-β-GGR must be cross linked. Standards, control, or unknown serum samples are pipetted into the appropriate microtitre wells coated with streptavidin, followed by application of a mixture of a biotinylated antibody and a peroxidase conjugated antibody. Then, a complex between CrossLaps antigens, biotinylated antibody and peroxidase conjugated antibody is generated, and this complex binds to the streptavidin surface via the biotinylated antibody. Following the one step incubation at room temperature, the wells are emptied and washed. 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, smoke or apply cosmetics 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

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.

HAMA interference

Some individuals have antibodies to mouse immunoglobulins (HAMA), which can cause interference in immunoassays that employ murine monoclonal antibodies, such as Serum CrossLaps. In rare cases, the content of HAMA exceeds the capacity of the blocking agent incorporated into Serum CrossLaps leading to a false-positive test result. Therefore, Serum CrossLaps values should be used only in conjunction with information available from the clinical evaluation of the patient.

Storage

Store the Serum CrossLaps® (CTX-I) ELISA kit upon receipt at 2-8°C. Under these conditions the kit is stable up to the expiry date stated on the box.

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

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.

For optimal results it is recommended to draw blood as fasting morning samples (18).

Also for monitoring the individual patient, follow up samples should be collected under same conditions as the baseline sample.

When analysing plasma, both heparia 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.

Streptavidin coated microtitre plate **MICROPLAT**

Microwell strips (12x8 wells) pre-coated with streptavidin. Supplied in a plastic frame.

CrossLaps Standard **CAL 0**

One vial (min. 5.0 mL/vial) of ready for use PBS buffered solution with protein stabiliser and preservative.

CrossLaps Standards **CAL 1 - 5**

Five vials (min. 0.4 mL/vial) of ready for use, CrossLaps standard in a PBS buffered solution with protein stabiliser and preservative. The exact value of each Standard is printed on the QC Report.

Control **CTRL 1 - 2**

Two vials (min. 0.4 mL/vial) of ready for use, desalted urinary antigens of human origin in a PBS buffered solution with protein stabiliser and preservative. Please refer to enclosed QC Report for control range.

Biotinylated Antibody **Ab BIOTIN**

One vial (min. 0.25 mL) of a concentrated solution of a biotinylated monoclonal murine antibody specific for degradation products of C-terminal telopeptides of Type I collagen, raised. Prepared in a buffered solution with protein stabiliser and preservative.

Peroxidase Conjugated Antibody **ENZYMCONJ**

One vial (min. 0.25 mL) of a concentrated solution of a peroxidase conjugated murine monoclonal antibody specific for degradation products of C-terminal telopeptides of Type I collagen. Prepared in a buffered solution with protein stabiliser and preservative.

Incubation Buffer **BUF**

One vial (min. 19 mL) of a ready for use buffered solution with protein stabiliser, 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 blueish.

Stopping Solution **H2SO4**

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

Washing Buffer **WASHBUF 50x**

One vial (min. 20 mL) of a concentrated washing buffer with detergent and preservative.

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 50-200 µL
- Distilled water
- Precision 8 or 12 channel multipipette to deliver 100 µL, and 150 µL
- Microwell mixing apparatus
- Microtiter plate reader

ASSAY PROCEDURE

Mix all reagents and samples before use (avoid foam)

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 immuno strips in the tightly closed foil bag with desiccant capsules.

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

1 Preparation of the Antibody Solution:

ATTENTION: Prepare the following Antibody Solution maximum 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.**

2 One Step incubation

Pipette 50 µL of either **Standards** **CAL 0 - 5**, **Control** **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 room temperature (18-22°C) on a microtitre plate mixing apparatus (300 rpm).

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 room temperature (18-22°C) in the dark on the mixing apparatus (300 rpm). 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 quadratic 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 0-5 (ordinate) against the corresponding CrossLaps concentrations (abscissa). Determine the CrossLaps concentration of the controls and each patient sample by interpolation.

Example of results obtained:

Standards/ Controls/ Samples	CrossLaps conc. (ng/mL)	A ₄₅₀₋₆₅₀ (nm) Obs 1/ Obs 2	Mean A ₄₅₀₋₆₅₀ (nm)	Interpolated CrossLaps conc. (ng/mL)
Standard 0	0.000	0.066 / 0.065	0.066	
Standard 1	0.178	0.210 / 0.209	0.210	
Standard 2	0.489	0.472 / 0.448	0.460	
Standard 3	0.960	0.844 / 0.819	0.832	
Standard 4	1.902	1.598 / 1.560	1.579	
Standard 5	2.494	2.061 / 2.004	2.033	
Control 1		0.349 / 0.354	0.352	0.355
Control 2		0.918 / 0.952	0.935	1.086
Sample I		0.140 / 0.138	0.139	0.091
Sample II		0.447 / 0.439	0.443	0.469
Sample III		1.305 / 1.303	1.304	1.555

Please note: The data above are for illustration only and should not be used to calculate the results of any run.

Performance characteristics

Detection limit: 0.020 ng/mL CrossLaps

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

Imprecision

The imprecision of the Serum CrossLaps® (CTX-I) 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 (%)
0.121	0.013	10.9
0.444	0.043	9.7
1.967	0.050	2.5

IntraAssay Variation (n=10)

Mean (ng/mL)	SD (ng/mL)	CV (%)
0.121	0.004	3.0
0.444	0.007	1.7
1.967	0.035	1.8

Dilution/Linearity

The Serum CrossLaps® (CTX-I) ELISA is linear in the range 0.020 ng/mL to 3.380 ng/mL of CrossLaps.

Serum samples with the concentration of 0.460 - 0.668 ng/mL CrossLaps were diluted with standard 0 and the concentration of CrossLaps were determined with Serum CrossLaps® (CTX-I) 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

Interference:

The interference of Ditaurobilirubin, Hemoglobin and IntraLipid on the measurement of CrossLaps in serum by Serum CrossLaps® (CTX-I) ELISA was investigated.

In the concentration listed below no interference was detected:

Ditaurobilirubin: 600 mg/L

Hemoglobin: 10 g/L

IntraLipid: 10 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. 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

Day to Day Individual Variation

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

Subject No	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Mean (ng/mL)	SD (ng/mL)	CV (%)
1	0,423	0,428	0,396	0,460	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	0,045	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	0,255	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

CLINICAL DATA

The Serum CrossLaps® (CTX-I) 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 Serum CrossLaps® (CTX-I) 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 Serum CrossLaps® (CTX-I) ELISA that is less than 40%.

Bisphosphonate studies

Below is shown the Serum CrossLaps® (CTX-I) 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

Serum CrossLaps® (CTX-I) ELISA

	Placebo group			Alendronate group		
	Mean (ng/mL)	SD	SEM	Mean (ng/mL)	SD	SEM
	(95% Confidence Int.)			(95% Confidence Int.)		
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

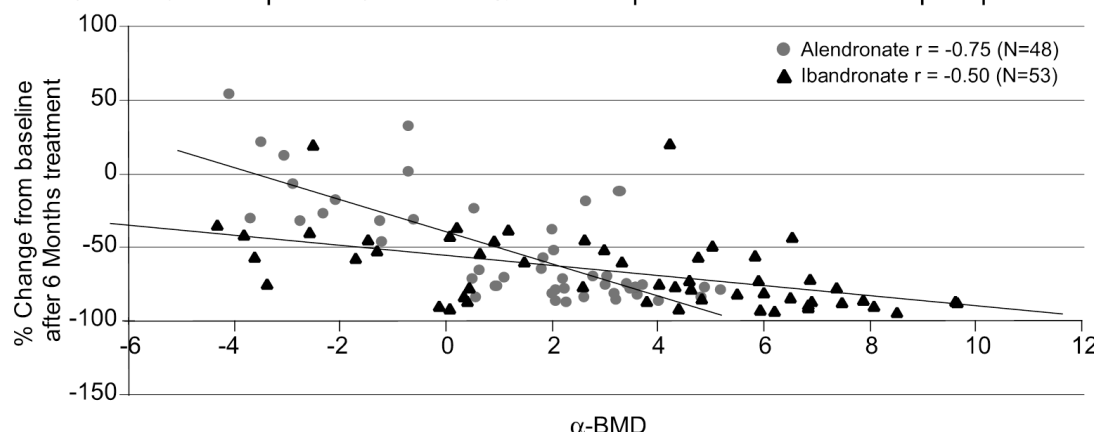
Ibandronate

- 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

Serum CrossLaps® (CTX-I) ELISA

	Placebo group			Ibandronate group		
	Mean (ng/mL)	SD	SEM	Mean (ng/mL)	SD	SEM
	(95% Confidence Int.)			(95% Confidence Int.)		
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

Serum CrossLaps® ELISA versus α -BMD for patients treated with bisphosphonates

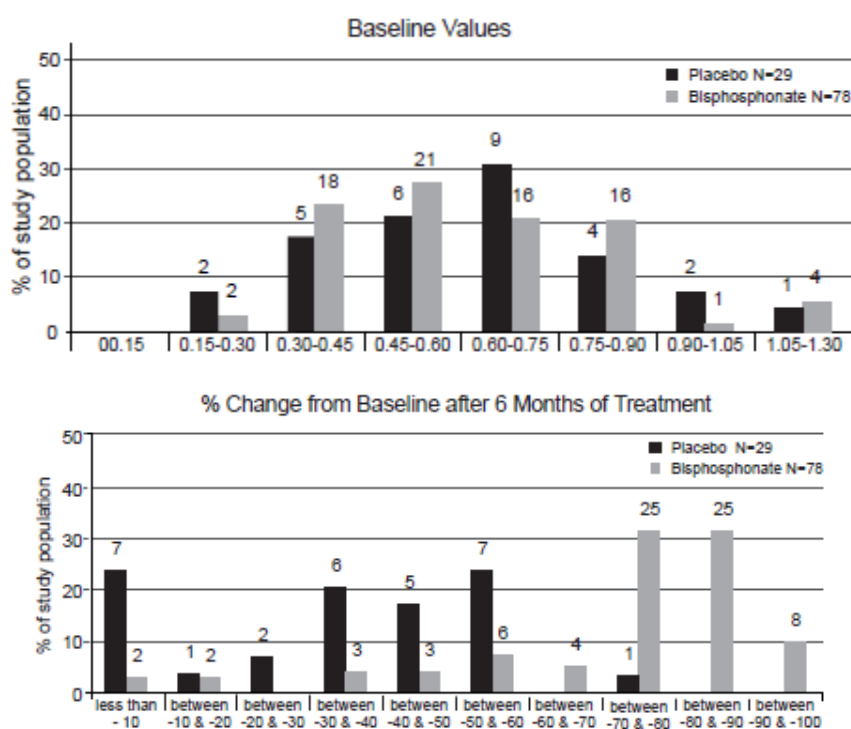


Using a cut-off for Serum CrossLaps® (CTX-I) ELISA of 40% change from baseline the following sensitivities, specificities and 95% confidence intervals are obtained

Below is indicated the actual fractions:

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



HRT Studies

Below is shown the Serum CrossLaps® (CTX-I) 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

Serum CrossLaps® (CTX-I) ELISA

	Placebo group			Tibolone group		
	Mean (ng/mL) (95% Confidence Int.)	SD	SEM	Mean (ng/mL) (95% Confidence Int.)	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.

Serum CrossLaps® (CTX-I) ELISA

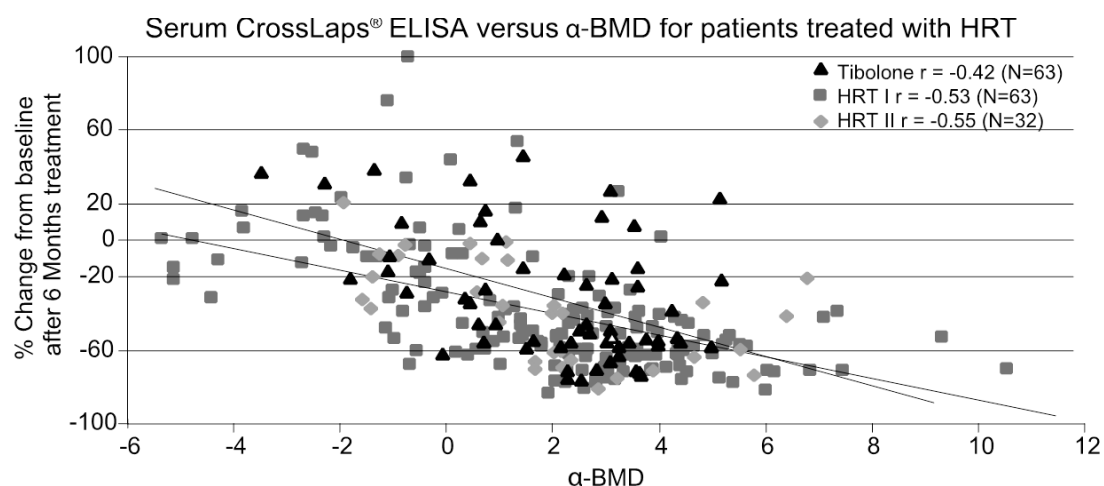
	Placebo group			HRT I group		
	Mean (ng/mL) (95% Confidence Int.)	SD	SEM	Mean (ng/mL) (95% Confidence Int.)	SD	SEM
Baseline	0.389(0.348-0.430)	0.136	0.021	0.411(0.386-0.437)	0.140	0.013
After 6 months treatment	0.396(0.349-0.443)	0.153	0.024	0.182(0.164-0.200)	0.098	0.009

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

Serum CrossLaps® (CTX-I) ELISA

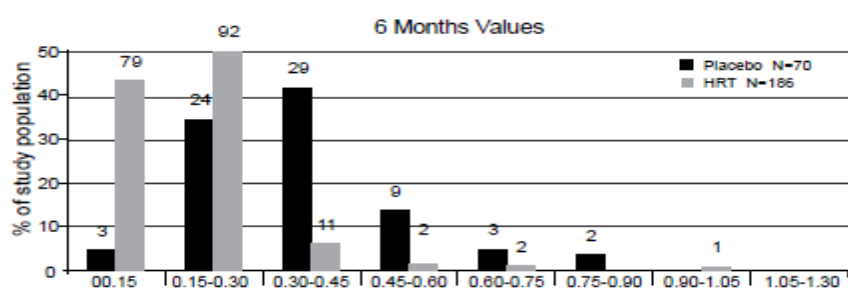
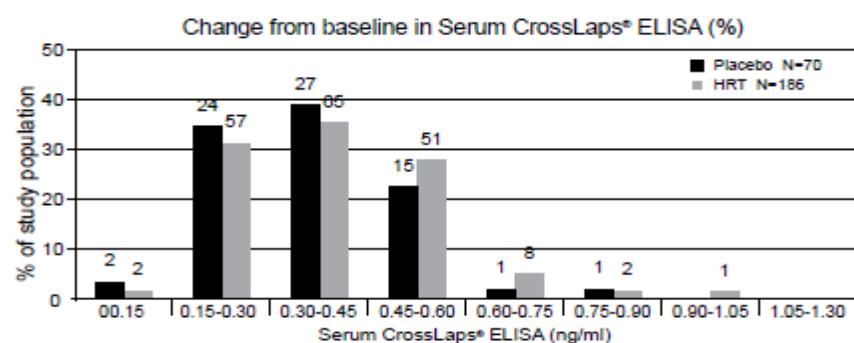
	Placebo group			HRT II group		
	Mean (ng/mL) (95% Confidence Int.)	SD	SEM	Mean (ng/mL) (95% Confidence Int.)	SD	SEM
Baseline	0.350(0.301-0.399)	0.099	0.025	0.371(0.306-0.436)	0.135	0.033
After 6 months treatment	0.293(0.240-0.346)	0.106	0.027	0.159(0.108-0.210)	0.106	0.026

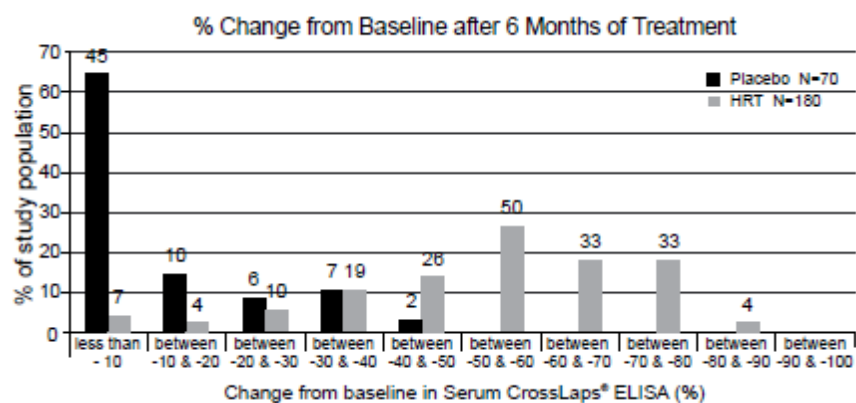


Using a cut off for Serum CrossLaps® (CTX-I) ELISA of 40% change from baseline the following sensitivities, specificities and 95% confidence intervals are obtained. Below is indicated the actual fractions

	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>1	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





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INTRODUCTION

But du dosage

Le kit Serum CrossLaps® (CTX-I) ELISA est une trousse pour le dosage immunoenzymatique d'une séquence C-terminal spécifique du collagène de type I dans le sérum et le plasma humains. Elle a fait l'objet d'un enregistrement en France auprès de l'Agence du Médicament sous la référence T87402. Le kit Serum CrossLaps® (CTX-I) ELISA a été développé pour un usage diagnostique in vitro ; il indique le degré de résorption des os humains et peut être utilisé en particulier dans les contextes suivants.

A. La mise en évidence des changements de la résorption osseuse en cas de :

1. Thérapie anti-résorptive chez la femme ménopausée.
2. Hormono-thérapie avec hormone ou analogue d'hormone
3. Thérapies aux biphosphonates

B. Prédiction de la réponse en terme de masse osseuse chez la femme post-ménopausée et suivant une thérapie anti-résorptive :

1. Hormono-thérapie avec hormone ou analogue d'hormone
2. Thérapies aux biphosphonates

Limitations

Le test n'a pas été validé pour prédire le développement de l'ostéoporose ou un futur risque de fracture. Il n'a pas été validé dans l'hyper-parathyroïdisme ou l'hyperthyroïdisme. Dans le cadre de suivi de thérapie, les résultats des tests doivent être confrontés aux données cliniques connues pouvant affecter la résorption osseuse telles que la présence de métastases osseuses, l'hyper-parathyroïdisme ou l'hyperthyroïdisme.

Les résultats du kit Serum CrossLaps® (CTX-I) ELISA doivent être interprétés avec les données cliniques et diagnostiques disponibles et ne doivent pas être utilisées seules pour décider d'un changement de thérapie. Ne pas échanger les données obtenues avec le kit Serum CrossLaps® (CTX-I) ELISA et le kit Urine CrossLaps® (CTX-I) ELISA.

Résumé

Le collagène de type I compte pour plus de 90% de la matrice organique osseuse et est synthétisé prioritairement par l'os (1). Pendant le renouvellement du squelette, ce collagène de type I est dégradé et de petits fragments de peptides provenant de l'os sont retrouvés dans la circulation sanguine. Ces fragments peuvent être dosés à l'aide de la trousse Serum CrossLaps® (CTX-I) ELISA (2). L'utilisation de ce kit a été décrite dans le suivi des traitements anti-résorptifs de patients avec désordres osseux métaboliques (3-17).

Principe

La trousse Serum CrossLaps® (CTX-I) ELISA contient deux anticorps monoclonaux hautement spécifiques dirigés contre le peptide qui possède la séquence d'acides aminés Glu-Lys-Ala-His-Asp-b-Gly-Gly-Arg, où le résidu (D) de l'acide aspartique est l'isomère b. Les deux chaînes de ce peptide doivent être liées pour obtenir un signal spécifique.

Les standards, contrôles et échantillons de sérum sont distribués dans les micropuits recouverts de streptavidine. Un mélange d'anticorps biotinylé et d'anticorps conjugués à la peroxydase est ajouté avec les contrôles et échantillons. Il y a alors formation d'un complexe entre l'antigène CrossLaps, l'anticorps biotinylé et l'anticorps conjugué à la peroxydase. Enfin ce complexe se fixe sur la surface des micropuits recouverte de streptavidine grâce à l'anticorps biotinylé. Après une étape unique d'incubation à température ambiante, les micropuits sont lavés. Une solution de substrat chromogène est ensuite ajoutée puis la réaction est arrêtée avec de l'acide sulfurique. L'absorbance est mesurée à 450 nm.

PRECAUTIONS

Les précautions suivantes doivent être observées dans le laboratoire :

- Ne pas effectuer les pipetages à la bouche.
- Ne pas fumer, boire ou manger dans les locaux où l'on manipule les échantillons ou les réactifs.
- Porter des gants à usage unique pendant la manipulation des réactifs ou des échantillons et se laver soigneusement les mains après.

- Couvrir l'espace de travail avec du papier absorbant jetable.

Interférence "HAMA"

Quelques patients ont des anticorps reconnaissant les immunoglobulines de souris (HAMA : Human Anti-Mouse Antibodies). Ces anticorps peuvent interférer dans les Immuno-tests utilisant des monoclonaux de souris, comme le kit Serum CrossLaps® (CTX-I) ELISA. Dans quelques rares cas, la quantité de "HAMA" peut dépasser la capacité de l'agent de blocage du kit Serum CrossLaps, produisant des résultats faussement positifs. Par conséquent, les résultats du Serum CrossLaps doivent être utilisés en conjonction avec les données cliniques du patient.

Avertissements

- Tous les réactifs, échantillons et équipements de laboratoire doivent être manipulés comme si ils étaient potentiellement infectieux.
- Ne pas utiliser les composants de la trousse au-delà de la date de péremption.
- Ne pas mélanger les réactifs provenant de lots différents.

Conservation

Le kit Serum CrossLaps® (CTX-I) ELISA doit être placé à 2-8°C dès réception. Dans ces conditions, le kit est stable jusqu'à la date de péremption indiquée sur l'emballage.

MATERIELS

Collecte des échantillons

Prélever le sang en évitant l'hémolyse. Séparer le sérum des cellules sanguines au maximum 3 heures après prélèvement. Il est recommandé de congeler immédiatement les échantillons et de les conserver à -18°C.

Pour un résultat optimum, il est recommandé de prélever le sang le matin à jeun.

Pour le suivi de patients, il est recommandé de prélever les différents échantillons dans les mêmes conditions et à la même heure que le premier échantillon.

Dans le cas d'utilisation de plasma, les prélèvements sur héparine et EDTA sont utilisables.

Composition de la trousse

Avant d'ouvrir le kit, veuillez lire le chapitre précautions.

Chaque trousse contient les quantités nécessaires pour 96 tests.

Barrettes de micropuits MICROPLAT

12 barrettes de micropuits (8 puits pour chaque) recouverts de streptavidine. Elles sont fournies dans un sachet plastique.

Standard CrossLaps CAL 0

1 flacon (min. 5.0 mL) de solution prête à l'emploi. Il contient du tampon PBS, des protéines stabilisatrices et un conservateur.

Standards CrossLaps CAL 1 - 5

5 flacons (min. 0.4 mL) de solution prête à l'emploi. Ils contiennent du standard CrossLaps d'origine humaine dans du tampon PBS, des protéines stabilisatrices et un conservateur. La valeur exacte de chaque standard est imprimée sur le rapport du contrôle de qualité.

Contrôle CTRL 1 - 2

Deux flacon (min. 0.4 mL) de solution prête à l'emploi. Il contient un antigène d'origine urinaire dans un tampon PBS, des protéines stabilisatrices et un conservateur. Se référer au document joint au kit pour les valeurs cibles.

Anticorps biotinylé Ab BIOTIN

1 flacon (min. 0.25 mL) de solution concentrée. Il contient un anticorps monoclonal murin biotinylé, spécifique des produits de dégradation des télopeptides C-terminaux du collagène de type I. Il contient une solution de tampon PBS, des protéines stabilisatrices et un conservateur.

Anticorps conjugué à la peroxydase **ENZYMCONJ**

1 flacon (min. 0.25 mL) de solution concentrée. Il contient un anticorps monoclonal murin conjugué à la peroxydase de raifort, spécifique des produits de dégradation des télopeptides C-terminaux du collagène de type I. Il contient une solution de tampon PBS, des protéines stabilisatrices et un conservateur.

Tampon d'incubation **BUF**

1 flacon (min. 19 mL) de solution prête à l'emploi. Il contient une solution tamponnée, des protéines animales, du détergent et un conservateur.

Solution substrat **SUBS TMB**

1 flacon (min. 12 mL) de solution prête à l'emploi. Il contient du substrat tétraméthylbenzidine (TMB) dans une solution acide. La solution de chromogène peut sembler légèrement bleue.

Solution d'arrêt **H2SO4**

1 flacon (min. 12 mL) d'une solution prête à l'emploi d'acide sulfurique 0,18 mol/l.

Solution de lavage **WASHBUF 50x**

1 flacon (min. 20 mL) d'une solution de lavage concentrée contenant un détergent et un conservateur.

Film adhésif

Film adhésif pour couvrir les micropuits pendant l'incubation.

Matériel et produits nécessaires mais non fournis

- Verrerie standard de laboratoire pour la préparation des solutions substrat, de lavage et d'arrêt
- Micropipettes de précision ou matériel similaire à embouts jetables permettant la distribution de 50 µL, 100 µL et 150 µL
- Eau distillée
- Agitateur rotatif automatique de microplaques (300 rpm)
- Laveur de plaque automatique ou pissette de laboratoire
- Lecteur de plaque avec des filtres à 450 nm et 650 nm

PROTOCOLE DU DOSAGE

Pour des performances optimales, il est important de se conformer aux instructions ci-dessous.

Protocole

Déterminer le nombre de barrettes nécessaire pour le dosage. Il est conseillé d'effectuer les mesures en double pour les standards, les contrôles et les échantillons. Il est donc nécessaire d'utiliser 16 micropuits pour les standards et contrôle lors de chaque dosage. Conserver les barrettes de micropuits non utilisées dans les sachets fermés en présence des déshydratants.

Avant utilisation, préparer et équilibrer toutes les solutions à température ambiante. **La technique doit être réalisée à température ambiante (18-22°C).**

1 Préparation de la solution d'anticorps

Attention : le mélange doit être préparé au maximum 30 minutes avant utilisation.

Mélanger l'anticorps biotinylé **Ab BIOTIN**, anticorps conjugué à la peroxydase **ENZYMCONJ** et tampon d'incubation **BUF**, dans un récipient vide, à raison de 1+1+100. Mélanger doucement en évitant la formation de mousse. Préparer le mélange avant chaque dosage.

2 Incubation en 1 étape

Distribuer 50 µL de standards **CAL 0 - 5** de contrôle **CTRL 1 - 2** ou d'échantillons dans les micropuits correspondants.

Ajouter 150 µL de solution d'anticorps anti-CrossLaps* dans tous les micropuits. Recouvrir la plaque avec le film adhésif et incuber 120±5 minutes à température ambiante (18-22°C) sous agitation par rotation (300 rpm).

3 Lavage

Laver les barrettes 5 fois manuellement avec 300 µL la solution de lavage diluée (**WASHBUF 50x**) dilué

1+50 dans l'eau distillée). S'assurer que les puits soient complètement vides après chaque série de lavage. Dans le cas d'un lavage avec un laveur automatique de plaques suivre les instructions du fabricant ou le guide du laboratoire. L'utilisation de 5 cycles de lavage est recommandée

4 Incubation de la solution substrat

Ajouter 100 µL de la solution substrat **SUBS TMB** dans chaque puit, recouvrir la plaque avec un film adhésif et incubé 15+2 minutes à 18-22°C, à l'abri de la lumière, sous agitation par rotation (300 rpm). Ne pas prélever la solution substrat directement dans le flacon mais transférer le volume nécessaire dans un tube propre. Le substrat transféré sera rejeté car il ne doit pas être reversé dans le flacon TMB.

5 Arrêt de la réaction colorée

Distribuer 100 µL de solution d'arrêt **H2SO4** dans chaque puits. Mélanger doucement.

6 Mesure de l'absorbance

Dans les 2 heures, mesurer l'absorbance à 450 nm en utilisant un lecteur de plaque. On recommande d'utiliser une lecture à 650 nm comme référence.

Limites de mesure

Si l'absorbance d'un échantillon dépasse celle du standard 5, l'échantillon doit être dilué dans le standard 0 et re-testé. A la fin du dosage, le résultat devra être multiplié par le facteur de dilution.

CONTROLE DE QUALITE

Les bonnes pratiques de laboratoire impliquent que des échantillons de contrôle soient utilisés dans chaque série de dosages pour s'assurer de la qualité des résultats obtenus. Ces échantillons devront être traités de la même façon que les prélèvements à doser et il est recommandé d'en analyser les résultats à l'aide de méthodes statistiques appropriées.

RESULTATS

Calcul des résultats

- Calculer la moyenne des absorbances des doublets. Construire une courbe d'étalonnage en quadratique, en reportant la moyenne des absorbances des six standards en ordonnée et les concentrations respectives de CrossLaps en abscisse. Tracer la courbe
- Déterminer la concentration en CrossLaps pour le contrôle et les échantillons par interpolation de la courbe
- La concentration de CrossLaps déterminée avec le contrôle doit tomber dans l'écart de concentration donné dans la fiche technique de la trousse

Exemple de résultats :

Standards / contrôles / échantillons	CrossLaps conc. (ng/mL)	A ₄₅₀₋₆₅₀ Obs 1/ Obs 2	Moyenne (nm)	Interpolated CrossLaps conc. (ng/mL)
Standard 0	0.000	0.066 / 0.065	0.066	
Standard 1	0.178	0.210 / 0.209	0.210	
Standard 2	0.489	0.472 / 0.448	0.460	
Standard 3	0.960	0.844 / 0.819	0.832	
Standard 4	1.902	1.598 / 1.560	1.579	
Standard 5	2.494	2.061 / 2.004	2.033	
Contrôle 1		0.349 / 0.354	0.352	0.355
Contrôle 2		0.918 / 0.952	0.935	1.086
Echantillon I		0.140 / 0.138	0.139	0.091
Echantillon II		0.447 / 0.439	0.443	0.469
Echantillon III		1.305 / 1.303	1.304	1.555

Note : Ces données sont utilisées à titre d'exemple et ne doivent en aucun cas être substituées aux résultats obtenus dans le

Laboratoire

Performance du dosage

Limites de détection : 0,020 ng/mL

Cette valeur correspond à 3 fois la déviation standard de la moyenne de 21 détermination du blanc (Standard 0 CrossLaps)

Imprécision

Elle a été évaluée à l'aide de 3 échantillons de sérum de concentration différentes .Les résultats sont résumés dans les tableaux suivants :

Variations inter essais (n=10)

Moyenne (ng/mL)	SD (ng/mL)	CV (%)
0.121	0.013	10.9
0.444	0.043	9.7
1.967	0.050	2.5

Variations intRA essais (n=10)

Moyenne (ng/mL)	SD (ng/mL)	CV (%)
0.121	0.004	3.0
0.444	0.007	1.7
1.967	0.035	1.8

VDilution / Linéarité

Le kit Serum CrossLaps® (CTX-I) ELISA est linéaire dans l'intervalle de 0.020 ng/mL à 3.380 ng/mL.

Des échantillons de sérum de concentrations variant de 0.460 à 0.668 ng/mL ont été dilués dans du standard 0 et dosés avec le kit. Les résultats sont présentés dans le tableau ci-dessous.

Dilution Procedure		
Sérum (%)	Standard 0 (%)	% de récupération
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

Interférences

Les interférences de la ditauro-bilirubine, de l'hémoglobine et des lipides ont été testées dans I kit Serum CrossLaps® (CTX-I) ELISA

Aux concentrations suivantes, aucune interférence n'a été constatée :

Ditauro-bilirubine 600 mg/L

Hémoglobine 10 g/L

Lipides 10 g/L

Valeurs attendues

Chaque laboratoire doit établir sa propre gamme de valeurs normales et pathologiques.

A titre d'exemple, des valeurs pour des populations normales et pathologiques sont présentés dans le tableau ci-dessous :

Populations	Nombre de sujets	Valeurs moyennes (ng/mL)	95% Range (ng/mL)
Femmes ménopausées	193	0.439	0.142 – 1.351
Femmes en pré-ménopause	226	0.287	0.112 – 0.738
Hommes	125	0.294	0.115 – 0.748

Variations circadiennes

Les variations circadiennes individuelles ont été évaluées sur les serum de 11 femmes ménopausées. Les prélèvements ont été réalisés le matin à jeun en 5 fois sur 2 semaines

Sujet No	Ech. 1	Ech. 2	Ech. 3	Ech. 4	Ech. 5	Moyenne (ng/mL)	Dev st (ng/mL)	CV (%)
1	0,423	0,428	0,396	0,460	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	0,045	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	0,255	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

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EINLEITUNG

Verwendungszweck

Der Serum CrossLaps® (CTX-I) ELISA ist ein enzymatimmunologischer Test zur Quantifizierung von Abbauprodukten C-terminaler Telopeptide des Typ-I Kollagens in menschlichem Serum und Plasma.

Der Serum CrossLaps® (CTX-I) ELISA Test wird vorgesehen für in vitro Diagnostik zur Indikation von humenem Knochenresorption und kann verwendet werden als Unterstützung bei:

A. Monitorieren der veränderten Knochenresorption bei:

1. Anti-resorptive Therapien bei postmenopausalen Frauen:
 - a. Hormonersatztherapie (HRT) mit Hormonen und Pharmaka mit hormonartiger Wirkung
 - b. Biphosphonattherapie
2. Anti-resorptive Therapien in Individuen mit Osteopenie:
 - a. Hormonersatztherapie (HRT) mit Hormonen und Pharmaka mit hormonartiger Wirkung
 - b. Biphosphonattherapie

B. Prognose der Knochen-Mineraldichte bei postmenopausalen Frauen unter antiresorptiver Therapie

- a. Hormonersatztherapie (HRT) mit Hormonen und Pharmaka mit hormonartiger Wirkung
- b. Bisphosphonattherapie

Einschränkungen

Der Gebrauch des Testes ist nicht etabliert worden, um die Entwicklung einer Osteoporose oder das zukünftige Frakturrisiko vorherzusagen. Der Gebrauch des Tests ist nicht etabliert bei Hyperparathyroidismus oder Hyperthyroidismus. Wenn der Test zur Therapieüberwachung eingesetzt wird, dann können die Ergebnisse falsch interpretiert werden bei Patienten mit klinischen Syndromen, die die Knochenresorption beeinflussen (wie bei Knochenmetastasen, Hyperparathyroidismus oder Hyperthyroidismus).

Ergebnisse des Serum CrossLaps® (CTX-I) ELISAs sollten im Zusammenhang mit klinischen Daten und anderen diagnostischen Ergebnissen interpretiert werden, und sollten nicht allein zur Beginn oder Veränderung einer Therapie benutzt werden.

Serum CrossLaps® (CTX-I) ELISA-Werte nicht mit Urine CrossLaps® (CTX-I) ELISA-Werten verwechseln!

Zusammenfassung und Erklärung des Tests

Das Typ I-Collagen macht mehr als 90% der organischen Matrix des Knochens aus und wird vorwiegend im Knochen synthetisiert (1). Während der Erneuerung des Skeletts wird das Typ I-Collagen abgebaut und kleine Peptidfragmente gelangen in den Blutstrom. Diese Fragmente können mit dem Serum CrossLaps® (CTX-I) ELISA wie beschrieben gemessen werden (2). Der Sandwich Test ist wie berichtet als geeignet zur Follow-up von anti-resorptiven Behandlung bei Patienten mit metabolischen Knochenerkrankungen geeignet (3-17).

Testprinzip

Der Serum CrossLaps® (CTX-I) ELISA basiert auf zwei hochspezifischen monoklonalen Antikörpern gegen die Aminosäuresequenz des EKAHD-β-GGR, in dem der Aspartatsäuretest (D) β-somerisiert ist. Um ein spezifisches Signal in dem Serum CrossLaps® (CTX-I) ELISA messen zu können, müssen zwei Ketten des EKAHD-β-GGR cross-linked werden.

Standard, Kontrollen und Serumproben werden in Microtiterwells pipettiert, die mit Streptavidin beschichtet sind. Anschließend erfolgt die Zugabe eines Gemisches von biotinyliertem Antikörper und einem peroxidase-konjugiertem Antikörper. Der sich bildende Komplex zwischen CrossLaps Antigen, biotinyliertem Antikörper und peroxidase-konjugiertem Antikörper bindet sich an die Streptavidinoberfläche über den biotinylierten Antikörper. Nach der Inkubation bei Raumtemperatur werden die Wells geleert und gewaschen. Ein chromogenes Substrat wird zugefügt und die Farbreaktion mit Schwefelsäure gestoppt. Zum Schluss wird die Absorption gemessen.

VORSICHTSMASSNAHMEN

Folgende Vorsichtsmassnahmen sollten im Labor eingehalten werden:

- Beim Gebrauch von immunodiagnostischen Materialien sollte nicht gegessen, getrunken, geraucht oder Kosmetik benutzt werden.
- Nicht mit dem Mund pipettieren.
- Bei der Verwendung immundiagnostischer Materialien sollten Handschuhe getragen werden. Hände anschliessend gründlich waschen.
- Die Arbeitsfläche sollte mit absorbierendem Papier abgedeckt werden.

HAMA Interferenz

Einige Individuen besitzen Antikörper gegen Maus-Immunoglobuline (HAMA), die in Immunoassays, die Maus-Antikörper enthalten (wie Serum CrossLaps® (CTX-I) ELISA), Interferenzen verursachen können. In seltenen Fällen überschreitet HAMA die Kapazität der im Serum CrossLaps enthaltenen Blocklösung, was zu falsch-positiven Testergebnissen führt. Deshalb sollten Serum CrossLaps Werte nur im Zusammenhang mit vorhandenen klinischen Evaluierungen des Patienten gebraucht werden.

Warnung

Nur für in-vitro-Anwendung.

- Alle Reagenzien und Ausrüstungen sollten wie infektiöses Material behandelt und entsorgt werden.
- Die Bestandteile des Kits sollten nicht über das Verfallsdatum hinaus verwendet werden und Reagenzien verschiedener Chargen sollten nicht gemischt werden.

Aufbewahrung

Das Serum CrossLaps® (CTX-I) ELISA Kit wird nach Gebrauch bei 2-8°C gelagert. Bei diesen Bedingungen ist der Kit stabil bis zum auf der Packungen angegebenen Haltbarkeitsdatum.

MATERIAL

Probengewinnung

Blut wird durch Venenpunktion unter Vermeidung von Hämolyse gewonnen. Das Serum sollte innerhalb 3 Stunden nach der Blutentnahme abgetrennt werden. Es wird empfohlen die Proben sofort einzufrieren (<-18°C). Es wird empfohlen, das Blut von nüchternen Patienten am Morgen zu entnehmen. Zum Patienten-Monitoring sollten die follow-up Blutproben unter identischen Bedingungen wie die Erstentnahmen erfolgen.

Es kann sowohl Heparin und EDTA zur Gewinnung von Plasma verwendet werden.

Beigefügtes Material

Ehe der Kit geöffnet wird, bitte den Abschnitt Vorsichtsmassnahmen lesen. Der Kit enthält ausreichend Reagenzien für 96 Bestimmungen.

Streptavidinbeschichtete Mikrotiterplatten **MICROPLAT**

Mikrowellstreifen (12 x 8 Wells) vorbeschichtet mit Streptavidin. Beigefügt in einem Plastikrahmen.

CrossLaps Standard **CAL 0**

Ein Fläschchen (min. 5.0 mL/Fläschchen) gebrauchsfertiger PBS-gepufferte Lösung mit Proteinstabilisatoren und Konservierungsmittel.

CrossLaps Standard **CAL 1 - 5**

Fünf Fläschchen (min. 0.4 mL/Fläschchen) gebrauchsfertige CrossLaps Standards in PBS-gepufferte Lösung mit Proteinstabilisatoren und Konservierungsmittel. Der genaue Wert für jeden Standard ist auf dem Qualitätskontrollbericht aufgedruckt.

Kontrolle **CTRL 1 - 2**

Zwei Fläschchen (min. 0.4 mL/Fläschchen) gebrauchsfertiger entsalzene Urinantigene menschlichen Ursprungs in einer PBS-gepufferten Lösung mit Proteinstabilisatoren und Konservierungsmittel. Die genaue Konzentration ist auf dem beigegefügttem Qualitätskontrollbericht entnehmen.

Biotinylierter Antikörper **Ab BIOTIN**

Ein Fläschchen (min. 0.25 mL) einer konzentrierten Lösung von biotinyliertem monoklonalen Mausantikörper spezifisch für Degradationsprodukte der C-terminalen Telopeptide vom Kollagen Typ-I. Zubereitet in einer gepufferten Lösung mit Proteinstabilisatoren und Konservierungsmittel.

Peroxidase-konjugierte Antikörper **ENZYMCNJ**

Ein Fläschchen (min. 0.25 mL) einer konzentrierter Lösung von peroxidase-konjugiertem monoklonalen Mausantikörper spezifisch für Degradationsprodukte der C-terminalen Telopeptide vom Kollagen Typ-I. Zubereitet in einer gepufferten Lösung mit Proteinstabilisatoren und Konservierungsmittel.

Inkubationspuffer **BUF**

Ein Fläschchen (min. 19 mL) gebrauchsfertiger gepufferten Lösung mit Proteinstabilisatoren, Detergenzien und Konservierungsmittel.

Substratlösung **SUBS TMB**

Ein Fläschchen (min. 12 mL) gebrauchsfertiger Tertamethylbenzidine (TMB) Substrat in einem sauren Puffer. Das chromogene Substrat kann leicht bläulich erscheinen.

Stopplösung **H2SO4**

Ein Fläschchen (min. 12 mL) gebrauchsfertige 0.18 mol/L Schwefelsäure.

Waschpuffer **WASHBUF 50x**

Ein Fläschchen (min. 20 mL) eines konzentrierten Waschpuffers mit Detergentien und Konservierungsmittel.

Klebestreifen

Adhesiver Film um die Wells während Inkubation zu bedecken.

Erforderliche Laborgeräte und Hilfsmittel

- Behälter zur Vorbereitung der Antikörperlösung und der Waschlösung
- Präzisionsmikropipetten für 50-200 µL
- Destilliertes Wasser
- Präzisionsmultikanalpipette für 100 µL und 150 µL
- Mikrotiterplatten-Schüttler
- Mikrotiterplattenreader (300 rpm)

TESTDURCHFÜHRUNG

Für die optimale Durchführung des Tests ist es wichtig die Instruktionen wie folgt zu befolgen Vor dem Gebrauch alle Lösungen vorbereiten und bei Raumtemperatur equilibrieren lassen. **Test bei 18-22°C durchführen.**

Die Anzahl der benötigten Immunostrips für den Test ermitteln. Es wird empfohlen alle Bestimmungen in Doppelwerten durchzuführen. Zusätzlich werden für jede Durchführung 16 Wells für die Standards und Kontrolle benötigt. Die Anzahl der benötigten Immunostrips in den Plastikrahmen plazieren. Unbenutzte Strips in die dicht verschlossene Folienbeutel mit Trockenkapseln aufbewahren.

Testdurchführung

Alle Reagenzien und Proben mischen vor dem Gebrauch (Schaumbildung vermeiden).

1 Vorbereitung der Antikörperlösung:

ACHTUNG: Die folgende Antikörperlösung maximal 30 Minuten vor Beginn der Testdurchführung ansetzen. Die Lösung biotinylierter Antikörper **Ab BIOTIN**, peroxidase-konjugierter Antikörper **ENZYMCNJ** und Inkubationspuffer **BUF** mit den Volumenverhältnissen 1+1+100 in einer leeren Schale mischen. Vorsichtig Mischen und Schaumbildung vermeiden. **Eine frische Lösung vor jeden benutzen des Tests ansetzen.**

2 Inkubation

50 µL von jeweils **Standard** **CAL 0 - 5**, **Kontrolle** **CTRL 1 - 2** oder unbekannte Probe in die dafür

vorgesehenen Wells pipettieren gefolgt von 150 µL der **Antikörperlösung**. Die Immunostrips mit Klebestreifen bedecken und für 120±5 Minuten bei 18-22°C auf einem Mikrotiterplatten-Schüttler (300 rpm) inkubieren.

3 Waschen

Die Immunostrips 5 mal manuell mit 300 µL **Waschpuffer**, (**WASHBUF 50x** 1+50 verdünnt in destilliertem Wasser), waschen. Bei Gebrauch von einem automatischen Plattenwäscher die Instruktionen des Herstellers oder den Richtlinien des Labors folgen. Gewöhnlich sind 5 Waschschrte ausreichend. Nach jedem manuellen oder automatischen Waschschrte sicherstellen, dass die Wells vollständig geleert sind nach jedemmanuellen oder automatischen Waschschrte.

4 Inkubation mit der chromogenen Lösung

100 µL der **Substratlösung** **SUBS TMB** in jedes Well pipettieren und für 15±2 Minuten bei 18-22°C im Dunklen auf dem Mikrotiterplatten-Schüttler (300 rpm) inkubieren. Gebrauche Klebestreifen. Nicht direkt von dem Fläschchen mit dem TMB Substrat pipettieren, sondern überführe das benötigte Volumen in einem sauberen Behältnis. Übriggebliebenes Substrat in dem Behältnis sollte verworfen und nicht in Fläschchen nr. 4 zurückgeschüttet werden.

5 Stoppen der Farbreaktion

100 µL der **Stopplösung** **H2SO4** in jedes Well pipettieren.

6 Absorption messen

Absorption bei 450 nm mit 650 nm als Referenz innerhalb zwei Stunden messen.

Einschränkungen des Tests

Wenn die Absorption einer Probe der des **Standard 5** übersteigt, sollte die Probe mit **Standard 0** verdünnt und nochmals gemessen werden.

QUALITÄTSKONTROLLE

Gute Labor Praxis (Good Laboratory Practice – GLP) verlangt den Gebrauch von Qualitätskontrollen, die bei jedem Testansatz mitgemessen werden, um die Durchführung des Test zu überprüfen.

Die Kontrollen sollten wie unbekannte Proben behandelt werden. Die Ergebnisse mit den appropriate statistischen Methoden analysieren.

ERGEBNISSE

Es sollte eine quadratische Kurvenanpassung verwendet werden.

Alternativ bestimmen Sie das Mittel aus den Doppelbestimmungen der optischen Dichten. Tragen Sie eine Standardkurve auf Millimeterpapier auf, indem Sie die durchschnittlichen optischen Dichten (Extinktionswerte) der sechs Standards 0 – 5 (Y-Achse) gegen die zugehörigen Crosslabs Konzentrationen (X-Achse) auftragen. Bestimmen Sie die Crosslabs Konzentration der Kontrollen und der jeweiligen Patientenprobe durch Interpolation.

Beispiel von ermittelten Resultaten:

Standards/ Kontrollen/ Proben	CrossLaps konz. (ng/mL)	A ₄₅₀₋₆₅₀ Obs 1/ Obs 2	Mittelwert A ₄₅₀₋₆₅₀ (nm)	Interpolierte CrossLaps konz. (ng/mL)
Standard 0	0.000	0.066 / 0.065	0.066	
Standard 1	0.178	0.210 / 0.209	0.210	
Standard 2	0.489	0.472 / 0.448	0.460	
Standard 3	0.960	0.844 / 0.819	0.832	
Standard 4	1.902	1.598 / 1.560	1.579	
Standard 5	2.494	2.061 / 2.004	2.033	
Kontrolle 1		0.349 / 0.354	0.352	0.355
Kontrolle 2		0.918 / 0.952	0.935	1.086
Probe I		0.140 / 0.138	0.139	0.091
Probe II		0.447 / 0.439	0.443	0.469

Probe III		1.305 / 1.303	1.304	1.555
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Bitte beachten: Die hier aufgeführten Ergebnisse sind ein Beispiel für eine Standardkurve. Sie dürfen nicht für die Auswertung des Assays verwendet werden

Testcharakteristika

Nachweisgrenze: 0.020 ng/mL CrossLaps

Dies entspricht der Konzentration von drei Standardabweichungen über dem Mittelwert von 21 Bestimmungen des Nullwertes („CrossLaps Standard 0“).

Präzision

Die Präzision des Serum CrossLaps® (CTX-I) ELISAs wurde ermittelt von drei Serum Proben. Die Ergebnisse sind in den Tabellen unten zusammengefasst:

InterAssay Variation (n=10)

Mittelwert (ng/mL)	SD (ng/mL)	CV (%)
0.121	0.013	10.9
0.444	0.043	9.7
1.967	0.050	2.5

Intra-Assay Variation (n=10)

Mittelwert (ng/mL)	SD (ng/mL)	CV (%)
0.121	0.004	3.0
0.444	0.007	1.7
1.967	0.035	1.8

Verdünnung/Linearität

Der Serum CrossLaps® (CTX-I) ELISA ist linear im Bereich von 0.020 ng/mL zu 3.380 ng/mL.

Serumproben mit einer Konzentration von 0.460-0.668 ng/mL CrossLaps werden mit Standard 0 verdünnt und die Konzentration von CrossLaps werden mit Serum CrossLaps® (CTX-I) ELISA bestimmt.
Die Serum-Ursprungsprobe wird als 100% gesetzt.

Die unten angegebenen Daten sind von 3 verschiedenen Durchläufen errechnet:

Verdünnungsprozedur		
Serum [%]	Standard 0 [%]	Wiederfindung [% des erwarteten Wertes]
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
Mittelwert		103

Interferenzen

Die Interferenz von Ditaurobilirubin, Hämoglobin und Intralipid auf die Messung von CrossLaps im Serum durch Serum CrossLaps® (CTX-I) ELISA wurde untersucht.

Bei den unten angegebenen Konzentrationen wurden keine Interferenzen festgestellt:

Ditaurobilirubin 600 mg/L

Hämoglobin 10 g/L

IntraLipid 10 g/L

Erwartete Ergebnisse

Es ist empfehlenswert, für ein Labor seine eigenen Normwert- und pathologischen Bereiche zu etablieren. Als Beispiel werden die Durchschnittswerte und Standardabweichungen für verschiedene Populationen unten angegeben. Für weitere Daten wird auf die Literaturliste hingewiesen. Alle Proben wurden morgens von gesunden, nüchternen Probanden gewonnen.

Populations	Anzahl	Mittelwert Serum CrossLaps (ng/mL)	95% Range (ng/mL)
Postmenopausale Frauen	193	0.439	0.142 – 1.351
Prämenopausale Frauen	226	0.287	0.112 – 0.738
Männer	125	0.294	0.115 – 0.748

Tag-zu-Tag individuelle Variation

Die Tag-zu-Tag intraindividuelle Variation wurde ermittelt durch die Analyse von Serumproben (morgens, nüchtern) von 11 gesunde postmenopausalen Frauen zu fünf Zeitpunkten über 2 Wochen

Individuum	Besuch 1	Besuch 2	Besuch 3	Besuch 4	Besuch 5	Mittelwert (ng/mL)	SD (ng/mL)	CV (%)
1	0,423	0,428	0,396	0,460	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	0,045	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	0,255	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

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INTRODUZIONE

Uso del kit

Il kit Serum CrossLaps® (CTX-I) ELISA è un dosaggio immunoenzimatico per la determinazione quantitativa dei prodotti di degradazione del telopeptide C-terminale del collagene umano di tipo I in siero e plasma.

Il kit Serum CrossLaps® (CTX-I) ELISA è per uso diagnostico in vitro come marcatore di riassorbimento osseo e può essere usato per:

A. Monitorare le modificazioni del riassorbimento osseo delle:

- 1) Terapie per la prevenzione del riassorbimento osseo in donne in postmenopausa:
 - a) Terapie ormonali sostitutive (HRT) con ormoni o sostanze ad azione ormonale
 - b) Terapie con difosfonati
- 2) Terapie anti riassorbimento osseo in soggetti con diagnosi di osteopenia:
 - a) Terapie ormonali sostitutive (HRT) con ormoni o sostanze ad azione ormonale
 - b) Terapie con difosfonati

B. Previsione della risposta scheletrica valutata con la densitometria ossea in donne in postmenopausa in terapia con farmaci che agiscono sul riassorbimento osseo

- a) Terapie ormonali sostitutive (HRT) con ormoni o sostanze ad azione ormonale
- b) Terapie con difosfonati

Limiti del metodo

L'uso di questo dosaggio non è stato validato per predire lo sviluppo di osteoporosi o di rischi futuri di fratture.

L'uso di questo dosaggio non è stato validato nell'iperparatiroidismo o nell'ipertiroidismo. Usando il kit per monitorare la terapia si possono ottenere risultati non corretti in pazienti con condizioni cliniche note per il loro effetto sul riassorbimento osseo quali metastasi ossee, iperparatiroidismo o ipertiroidismo.

I risultati ottenuti con il kit Serum CrossLaps® (CTX-I) ELISA devono essere interpretati insieme ai riscontri clinici e ai risultati di altri test diagnostici e non devono essere usati da soli per iniziare o modificare una terapia. I valori ottenuti con il kit Serum CrossLaps® (CTX-I) ELISA non possono essere interpolati con i valori ottenuti con il kit Urine CrossLaps® (CTX-I) ELISA.

Descrizione del metodo

Il collagene di tipo I costituisce più del 90% della matrice organica dell'osso dove è prevalentemente sintetizzato.

(1). Durante il rimaneggiamento scheletrico, viene degradato il collagene di tipo I e vengono liberati nel torrente circolatorio piccoli frammenti peptidici. Questi frammenti possono essere dosati con il kit Serum CrossLaps® (CTX-I) ELISA. La misura dei prodotti specifici di degradazione del collagene di tipo I con i metodi competitivi CrossLaps è stata descritta sia nelle urine (2) che nel siero (3).

Il metodo sandwich si è dimostrato utile nel monitoraggio degli effetti della terapia anti riassorbimento osseo in pazienti con malattie metaboliche dell'osso (3-17).

Principio del metodo

Il metodo è basato sull'uso di due anticorpi monoclonali altamente specifici, diretti contro la sequenza aminoacidica di EKAHD-β-GGR, nella quale il residuo di acido aspartico (D) è isomerizzato in β. Per ottenere un segnale specifico con questo kit, le due catene di EKAHD-β-GGR devono essere legate tra loro.

Standard, controlli e campioni vengono pipettati in pozzetti di una micropiastra in cui è legata streptavidina.

Viene quindi aggiunta una soluzione contenente un anticorpo biotinilato e un anticorpo coniugato con perossidasi; si forma così un complesso tra antigeni CrossLaps, anticorpo biotinilato e anticorpo coniugato con perossidasi che si lega alla fase solida attraverso l'interazione tra biotina e streptavidina. Al termine dell'incubazione, si lava la micropiastra e si aggiunge il substrato; dopo 15 minuti si blocca la reazione con acido solforico e si misura l'assorbanza dei pozzetti.

PRECAUZIONI

In laboratorio devono essere sempre osservate le seguenti precauzioni:

- Non mangiare, bere, fumare, applicare cosmetici durante la manipolazione di reattivi immunodiagnostici
- Non usare pipette a bocca
- Quando si manipolano reattivi immunodiagnostici indossare sempre guanti protettivi; terminato il lavoro lavarsi accuratamente le mani con acqua e sapone.
- Coprire l'area di lavoro con fogli di carta assorbente

Interferenza da HAMA

Alcuni soggetti presentano anticorpi diretti contro le immunoglobuline di topo (HAMA); questi anticorpi possono provocare interferenze nei dosaggi immunometrici che utilizzano anticorpi monoclonali da topo, come il kit Serum CrossLaps® (CTX-I) ELISA. In rari casi, la concentrazione di HAMA supera le capacità neutralizzanti dell'agente bloccante inserito nel kit Serum CrossLaps, provocando risultati falsamente positivi nel dosaggio. Per questo motivo i valori ottenuti con il kit Serum CrossLaps devono essere utilizzati insieme alle informazioni derivate dalla valutazione clinica del soggetto.

Attenzione

Solo per uso in vitro.

- I reattivi e il materiale di laboratorio deve essere manipolato ed eventualmente eliminato come se fosse potenzialmente infettivo
- Non usare i componenti del kit oltre la data di scadenza e non utilizzare insieme reattivi di lotti diversi

Conservazione dei reattivi

Il kit Serum CrossLaps® (CTX-I) ELISA deve essere conservato a 2-8°C. In queste condizioni è stabile fino alla data di scadenza riportata sulla confezione.

MATERIALI

Raccolta dei campioni

Prelevare sangue venoso facendo attenzione a non emolizzare il campione. Separare il siero dalla parte corpuscolata entro tre ore dal prelievo. Congelare immediatamente a < -18°C i campioni. Eseguire il prelievo il mattino con il paziente a digiuno. Se si eseguono prelievi seriali dello stesso paziente, si consiglia di raccogliere i campioni successivi nelle stesse condizioni del campione basale. E' possibile utilizzare anche plasma da EDTA o da eparina.

Reattivi forniti

Prima di utilizzare il kit, leggere la sezione **Precauzioni** di questo manuale. Il kit contiene reattivi sufficienti per 96 determinazioni.

Micropiastra **MICROPLAT**

12 strip da 8 pozzetti ciascuna fornite in un telaio di plastica, sensibilizzate con streptavidina.

Standard **CAL 0**

1 flacone da min. 5.0 mL. Tampone PBS pronto per l'uso, contenente una proteina stabilizzante e conservanti.

Standard **CAL 1 - 5**

5 flaconi da min. 0.4 mL. Standard a 5 livelli di CrossLaps in tampone PBS pronto per l'uso, contenente una proteina stabilizzante e conservanti. Il valore esatto di ciascuno standard è stampato sul rapporto del controllo di qualità.

Controllo **CTRL 1 - 2**

1 flacone da min. 0.4 mL. Antigeni urinari umani desalificati in tampone PBS, contenente una proteina stabilizzante e conservanti.

I valori di riferimento sono riportati sul rapporto del controllo di qualità accluso al kit.

Anticorpo biotinilato **Ab BIOTIN**

1 flacone da min. 0.25 mL. Anticorpo monoclonale concentrato specifico per i prodotti di degradazione del telopeptide C-terminale del collagene umano di tipo I, in tampone contenente una proteina stabilizzante e conservanti.

Anticorpo coniugato con perossidasi **ENZYMCONJ**

1 flacone da min. 0.25 mL. Anticorpo monoclonale concentrato, coniugato con HRP, specifico per i prodotti di degradazione del telopeptide C-terminale del collagene umano di tipo I, in tampone contenente una proteina stabilizzante e conservanti.

Tampone di incubazione **BUF**

1 flacone min. 19 mL. Tampone pronto per l'uso, contenente una proteina stabilizzante, detergenti e conservanti.

Substrato **SUBS TMB**

1 flacone min. 12 mL. Soluzione pronta per l'uso di trimetilbenzidina (TMB) in tampone acido. Il cromogeno presenta una leggera colorazione blu.

Soluzione di stop **H2SO4**

1 flacone min. 12 mL. Soluzione pronta per l'uso di Acido solforico 0,18 M.

Tampone di lavaggio **WASHBUF 50x**

1 flacone min. 20 mL. Tampone di lavaggio concentrato, contenente detergenti e conservanti.

Copripietra adesivo

Materiale richiesto ma non fornito

- Vetreria per preparare la soluzione di lavoro dell'anticorpo e la soluzione di lavaggio
- Micropipette di precisione per 50-200 µL
- Acqua distillata
- Multipipette di precisione a 8 o 12 canali per 100 µL e 150 µL
- Agitatore rotante per micropiastre (300 rpm)
- Lettore di micropiastre

METODO DEL DOSAGGIO

Prima dell'uso preparare ed equilibrare tutte le soluzioni a temperatura ambiente. **Eseguire il dosaggio a temperatura ambiente (18-22°C).**

1 Preparazione della soluzione di lavoro dell'anticorpo

ATTENZIONE: Preparare la **soluzione di lavoro** dell'anticorpo non più di 30 minuti prima di iniziare il dosaggio. Aggiungere in un flacone pulito 1 parte di anticorpo biotinilato **Ab BIOTIN**, 1 parte di anticorpo coniugato con perossidasi **ENZYMCONJ** e 100 parti di tampone di incubazione **BUF**. Mescolare con cura, evitando la formazione di schiuma. **Preparare per ogni dosaggio una soluzione fresca. Al termine del dosaggio scartare la soluzione di lavoro avanzata.**

2 Incubazione simultanea

Pipettare 50 µL di Standard **CAL 1 - 5**, Controllo **CTRL 1 - 2** o campioni nei rispettivi pozzetti e 150 µL di soluzione di lavoro dell'anticorpo. Coprire con il copripietra e incubare 120±5 minuti a temperatura ambiente (18-22°C) su un agitatore rotante per micropiastre (300 rpm).

3 Lavaggio

Lavare 5 volte le strisce con 300 µL tampone di lavaggio (**WASHBUF 50x** diluito 1+50 con acqua distillata). Se si usa un lavatore automatico, seguire le istruzioni del produttore. Di solito 5 cicli di lavaggio sono sufficienti. Verificare che tra un ciclo e l'altro di lavaggio i pozzetti vengano aspirati completamente.

4 Incubazione con il substrato

Pipettare 100 µL di **soluzione di substrato** **SUBS TMB** in tutti i pozzetti e incubare 15±2 minuti a temperatura ambiente (18-22°C) al buio su agitatore rotante (300 rpm). Coprire con un copripietra. Per evitare contaminazioni del substrato, trasferire la quantità necessaria di substrato in un contenitore pulito. Dopo l'uso scartare la soluzione avanzata nel contenitore.

5 Arresto della reazione

Pipettare 100 µL di soluzione di stop **H₂SO₄** in tutti i pozzetti.

6 Misura dell'assorbanza

Misurare l'assorbanza a 450 nm contro 650 nm entro due ore.

Limiti del metodo

Se l'assorbanza di un campione è superiore a quella dello standard più elevato (standard 5), è necessario diluire il campione con lo standard 0 e ridosarlo

CONTROLLO DI QUALITA'

La "Good Laboratory Practice" (GLP) richiede che vengano inseriti in ogni esperimento sieri di controllo per verificare le prestazioni del dosaggio. I controlli devono essere trattati come i campioni e i risultati devono essere analizzati con metodi statistici appropriati.

RISULTATI

Calcolo dei risultati

Per il calcolo dei risultati utilizzare l'interpolazione quadratica.

In alternativa, si può calcolare la media delle assorbanze dei duplicati e costruire una curva standard su carta millimetrata, ponendo in ordinata la media delle assorbanze dei 6 standard e in ascissa le concentrazioni di CrossLaps corrispondenti. Determinare per interpolazione la concentrazione di CrossLaps in campioni e controlli.

Esempio dei risultati che si ottengono in un dosaggio:

Standard/ Controlli/ Campioni	Conc. di CrossLaps (ng/mL)	A ₄₅₀₋₆₅₀ Obs 1/ Obs 2	Media A ₄₅₀₋₆₅₀ (nm)	Conc. interpolata di CrossLaps (ng/mL)
Standard 0	0.000	0.066 / 0.065	0.066	
Standard 1	0.178	0.210 / 0.209	0.210	
Standard 2	0.489	0.472 / 0.448	0.460	
Standard 3	0.960	0.844 / 0.819	0.832	
Standard 4	1.902	1.598 / 1.560	1.579	
Standard 5	2.494	2.061 / 2.004	2.033	
Control 1		0.349 / 0.354	0.352	0.355
Control 2		0.918 / 0.952	0.935	1.086
Sample I		0.140 / 0.138	0.139	0.091
Sample II		0.447 / 0.439	0.443	0.469
Sample III		1.305 / 1.303	1.304	1.555

Nota: I dati sopra riportati sono solo esemplificativi e non devono essere utilizzati per calcolare i risultati dei singoli dosaggi.

Caratteristiche del metodo

Concentrazione minima rilevabile: 0.020 ng/mL di CrossLaps

La concentrazione minima rilevabile è la concentrazione di CrossLaps corrispondente a tre DS oltre la media delle assorbanze di 21 determinazioni dello standard zero ("CrossLaps Standard 0")

Imprecisione

E' stata l'imprecisione del kit Serum CrossLaps® (CTX-I) ELISA dosando 3 campioni di siero. I risultati sono riportati nella successiva tabella.

Variazione intrasaggio (n=10)

Variazione inter saggio (n=10)

Media (ng/mL)	DS (ng/mL)	CV (%)
0.121	0.013	10.9
0.444	0.043	9.7
1.967	0.050	2.5

Media (ng/mL)	DS (ng/mL)	CV (%)
0.121	0.004	3.0
0.444	0.007	1.7
1.967	0.035	1.8

Diluizione/Linearità

Il kit Serum CrossLaps® (CTX-I) ELISA è lineare nell'intervallo compreso tra 0.020 ng/mL e 3.380 ng/mL di CrossLaps.

Campioni di siero con concentrazioni di CrossLaps comprese tra 0.460 e 0.668 ng/mL sono stati diluiti con lo standard zero (standard 0) e la loro concentrazione di CrossLaps è stata determinata con il kit Serum CrossLaps® (CTX-I) ELISA. Il siero non diluito non diluito è riportato come 100%.

I dati riportati in tabella sono stati ricavati da 3 esperimenti:

Test di diluizione		
Siero [%]	Standard 0 [%]	Recupero [% del valore atteso]
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
Media		103

Interferenze:

E' stata valutata l'interferenza di Ditaurobilirubina, Emoglobina e IntraLipid nella misura dei CrossLaps nel siero con il kit Serum CrossLaps® (CTX-I) ELISA.

Per le concentrazioni sotto riportate delle sostanze in esame non è stata trovata alcuna interferenza:

Ditaurobilirubina: 600 mg/L

Emoglobina: 10 g/L

IntraLipid: 10 g/L

Valori attesi

Ogni laboratorio deve stabilire propri intervalli di riferimento per soggetti normali e patologici. I valori riportati nella seguente tabella sono stati determinati con una sperimentazione indipendente e vengono forniti a titolo di esempio. Per approfondimenti si rimanda alla bibliografia. Tutti i campioni sono stati prelevati al mattino da soggetti apparentemente sani, a digiuno dalla mezzanotte.

Popolazione	Numero di soggetti	Valore medio (ng/mL)	95% Range (ng/mL)
Donne in post-menopausa	193	0.439	0.142 – 1.351
Donne in pre-menopausa	226	0.287	0.112 – 0.738
Uomini	125	0.294	0.115 – 0.748

Variazione giornaliera individuale:

E' stata valutata la variazione giornaliera individuale dosando 5 campioni di siero prelevati il mattino in giorni differenti in due settimane da 11 donne in post menopausa apparentemente sane e a digiuno dalla mezzanotte

Soggetto	Visita 1	Visita 2	Visita 3	Visita 4	Visita 5	Media (ng/mL)	DS (ng/mL)	CV (%)
1	0,423	0,428	0,396	0,460	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	0,045	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	0,255	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

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INTRODUCCION

El Serum CrossLaps® (CTX-I) ELISA es un test Inmuno enzimático para la cuantificación de la degradación de los telopéptidos C-Terminal del colágeno Tipo I en suero y plasma.

El Serum Crosslaps® (CTX-I) ELISA es para diagnóstico in vitro. Se utiliza para cuantificar los cambios de resorción ósea en los siguientes casos:

A. Monitorización de los cambios de resorción ósea en

1. Terapias Anti- resortivas en mujeres postmenopáusicas
 - a. En tratamientos con terapias hormonales sustitutorias HRT
 - b. Terapias con Bifosfonatos
2. Terapias Anti-resortivas en pacientes con Osteopenia

B. Cambios en la Densidad Osea en mujeres Post menopáusicas con terapias Anti resortivas

- a. Terapias hormonales sustitutorias
- b. Terapias con Bifosfonatos

Limitaciones

El empleo del Test no ha sido establecido para predecir el desarrollo de Osteoporosis o riesgo de fracturas. Tampoco en caso de hiperparatoidismo o hipertiroidismo. Los resultados obtenidos con el Test Serum CrossLaps® (CTX-I) ELISA deberán interpretarse en el contexto clínico del paciente, de manera que pacientes con metástasis, hiperparatoidismo o hipertiroidismo pueden interferir en los resultados del Test. Los valores del Serum CrossLaps® (CTX-I) ELISA no son intercambiables con los valores obtenidos con los del Test Urine CrossLaps® ELISA.

Explicaciones del test

El colágeno Tipo I representa el 90% de la matriz ósea. Es sintetizado en primer lugar en el hueso. Durante la renovación del esqueleto, el colágeno Tipo I es degradado en pequeños fragmentos y excretado al torrente sanguíneo. Estos fragmentos pueden ser medidos mediante el Serum Crosslaps® (CTX-I) ELISA, tal y como hemos descrito.

Principio del ensayo

El Serum CrossLaps® (CTX-I) ELISA está basado en dos anticuerpos monoclonales altamente específicos cuya secuencia aminoacídica es EKAHD-β-GGR, donde el residuo del ácido aspártico está β-isomerizado.

El Serum Crosslaps® (CTX-I) ELISA utiliza un péptido sintético estreptavidina inmovilizado en la pared de la microplaca. Se añade el estándar, el control, la muestra de suero y la solución con los anticuerpos : Anticuerpo conjugado con la peroxidasa, Anticuerpo conjugado con Biotina. Se procede a la incubación a Temperatura ambiente. Se procede a lavar. Se añade el sustrato cromogénico. La reacción colorimétrica se para con ácido sulfúrico. Finalmente se mide la absorbancia.

PRECAUCIONES

- No comer, no beber, no fumar ni aplicar cosméticos donde los materiales inmunoenzimáticos son manipulados
- No pipetear con la boca
- Utilizar guantes para la manipulación, y lavar las manos después
- Cubrir la zona de trabajo, con papel absorbente

Interferencia HAMA.

Algunos individuos tienen anticuerpos a las Inmunoglobulinas de ratón (HAMA), lo cual puede causar interferencias en los ensayos inmuno enzimáticos empleando anticuerpos monoclonales murine, como el Suero CrossLaps.

Es casos excepcionales, el contenido de HAMA excede la

capacidad del agente bloqueante incorporado al Suero CrossLaps, dando un falso positivo en el resultado del test.

No obstante los valores del suero CrossLaps deberán ser empleados, en conjunción con la información clínica del paciente.

Advertencia

Para uso In vitro.

- Los materiales o reactivos, procedentes de muestras infecciosas, serán eliminados
- No utilizar Kit caducados. No mezclar reactivos de lotes diferentes

MATERIALES

Recolección de la muestra

Recoger la sangre con una jeringa, evitando la hemólisis. Separar el suero de las Células, nunca después de las 3 horas de la recogida de la muestra. Es recomendable congelar la muestra a -18°C inmediatamente. Para obtener mejores resultados se recomienda recoger la sangre a primera hora de la mañana. Para una correcta monitorización, se deberán obtener las muestras de los pacientes en condiciones basales. Cuando se analice el Plasma, se utilizará heparina y EDTA.

Materiales suministrados en el Kit

Antes de abrir el Kit, leer las precauciones. El Kit contiene 96 determinaciones.

Estreptavidine, unida a la microplaca MICROPLAT

8 X 12 pocillos unidos a la estreptavidine.

Estándar Crosslaps CAL 0

Un vial (min 5.0 mL/vial) listo para usar solución PBS Buffer con proteína estabilizada

Estándar CrossLaps CAL 1 - 5

Cinco viales(min. 0.4 mL/vial) de estándar Crosslaps listo para usar, en solución Buffer-PBS, con proteína estabilizada. El valor exacto de cada estándar está indicado en el informe de control de calidad.

Control CTRL 1- 2

Un vial (min. 0.4 mL/vial) listo para usar, de antígenos humanos procedentes de la orina, en Solución Buffer PBS, con proteína estabilizada y conservante. Ver el informe de control de calidad para el rango de los controles.

Anticuerpo conjugado con Biotina Ab BIOTIN

Un vial de (min. 0.25 mL) solución concentrada del anticuerpo conjugado con biotina. Preparado en solución Buffer con proteína estabilizada y conservante.

Anticuerpo conjugado con Peroxidasa ENZYMCONJ

Un vial de (min. 0.25 mL) solución concentrada del anticuerpo conjugado con peroxidasa. Preparado en solución Buffer con proteína estabilizada y conservante.

Incubación Buffer BUF

Un vial (min. 19 mL) de solución buffer lista para usar con proteína estabilizada. Detergente y conservante.

Solución sustrato SUBS TMB

Un vial (min. 12 mL) listo para usar de TMB sustrato en un buffer ácido.
El sustrato cromogénico debe aparecer como azul muy claro.

Solución para parar la reacción H2SO4

Un vial (min. 12 mL) listo para usar 0.18 mol/L ácido sulfúrico

Buffer de lavado WASHBUF 50x

Un vial (min. 20 mL) buffer de lavado concentrado con detergente y conservante.

Plástico Adhesivo, para cubrir la microplaca durante la incubación.

Materiales Necesarios y no suministrados

- Recipientes para preparar la solución con el anticuerpo y la solución de lavado
- Pipetas 50-200 µL
- Agua destilada
- Multipipeta de 8-12 canales para suministrar 100 µL y 150 µL

PROCEDIMIENTO DEL ENSAYO

Mezclar todos los reactivos y muestras antes de usarlos.

Antes de usarlo , preparar y equilibrar la solución temperatura ambiente 18-22°C.

Determinar el número de tiras que se requieren para el ensayo. Se recomienda que todas las muestras se analicen por duplicado. Además, se necesita un total de 16 pocillos por ensayo para los estándares y los controles. Colocar el número de tiras que se necesiten en su soporte. Colocar las tiras sin usar en la bolsa de aluminio bien cerrada con el desecante en su interior.

Evitar hacer espuma

1. Preparación de la Solución con el anticuerpo

Atención: Preparar la solución con el anticuerpo 30 minutos máximo antes de comenzar el procedimiento. Mezclar la Anticuerpo conjugado con Biotina **Ab BIOTIN** , Anticuerpo conjugado con Peroxidasa **ENZYMCONJ** y Buffer Incubación **BUF** , en la siguiente proporción volumétrica 1+1+100 en un recipiente vacío. Mezclar cuidadosamente y evitar la formación de espuma. **Preparar una solución nueva antes, de proceder con cada ensayo.**

2. Incubación en un paso

Pipetear 50 µL de cada estándar **CAL 0 - 5**, Control **CTRL 1 - 2** o la muestra desconocida en los pocillos y 150 µL de la solución del Anticuerpo. Cubrirlo y y incubarlo durante 120+5 minutos a temperatura ambiente (18-22°C) y centrifugarlo a 300 rpm.

3. Lavado

Se procede a lavar con 300 µL solución Buffer (**WASHBUF 50x** diluida 1+50 en agua destilada). Utilizando un plato de lavado automático. Emplear 5 ciclos de lavado. Es importante asegurarse de que los pocillos, quedan completamente vacíos antes de iniciar un ciclo automático de lavado.

4. Incubación con el sustrato cromogénico

Pipetear 100 µL de Solución sustrato **SUBS TMB** dentro de cada pocillo y incubar Durante 15+2 minutos a temperatura ambiente (18-22°C) en ausencia de luz, centrifugar a 300 rpm., cubriéndolo con un plástico. No pipetearlo directamente desde el vial que contiene el sustrato TMB. Depositar un volumen necesario a un recipiente limpio. La solución de sustrato que sobre, deberá ser desechada.

5. Solución para parar la reacción

Pipetear 100 µL de la solución de parade **H2SO4** en cada pocillo.

6. Medida de la absorbancia

Medida de la absorbancia de 450nm a 650nm como referencia dentro de las dos horas.

Limitaciones del procedimiento

Si la absorbancia de la muestra supera al **estándar 5**, la muestra deberá ser diluida en el **Estándar 0**, y se volverá a analizar.

CONTROL DE CALIDAD

La buena práctica del Laboratorio requiere la utilización de muestras como control de calidad en cada serie de procedimientos, de manera que podamos chequear el rendimiento del ensayo. Los controles se deberán hacer

con muestras desconocidas, y los resultados analizados con métodos estadísticos apropiados.

RESULTADOS

Calculo de resultados

Se calcula la media de las determinaciones de absorbancia obtenidas por duplicado.
Se representa la media de la absorbancia de los seis estándares en las ordenadas, y en las abscisas la concentración de CrossLaps. Se determina la concentración de CrossLaps de los controles y de cada una de las muestras del paciente por interpolación.

Ejemplo de los resultados obtenidos:

Standards/ Controls/ Samples	CrossLaps conc. (ng/mL)	A ₄₅₀₋₆₅₀ Obs 1/ Obs 2	Media (nm)	Interpolación CrossLaps conc. (ng/mL)
Estándar 0	0.000	0.066 / 0.065	0.066	
Estándar 1	0.178	0.210 / 0.209	0.210	
Estándar 2	0.489	0.472 / 0.448	0.460	
Estándar 3	0.960	0.844 / 0.819	0.832	
Estándar 4	1.902	1.598 / 1.560	1.579	
Estándar 5	2.494	2.061 / 2.004	2.033	
Controles 1		0.349 / 0.354	0.352	0.355
Controles 2		0.918 / 0.952	0.935	1.086
muestra I		0.140 / 0.138	0.139	0.091
muestra II		0.447 / 0.439	0.443	0.469
muestra III		1.305 / 1.303	1.304	1.555

NOTA: Los datos que aparecen en la tabla, sirven solamente para ilustrar el modo de hacer el cálculo.

Representación de las características

Límite de detección: 0.020 ng/mL CrossLaps
Esta es la concentración correspondiente a tres desviaciones estándar, la Media de 21 determinaciones (CrossLaps Estándar 0).

Imprecisión

La imprecisión del Serum CrossLaps® (CTX-I) ELISA fue evaluada para tres muestras de suero.
Los resultados son resumidos en la tabla de abajo:

Variación Inter ensayo (n=10)

Intra-Assay Variation (n=10)

Media (ng/mL)	SD (ng/mL)	CV (%)		Media (ng/mL)	SD (ng/mL)	CV (%)
0.121	0.013	10.9		0.121	0.004	3.0
0.444	0.043	9.7		0.444	0.007	1.7
1.967	0.050	2.5		1.967	0.035	1.8

Dilución / Linealidad

El Serum CrossLaps® (CTX-I) ELISA es lineal en el rango 0.020ng/mL a 3.380 ng/mL de CrossLaps.

Las muestras de suero con la concentración de 0.460-0.668 ng/mL CrossLaps fueron diluidas con el Estándar 0 y la concentración de CrossLaps fue determinada con Serum CrossLaps® (CTX-I) ELISA.
La muestra de suero está ajustada al 100%

El dato que aparece en la tabla de abajo está calculado de 3 maneras diferentes

Procedimiento de dilución		
Suero (%)	Standard 0 (%)	Recuperación (% valores esperados)
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
Media		103

Interferencia:

La interferencia de Ditaurobilirrubina, Hemoglobina y Lípidos en la medida de CrossLaps en suero por Serum CrossLaps® (CTX-I) ELISA fué investigada.

En la lista de concentraciones que aparece, en la tabla de abajo no fué detectada

Interferencia:

Ditaurobilirrubina: 600 mg/L

Hemoglobina: 10 g/L

IntraLip: 10 g/L

Valores Esperados

El laboratorio establecerá sus propios valores patológicos y normales. En la tabla de abajo se dan valores medios y desviaciones estándar en varias Poblaciones. Todas la muestras fueron recogidas a primera hora de la mañana y en mujeres sanas.

Población	Número de individuos	Valores medios de Suero CrossLaps (ng/mL)	95% Range (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

Variación individual día a día

La variación individual día a día fué calculada analizando las muestras de suero (primera hora de la mañana) de 11 mujeres sanas post menopausicas, cinco veces durante 2 semanas.

Subject No	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Mean (ng/mL)	SD (ng/mL)	CV (%)
1	0,423	0,428	0,396	0,460	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	0,045	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	0,255	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

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