Urine CartiLaps® (CTX-II) EIA

For the quantification of degradation products of C-terminal telopeptides of type II collagen (CTX-II) in urine
Immunodiagnostic Systems Limited and BioVendor LLC are not responsible for any other use of the kit or consequence hereof than the one specified above. Neither for misuse e.g. use deviating from the procedure described in this manual. Furthermore, Immunodiagnostic Systems Limited and it's distributor, BioVendor LLC, are not to be made responsible for any diagnoses or conclusions made by the user or third party based on the results obtained with the UrineCartiLaps® (CTX-II) EIA kit nor for any consequences such interpretations may cause.
INTRODUCTION

Intended use
The UrineCartiLaps® (CTX-II) EIA detects degradation products of C-terminal telopeptides of type II collagen. It is intended for in vitro diagnostic use as an indication of degradation of cartilage and may be used as an aid for:
• quantitative assessment of disease activity (structural damage of articular cartilage) in patients with RA and OA
• prognosis of disease activity in patients with RA and OA, and
• early assessment of long-term effect of therapy in patients with RA

Background
Disruption of the structural integrity of cartilage is the major histological finding in osteoarthritis and rheumatoid arthritis. Type II collagen is the major organic constituent of cartilage and fragments of type II collagen (CTX-II) are being released into circulation and subsequently secreted into urine following degradation of cartilage. In urine, the CTX-II fragments can be quantified by UrineCartiLaps® (CTX-II) EIA.

The UrineCartiLaps® (CTX-II) EIA has been reported to be useful in prediction of progression of osteoarthritis (Reijman (2003), Garnero (2003)) and in other clinical and pre-clinical investigations (please refer to REFERENCES).

Principle of the procedure
UrineCartiLaps® (CTX-II) EIA (Christgau (2001) is based on the competitive binding of a monoclonal antibody to urinary fragments of type II collagen or to biotinylated, synthetic peptides bound to the surface of microtitre plates coated with streptavidin.

Initially, biotinylated, synthetic peptides are bound to the surface of streptavidin-coated wells of the microtitre plate. After washing, standards, controls, and urine samples are pipetted into the wells followed by addition of a solution of the monoclonal antibody. The wells are washed, and a solution of peroxidase-conjugated anti-mouse immunoglobulin (rabbit) is added to the wells. Following the second washing step, a chromogenic substrate is added to all wells and the colour reaction is stopped with sulphuric acid and the absorbance is measured.

PRECAUTIONS

The following precautions should be observed in the laboratory:
• Do not eat, drink or smoke where immunodiagnostic materials are being handled.
• Do not pipette by mouth.
• Wear gloves when handling immunodiagnostic materials.
• Do not use kit components beyond their expiration date and do not mix reagents from different lots of kits.
• Cover working area with disposable absorbent paper.
• Always use clean containers.

Warnings
For in vitro use only.
• All reagents and laboratory equipment should be handled and disposed of as if they were infectious.

Storage
Store the UrineCartiLaps® (CTX-II) EIA kit at 2-8°C upon receipt. Under these conditions the kit is stable up to the expiry date stated on the box.

MATERIALS

Specimen collection
It is recommended to use second morning void urine specimens, but any spot urine sample may be used. Urine samples are stable for 24 hours at 4°C and should be stored frozen (<-18°C) for longer storage. Urine samples are stable for at least 10 freeze-thaw cycles.
Prior to use, urine specimens should be shaken and sedimentation allowed for a minimum of 30 minutes.
**Materials supplied**
Before using the kit, please read the section on **Precautions**. The kit contains reagents sufficient for 96 determinations.

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

**CartiLaps Standard**
One vial (min. 3.0 mL) of a ready-for-use TRIS-buffered solution containing protein stabilizer, detergent, and preservative.

**CartiLaps Standards**
Five vials (min. 0.4 mL each) of ready-for-use synthetic peptide in a TRIS-buffered solution containing protein stabilizer, detergent, and preservative. The exact value of each Standard is printed on the QC Report.

**CartiLaps Control**
Two vials (min. 0.4 mL) of ready-for-use synthetic peptide in a TRIS-buffered solution containing protein stabilizer, detergent, and preservative. Please refer to enclosed QC Report for control range.

**Biotinylated CartiLaps Antigen**
One vial (min. 12.0 mL) of ready-for-use biotinylated, synthetic peptide in a PBS-buffered solution containing protein stabilizer, detergent, and preservative.

**Primary Antibody**
One vial (min. 12.0 mL) of ready-for-use monoclonal antibody in a TRIS-buffered solution containing protein stabilizer, detergent, preservative, and a red dye.

**Peroxidase Conjugated Antibody**
One vial (min. 12.0 mL) of ready-for-use peroxidase-conjugated anti-mouse immunoglobulins (rabbit) in a TRIS-buffered solution with protein stabilizer, detergent, preservative, and a blue dye.

**Substrate Solution**
One vial (min. 12.0 mL) of a ready-for-use tetramethylbenzidine (TMB) substrate in an acidic solution. Please note that the chromogenic substrate might appear slightly bluish.

**Stopping Solution**
One vial (min. 12.0 mL) of ready-for-use 0.18 M sulfuric acid.

**Washing Solution**
One vial (min. 20.0 mL) of a concentrated washing buffer with detergent and preservative.

**Sealing tape**
Adhesive film for covering wells during incubation.

**Materials required — not supplied**
- Container for preparing the Washing Solution.
- Precision micropipette to deliver 40 µL.
- Precision 8 or 12-channel multipipette to deliver 100 µL.
- Distilled water.
- Refrigerator (2-8°C).
- Microtiter plate reader for reading at both 450 nm and 650 nm.
ASSAY PROCEDURE

For optimal performance of the assay, it is important to comply with the instructions given below. Equilibrate all reagents to room temperature (18-22°C) prior to use. 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 standards and controls. Place the appropriate number of strips in the plastic frame. Store unused immunostrips in the tightly closed foil bag with desiccant capsules.

Assay Procedure

1 **Pre-incubation**
   Add 100 µL of Biotinylated Urine CartiLaps Antigen \[^{Ag\ BIOTIN}\] to each well, cover with sealing tape, and incubate for 30±5 minutes at room temperature (18-22°C) without shaking.

2 **Washing**
   Wash the immuno strips 5 times manually with 300 µL Washing Solution \[^{WASHBUF\ 50x\ diluted\ in\ distilled\ water}\]. 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 automated washing cycle.

3 **Primary incubation**
   Pipette 40 µL of either Urine CartiLaps Standards \[^{CAL\ 0-5}\] or Controls \[^{CTRL}\] or unknown urine samples into appropriate wells followed by 100 µL Primary Antibody \[^{Ab}\]. Cover the immunostrips with sealing tape and incubate for 21±3 hrs in a refrigerator (2-8°C) without shaking.

4 **Washing**
   See step 2.

5 **Secondary incubation**
   Add 100 µL of the Peroxidase-Conjugated Antibody solution \[^{ENZYMCONJ}\] to each well. Cover the immunostrips with sealing tape and incubate for 60±5 minutes at room temperature (18-22°C) without shaking.

6 **Washing**
   See step 2.

7 **Incubation with chromogenic substrate solution**
   Pipette 100 µL of the Substrate Solution \[^{SUBS\ TMB}\] into each well, cover the immunostrips with sealing tape and incubate for 15±2 minutes in the darkness at room temperature (18-22°C) without shaking.

8 **Stopping of color reaction**
   Pipette 100 µL of the Stopping Solution \[^{H2SO4}\] into each well.

9 **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 is lower than 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
Construct a standard curve using a four-parametric logistic curve fit, and determine the Urine CartiLaps concentration of the Controls and each of the patient specimens by interpolation on the curve.

Example of results obtained:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Urine CartiLaps concentration (µg/L)</th>
<th>Abs_{450-650nm} Obs 1 / Obs 2 (Abs.)</th>
<th>Mean absorbance (Abs.)</th>
<th>Interpolated Urine CartiLaps concentration (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 0</td>
<td>0.00</td>
<td>1.914/1.879</td>
<td>1.897</td>
<td></td>
</tr>
<tr>
<td>Standard 1</td>
<td>0.73</td>
<td>1.069/1.046</td>
<td>1.058</td>
<td></td>
</tr>
<tr>
<td>Standard 2</td>
<td>1.32</td>
<td>0.822/0.806</td>
<td>0.814</td>
<td></td>
</tr>
<tr>
<td>Standard 3</td>
<td>2.50</td>
<td>0.538/0.529</td>
<td>0.534</td>
<td></td>
</tr>
<tr>
<td>Standard 4</td>
<td>5.00</td>
<td>0.317/0.300</td>
<td>0.309</td>
<td></td>
</tr>
<tr>
<td>Standard 5</td>
<td>10.06</td>
<td>0.186/0.171</td>
<td>0.179</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>0.792/0.754</td>
<td>0.773</td>
<td>1.40</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>0.369/0.348</td>
<td>0.359</td>
<td>4.31</td>
</tr>
<tr>
<td>Sample 1</td>
<td></td>
<td>0.998/0.967</td>
<td>0.983</td>
<td>0.88</td>
</tr>
<tr>
<td>Sample 2</td>
<td></td>
<td>0.407/0.433</td>
<td>0.420</td>
<td>3.52</td>
</tr>
<tr>
<td>Sample 3</td>
<td></td>
<td>0.238/0.227</td>
<td>0.233</td>
<td>7.23</td>
</tr>
</tbody>
</table>

Note: The data above are for illustration only and should not be used for calculation of results.

Correction with creatinine
The CTX-II value determined as described above should be corrected with creatinine concentration.

Determine the concentration of creatinine (mmol/L) in the sample using an enzymatic colorimetric routine method for clinical chemistry analysers and perform the correction using the equation:

Corrected CTX-II Value (ng/mmol) = \frac{1000 \times \text{Urine CartiLaps (µg/L)}}{\text{Creatinine (mmol/L)}}

Performance characteristics
Lot-to-Lot variability \(<7.0\%

The lot-to-lot variability was determined by testing three urine samples in three different lots of Urine CartiLaps® (CTX-II) EIA

<table>
<thead>
<tr>
<th></th>
<th>Mean (µg/L)</th>
<th>SD (µg/L)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOW</td>
<td>0.47</td>
<td>0.033</td>
<td>7.0</td>
</tr>
<tr>
<td>MEDIUM</td>
<td>1.87</td>
<td>0.064</td>
<td>3.4</td>
</tr>
<tr>
<td>HIGH</td>
<td>5.44</td>
<td>0.136</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Detection limit \(0.20 \text{ µg/L}\)

The detection limit was determined to 0.20 ng/mL, which is the concentration corresponding to three standard deviations below the mean of 21 determinations of the absorbance of the Urine CartiLaps Standard 0.

Precision \(\leq 12.2\%\)

The precision was determined using ten analytical runs, each with duplicate determinations of urine samples.
### Sample Mean (µg/L)

<table>
<thead>
<tr>
<th>Sample</th>
<th>SD (µg/L)</th>
<th>CV (%)</th>
<th>SD (µg/L)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOW</td>
<td>0.04</td>
<td>7.8</td>
<td>0.06</td>
<td>12.2</td>
</tr>
<tr>
<td>MEDIUM</td>
<td>0.08</td>
<td>4.6</td>
<td>0.20</td>
<td>10.8</td>
</tr>
<tr>
<td>HIGH</td>
<td>0.28</td>
<td>5.2</td>
<td>0.38</td>
<td>6.9</td>
</tr>
</tbody>
</table>

### Dilution/Linearity 96%

The dilution recovery of the Urine CartiLaps® (CTX-II) EIA was determined to 96%. Four urine samples were diluted in Urine CartiLaps Standard 0, the concentration of CTX-II was determined in the Urine CartiLaps® (CTX-II) EIA and the recovery calculated after correction with the dilution factor.

### Interference

No interference could be detected in Urine CartiLaps® (CTX-II) EIA with addition of the following compounds into human urine samples:

- Urea up to 30 g/L
- Ibuprofen up to 50 g/L
- Creatinine up to 10 mg/L
- Acetyl-salicylic acid up to 50 g/L
- Glucose up to 5 mg/L
- Paracetamol up to 50 g/L
- Ascorbic acid up to 5 mg/L
- Albumin up to 50 mg/L

### Specificity

The epitope being detected in the Urine CartiLaps® (CTX-II) EIA is highly conserved and therefore the test can be applied to urine samples from most other species, including non-human primates, bovines, horses, pigs, rabbits, rats, and mice.

### Expected values

It is advisable for each laboratory to establish its own range of healthy and pathological CTX-II values. As an example, the geometric mean values and 95% confidence interval (CI) for various populations are given below. For further information, please refer to the reference list at the end of these instructions.

<table>
<thead>
<tr>
<th>Populations</th>
<th>Number of subjects</th>
<th>Age (years)</th>
<th>Mean CTX-II (ng/mmol)</th>
<th>95% CI (ng/mmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All women</td>
<td>459</td>
<td>20-85</td>
<td>299</td>
<td>79-1137</td>
</tr>
<tr>
<td>Pre 20-30 yrs</td>
<td>38</td>
<td>20-30</td>
<td>464</td>
<td>103-2086</td>
</tr>
<tr>
<td>Pre 30-60 yrs</td>
<td>165</td>
<td>30-59</td>
<td>200</td>
<td>65-618</td>
</tr>
<tr>
<td>Post</td>
<td>256</td>
<td>46-85</td>
<td>363</td>
<td>112-1172</td>
</tr>
<tr>
<td>Pre 48-53 yrs</td>
<td>28</td>
<td>48-53</td>
<td>164</td>
<td>66-410</td>
</tr>
<tr>
<td>Post 48-53 yrs</td>
<td>38</td>
<td>48-53</td>
<td>318</td>
<td>89-1132</td>
</tr>
<tr>
<td>All male</td>
<td>247</td>
<td>22-87</td>
<td>278</td>
<td>87-895</td>
</tr>
<tr>
<td>Male 20-30 yrs</td>
<td>27</td>
<td>20-30</td>
<td>501</td>
<td>214-1171</td>
</tr>
<tr>
<td>Male 30-60 yrs</td>
<td>141</td>
<td>30-60</td>
<td>236</td>
<td>89-628</td>
</tr>
<tr>
<td>Male &gt; 60 yrs</td>
<td>79</td>
<td>60-87</td>
<td>305</td>
<td>85-1096</td>
</tr>
</tbody>
</table>
REFERENCES

1. Ceunick F De. et al., Urinary collagen type II C-telopeptide fragments are sensitive markers of matrix metallo-proteinase dependent cartilage degradation in rat adjuvant induced arthritis. *J Rheumatol* (2003); 30: 1561-1564.
15. Mazières B. et al., Molecular markers of cartilage breakdown and synovitis are strong independent predictors of structural progression of hip osteoarthritis (OA). the ECHODIAH cohort. *ACR 2003*.
GB Catalogue number
DE Bestellnummer
ES Número de catálogo
IT Numero di catalogo
FR Référence du catalogue
NL Catalogus nummer
CZ Katalogová číslo
SK Katalógové číslo
GR Αριθμός καταλόγου
PT Referência de catálogo
HU Katalógusszám
PL Numer katalogowy
GB Manufacturer
DE Hersteller
ES Fabricante
IT Fabbricante
FR Fabricant
NL Fabrikant
DK Producent
CZ Výrobce
SK Výrobca
GR Κατασκευαστής
PT Fabricante
HU Gyártó
SE Tillverkare
PL Producent

GB Contains sufficient for <n> tests
DE Inhalt ausreichend für <n> Prüfungen
ES Contenido suficiente para <n> ensayos
IT Contenuto sufficiente per "n" saggi
FR Contenu suffisant pour "n" tests
NL Inhoud voldoende voor "n" testen
DK Indeholder tilstrækkeligt til "n" test
CZ Obsah postačuje na "n" testů
SK Obsah poskytuje na "n" stanovení
GR Περιεχόμενο επαρκές για "n" εξέτασες
PT Conteúdo suficiente para "n" ensaios
HU A doboz tartalma "n" vizsgálat elégéhez
SE Räcker till "n" antal tester
PL Wystarczy na wykonanie "n" testów

GB Temperature limitation
DE Temperaturbegrenzung
ES Límites de temperatura
IT Limiti di temperatura
FR Limites de température
NL Temperatuurlimiet
DK Temperaturbegrænsning
CZ Teplotní rozmezí od do
SK Teplotné rozmedzie od do
GR Περιορισμοί θερμοκρασίας
PT Limites de temperatura
HU Hőmérséklettartomány
SE Temperatursgränser
PL Przezstrzegać zakresu temperatury
GB Batch code
DE Chargenbezeichnung
ES Código de lote
IT Codice del lote
FR Code du lot
NL Lot nummer
DK Lotnummer
CZ Číslo šarže
SK Číslo šarže
GR Αριθμός Παριόδου
PT Código do lote
HU Szákszám
SE Lot number
PL Kod parti

GB In Vitro Diagnostic Medical Device
DE In-Vitro-Diagnostikum
ES Producto sanitario para diagnóstico in vitro
IT Dispositivo medico-diagnostico in vitro
FR Dispositif médical de diagnostic in vitro
NL Medisch hulpmiddel voor in-vitro diagnostiek
DK Medicinsk udstyr til in vitro-diagnostik
CZ In Vitro diagnostický zdravotnický prostředek
SK Zdravotnícka pomôcka in vitro
GR In Vitro Διαγνωστικό Ιατροτεχνολογικό προϊόν
PT Dispositivo médico para diagnóstico in vitro
HU In vitro diagnosztikum
SE Medicintekniska produkter för in vitro diagnostik
PL Wyrób do diagnostyki In Vitro

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