Intended Use
For In Vitro Diagnostic Use
The IDS-iSYS CTX-I (CrossLaps®) assay is intended for the quantitative determination of degradation products of C-terminal telopeptides of Type I collagen (CTX-I) in human serum or plasma on the IDS-iSYS Multi-Discipline Automated System. Results are to be used in conjunction with other clinical and laboratory data to assist the clinician. CTX-I can be used as an indicator of bone resorption status as well as an aid in monitoring bone resorption changes during hormone replacement and Bisphosphonate therapies.

Summary and Explanation
Type I collagen accounts for more than 90% of the organic matrix of bone and is synthesized primarily in bone (1). During renewal of the skeleton, Type I collagen is degraded and small peptide fragments are excreted into the bloodstream. These fragments can be measured by the IDS-iSYS CTX-I Kit.

The measurements of the specific degradation products of Type I collagen in both urine (2) and serum (3) by competitive CrossLaps assays 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).

Method Description
The CTX-I assay is based on chemiluminescence technology. Two highly specific monoclonal antibodies are used. The antibodies are against the amino acid sequence of EKAHD-β-GGR, where the aspartic acid residue is (D) β-isomerized. In order to obtain a specific signal in IDS iSYS CTX-I assay, two chains of of EKAHD-β-GGR must be cross-linked.

Samples are incubated with both labelled antibodies for a period of time. Streptavidin coated magnetic particles are then added and following a further incubation step, the particles are “captured” using a magnet. After a washing step and addition of trigger reagents, the light emitted by the acridinium label is directly proportional to the concentration of CTX-I in the original sample.

Warnings and Precautions
The IDS-iSYS CTX-I (CrossLaps®) assay is for in vitro diagnostic use only and is not for internal use in humans or animals. This product must be used strictly in accordance with the instructions set out in these Instructions for Use (IFU). IDS Ltd. will not be held responsible for any loss or damage (except as required by statute), howsoever caused arising out of non-compliance with the instructions provided.

CAUTION: This kit contains material of animal origin. Handle kit reagents as if capable of transmitting an infectious agent.

Appropriate precautions and good laboratory practice must be used in the storage, handling and disposal of the kit reagents. Disposal of kit reagents should be in accordance with local regulations.

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

Handling Precautions
The reagents are ready to use. Before a new cartridge is loaded onto the Analyser, the magnetic particle container requires mixing by the operator with a brisk rotation motion. This will resuspend the magnetic particles that have settled during shipment. It is very important to avoid any foam formation.

Sample Collection and Storage
The assay should be performed using serum (standard sampling tubes or tubes containing serum separating gel) or plasma (lithium heparin, sodium heparin, potassium EDTA) samples. Samples should be separated as soon as possible after collection.

Store samples at -20 °C. Avoid repeated freeze-thaw of samples.

Samples containing particulate matter must be centrifuged before performing the assay.

Do not use heat-inactivated samples.

To minimise possible evaporation effects, samples, calibrators, and controls should be measured within 3 hours after being placed on the analyser.

Before performing the assay, make sure that samples, calibrators and controls are at room temperature (20 - 25 °C).

Note: Some sample collection tubes that are commercially available might affect the results of testing in particular cases.

It is recommended to follow the instructions of the tube manufacturer especially when processing samples in primary tubes.

Procedure
Materials Provided
Reagent Cartridge
MP33
Magnetic particles coated with Streptavidin in a phosphate buffer containing sodium azide as preservative (<0.1 %), 1 bottle, 2.6 mL.

CONJ
Anti-CTX-I labelled with an acridinium ester derivative, in buffer containing bovine and mouse proteins with sodium azide as preservative (<0.1 %), 1 bottle, 5 mL.

Ab-BIOT
Anti-CTX-I labelled with biotin, in buffer containing bovine and mouse proteins with sodium azide as preservative (<0.1 %), 1 bottle, 5 mL.

Assay Bu
A phosphate BSA buffer containing bovine and mouse proteins, horse serum and sodium azide as preservative (<0.1 %), 1 bottle, 8.4 mL.
A phosphate BSA buffer containing bovine and mouse proteins, horse serum and sodium azide as preservative (<0.1 %), 1 bottle, 8.4 mL.

Calibrators

<table>
<thead>
<tr>
<th>CAL A</th>
<th>Horse serum containing CTX-I and sodium azide as preservative (&lt;0.1 %), 1 each of 2 concentration levels, 2.5 mL.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL B</td>
<td>Mini CD Contains IFU for IDS-iSYS reagents, control ranges and CRY files.</td>
</tr>
</tbody>
</table>

Materials Required But Not Provided

IDS-iSYS Multi-Discipline Automated System: IS-310400
IDS-iSYS CTX-I Control Set: IS-3030, 2 x 2.5 mL each of level 1, 2, and 3.
IDS-iSYS Cuvettes Cube: IS-CC100, box of 960 cuvettes.
IDS-iSYS System Liquid (Syst. I): IS-CS100, 5 L container, ready to use.
IDS-iSYS Wash Solution (Wash S): IS-CW100, 10 L container, ready to use.
IDS-iSYS Trigger Set: IS-CT100, 2 x 250 mL per bottle, ready to use.
IDS-iSYS Cartridge Check System (CCS): IS-6010, ready to use.
Sample Cups (500 µL): IS-SC105.

Assay Procedure

Reagent Cartridge

The reagents provided in the cartridge are ready to use. The Analyser automatically performs the mixing of magnetic particles to maintain homogeneity. Before a new cartridge is loaded on board the analyser, mix the magnetic particles container by brisk rotation motion. Avoid foam formation.

The barcode is read when the cartridge is loaded on the reagent tray. If the label cannot be read by the analyser barcode reader, a manual procedure exists to enter the barcode data (see the IDS-iSYS User Manual).

Load the cartridge on the reagent tray and wait for at least 40 minutes before starting the assay.

If the cartridge is removed from the reagent tray, store the cartridge vertically at 2 - 8 °C in the dark.

Calibrators

The CTX-I calibrators are ready to use. Leave the calibrators at room temperature for 10 minutes and gently mix the bottles by hand. Avoid formation of foam. Pipette approximately 400 µL of calibrators into sample cups and place on the machine. Discard the material in the sample cups after use. DO NOT return material to the calibrator vial.

Analyser Calibration

The two CTX-I calibrators are required to perform the adjustment of the master curve. The calibrators are supplied with the kit and calibrators from another lot must not be used.

Note that to perform a master curve adjustment controls MUST be run at the same time as the calibrators.

All data required for the calibration of the cartridge batch can be found on the mini CD. Use calibrator levels A and B to adjust the master curve to the reagents on board the Analyser. Check for the presence of a CTX-I cartridge on the reagent tray and the availability of the cartridge master curve in the database. If the data for the lot of calibrators is not available on board the Analyser, load the data using the mini CD provided with the calibrator.

Start the immunoassay calibration on the IDS-iSYS Analyser according to the IDS-iSYS User Manual. The calibration is carried out in triplicate. One replicate may be removed to meet the calibration requirements. As stated above, please note that controls must also be run. Verify and approve the calibration according to the calibration status displayed in the calibration windows and discard the calibrator from the sample tray after use.

Calibration

The IDS-iSYS CTX-I assay is standardized against in-house reference standards (CTX-I in horse serum).

Calibration Frequency

A new calibration is required:

- Each time a new lot of cartridges is loaded on board
- Each time a new lot of trigger or cuvettes is used
- When the control values do not fall within the defined ranges
- When the calibration has expired
- After Analyser service.

Verification of the calibration is automatic and managed by the Analyser.

Quality Control

Use the IDS-iSYS CTX-I (CrossLaps®) Control Set for quality control. To ensure validity of results at least three controls with varying levels of CTX-I should be measured. Other suitable control material can be used in addition to the IDS-iSYS CTX-I (CrossLaps®) Control Set. Controls should be tested at (or near) the beginning of every run containing patient samples and also during calibrations or according to local regulations. It is recommended that the controls be routinely run in duplicate. Laboratories should test controls at least once per shift.

Refer to the IDS-iSYS CTX-I Control Set IFU for preparation and handling instructions.

Determination of Sample CTX-I levels

Process samples according to the IDS-iSYS User Manual.

Calculation of Results

The CTX-I concentration of each sample is calculated automatically. The display of the concentrations (screen or printed) is produced upon user request.

The IDS-iSYS CTX-I assay uses a 4-parameter logistic curve fit (4PL) to calculate the CTX-I concentrations.

Measurement Range (Reportable Range)

The reportable range of the assay is 0.033 - 6.000 ng/mL. Any value that reads below 0.033 ng/mL should be reported as “< 0.033 ng/mL”.
Dilution
Samples with CTX-I concentrations above the reportable range should be diluted manually with a low concentration sample human serum sample in a ratio of 1 in 2. The results for diluted samples must be multiplied by the dilution factor 2 and corrected for the concentration of the low sample.

Limitations of Use
1. As in the case of any diagnostic procedure, results must be interpreted in conjunction with the patient's clinical presentation and other information available to the physician.
2. The performance characteristics of this assay have not been established in a paediatric population.
3. The following substances do not interfere in the IDS-iSYS CTX-I assay when the concentrations presented in the following table are below the stated threshold.

<table>
<thead>
<tr>
<th>Potentially Interfering Agent</th>
<th>Threshold Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid</td>
<td>3000 mg/dL</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>20 mg/dL</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>500 mg/dL</td>
</tr>
<tr>
<td>Red blood cells</td>
<td>0.4 %</td>
</tr>
<tr>
<td>Biotin</td>
<td>300 nmol/L</td>
</tr>
</tbody>
</table>

4. The hook effect was tested using concentrations of CTX-I up to 145 ng/mL. No hook effect was observed.

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. All samples were morning fasting samples from healthy individuals.

<table>
<thead>
<tr>
<th>Populations</th>
<th>Number of subjects</th>
<th>Mean Values (ng/mL)</th>
<th>95% Confidence Interval (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>125</td>
<td>0.294</td>
<td>0.115 – 0.748</td>
</tr>
<tr>
<td>Pre-menopausal women</td>
<td>226</td>
<td>0.287</td>
<td>0.112 – 0.738</td>
</tr>
<tr>
<td>Post-menopausal women</td>
<td>193</td>
<td>0.439</td>
<td>0.142 – 1.351</td>
</tr>
</tbody>
</table>

Performance Data
Representative laboratory data are shown. Results obtained at individual laboratories may vary.

Sensitivity
The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) were determined with guidance from CLSI EP17-A, “Protocols for Determination of Limits of Detection and Limits of Quantitation” using 60 blanks and 144 low level samples.

<table>
<thead>
<tr>
<th></th>
<th>LoB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.009 ng/mL</td>
</tr>
</tbody>
</table>

Precision
Precision was evaluated in accordance with a modified protocol based on CLSI EP-5A2, “Evaluation of Precision Performance of Quantitative Measurement Methods”. Five serum controls were assayed using three lots of reagents in duplicate twice per day for 20 days on three analysers.

<table>
<thead>
<tr>
<th>Control</th>
<th>n</th>
<th>Mean (ng/mL)</th>
<th>SD</th>
<th>CV%</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>0.216</td>
<td>0.010</td>
<td>4.9</td>
<td>0.019</td>
<td>8.8</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>0.639</td>
<td>0.021</td>
<td>3.2</td>
<td>0.040</td>
<td>6.3</td>
</tr>
<tr>
<td>3</td>
<td>80</td>
<td>0.877</td>
<td>0.018</td>
<td>2.1</td>
<td>0.045</td>
<td>5.1</td>
</tr>
<tr>
<td>4</td>
<td>80</td>
<td>2.270</td>
<td>0.054</td>
<td>2.4</td>
<td>0.107</td>
<td>4.7</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td>4.273</td>
<td>0.149</td>
<td>3.5</td>
<td>0.251</td>
<td>5.9</td>
</tr>
</tbody>
</table>

Linearity
Linearity was evaluated based on CLSI EP-6A, “Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach”. Samples containing varying concentrations of CTX-I were assayed in quadruplicate. The resulting mean concentrations were compared to predicted concentrations. Samples were prepared by diluting a high patient sample with a low patient sample prior to assay.

<table>
<thead>
<tr>
<th>Predicted Concentration (ng/mL)</th>
<th>Measured Concentration (ng/mL)</th>
<th>Variation (ng/mL)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.245</td>
<td>0.229</td>
<td>-0.016</td>
<td>-6%</td>
</tr>
<tr>
<td>0.876</td>
<td>0.873</td>
<td>-0.003</td>
<td>0%</td>
</tr>
<tr>
<td>1.507</td>
<td>1.532</td>
<td>0.025</td>
<td>2%</td>
</tr>
<tr>
<td>2.138</td>
<td>2.174</td>
<td>0.036</td>
<td>2%</td>
</tr>
<tr>
<td>2.769</td>
<td>2.794</td>
<td>0.025</td>
<td>1%</td>
</tr>
<tr>
<td>3.400</td>
<td>3.406</td>
<td>0.006</td>
<td>0%</td>
</tr>
<tr>
<td>4.031</td>
<td>3.945</td>
<td>-0.086</td>
<td>-2%</td>
</tr>
<tr>
<td>4.662</td>
<td>4.525</td>
<td>-0.137</td>
<td>-3%</td>
</tr>
<tr>
<td>5.293</td>
<td>5.441</td>
<td>0.148</td>
<td>3%</td>
</tr>
</tbody>
</table>

Method Comparison
The IDS-iSYS CTX-I assay was compared against the IDS Serum CrossLaps® ELISA for the quantitative determination of CTX-I, following CLSI EP-9A2, “Method Comparison and Bias Estimation Using Patient Samples”. A total of 322 samples, selected to represent a wide range of CTX-I concentrations [0.060 - 1.415 ng/mL], was assayed by each method. Passing-Bablok analysis were performed on the comparative data: IDS-iSYS = 0.987 (x) - 0.019 (95% CI of the slope and intercept were 0.954 to 1.025, and -0.031 to -0.004, respectively); correlation coefficient (r) = 0.96.

Bibliography


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