Intended Use

For In Vitro Diagnostic Use

The IDS-iSYS N-Mid® Osteocalcin Assay is intended for the quantitative determination of Osteocalcin in human serum or plasma on the IDS-iSYS Multi-Discipline Automated System (System). Results are to be used in conjunction with other clinical and laboratory data to assist the clinician. Osteocalcin is an indicator of osteoblastic activity in human serum and plasma and is intended to be used as an aid in the prevention of osteoporosis.

Summary and Explanation

Osteocalcin, or bone Gla protein (BGP), is the major non-collagenous protein of bone matrix. It has a molecular weight of approximately 5800 Daltons and consists of 49 amino acids, including three residues of gamma-carboxyglutamic acid.

Osteocalcin is synthesised in bone by osteoblasts. After production, it is partly incorporated into the bone matrix and partly delivered to the circulatory system. A large number of studies have shown that the circulating level of osteocalcin reflects the rate of bone formation (1-14).

Determination of serum osteocalcin has proved to be valuable as an aid in identifying women at risk of developing osteoporosis, for monitoring bone metabolism during the perimenopause and postmenopause and during antiresorptive therapy.

Method Description

The IDS-iSYS N-Mid® Osteocalcin assay is based on chemiluminescence technology. Two highly specific monoclonal antibodies against human osteocalcin are utilised. An antibody recognising the mid-region (amino acids 20-29) is used as the capture antibody and for detection an acridinium conjugated antibody recognising the N-terminal region (amino acids 10-16) is used. In addition to intact osteocalcin, (amino acid 1-49) the N-terminal-Mid fragment (amino acids 1-43) will also be detected.

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 osteocalcin in the original sample.

Warnings and Precautions

The IDS-iSYS N-Mid® Osteocalcin 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 Limited 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.

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 and sodium citrate) samples. Samples should be separated as soon as possible after collection. Store samples at -20 °C or below. 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 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

Magnetic particles coated with Streptavidin in a phosphate buffer containing sodium azide as preservative (<0.1%), 1 bottle, 2.7 mL.

CONJ

Anti-osteocalcin labelled with an acridinium ester derivative, in buffer containing bovine proteins, horse and mouse sera with sodium azide as preservative (<0.1%), 1 bottle, 12.5 mL.

Ab-BIOT

Anti-osteocalcin labelled with biotin, in buffer containing bovine and mouse proteins with sodium azide as preservative (<0.1%), 1 bottle, 12.5 mL.

Calibrators

CAL A

A phosphate BSA matrix containing osteocalcin and sodium azide as preservative (<2%), 2 each of 2 concentration levels, 1 mL.

Mini CD

Contains IFU for IDS-iSYS reagents, control ranges and CRY files.

Materials Required But Not Provided

IDS-iSYS Multi-Discipline Automated System: IS-310400
IDS-iSYS N-Mid® Osteocalcin Control Set: IS-2930, 4 x 1 mL, each of level 1, 2, and 3.
IDS-iSYS Cuvettes: IS-CC100, box of 960 cuvettes.
IDS-iSYS System Liquid (Syst. L): 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-CSC105.

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 osteocalcin calibrators are lyophilised. Reconstitute immediately before use. Add 1.0mL of distilled or deionised water to each bottle. Replace the stopper. Leave for 10 minutes to reconstitute with occasional gentle mixing by hand. Avoid formation of foam. Pipette approximately 300 µL of calibrators into sample cups and place on the machine. Proceed according to the instructions of the IDS-iSYS User Manual. DO NOT return material to the calibrator vial.

If calibrators are to be used more than once, they should be aliquotted and stored at -20 °C or lower within 15 minutes of reconstitution. When re-using frozen calibrators, thaw at room temperature and mix well. Ensure that calibrators are at room temperature before they are placed on the machine. Calibrators should be placed on the machine within 30 minutes of thawing. Aliquots should not be re-frozen.

Analyser Calibration

The two osteocalcin 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 Osteocalcin 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 N-Mid® Osteocalcin assay has been standardized against in-house reference standards (osteocalcin in analyte-free human serum matrix).

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 N-Mid® Osteocalcin Control Set for quality control. To ensure validity of results at least three controls with varying levels of osteocalcin should be measured. Other suitable control material can be used in addition to the IDS-iSYS N-Mid® Osteocalcin 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 N-Mid® Osteocalcin Control Set IFU for preparation and handling instructions.

Determination of Sample Osteocalcin levels

Process samples according to the IDS-iSYS User Manual.

Calculation of Results

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

The IDS-iSYS N-Mid® Osteocalcin Assay uses a 4-parameter logistic curve fit (4PL) to calculate the osteocalcin concentrations.

Measurement Range (Reportable Range)

The reportable range of the assay is 2-200 ng/mL. Any value that reads below 2 ng/mL should be reported as <2 ng/mL.

Dilution

Samples with osteocalcin concentrations above the reportable range should be diluted manually with a low concentration 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 N-Mid® Osteocalcin 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>Biotin</td>
<td>300 nmol/L</td>
</tr>
</tbody>
</table>

4. The hook effect was tested using concentrations of osteocalcin up to 5000 ng/mL. No hook effect was observed.

Expected Values

Each laboratory should determine ranges for their local population. The following range was determined using the IDS-iSYS N-Mid® Osteocalcin Assay and is provided for information only. The 95% reference interval for the following groups, was calculated by a non-parametric method following the NCCLS guideline C28-A2, “How to Define and Determine Reference Intervals in the Clinical Laboratory”.

Normal Adults 10.4 - 45.6 ng/mL (n=149)

Performance Data

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

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>LoB</td>
<td>0.27 ng/mL</td>
</tr>
<tr>
<td>LoD</td>
<td>0.27 ng/mL</td>
</tr>
<tr>
<td>LoQ</td>
<td>1.57 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”. Six serum controls were assayed using three lots of reagents in duplicate twice per day for 20 days on two instruments.

<table>
<thead>
<tr>
<th>Control n</th>
<th>mean (ng/mL)</th>
<th>Within-run SD</th>
<th>Total SD</th>
<th>Total CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>5.9</td>
<td>0.22</td>
<td>3.8</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>15.6</td>
<td>0.49</td>
<td>3.2</td>
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<td>3</td>
<td>80</td>
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<td>4</td>
<td>80</td>
<td>72.2</td>
<td>1.3</td>
<td>1.8</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td>131</td>
<td>2.7</td>
<td>2.1</td>
</tr>
<tr>
<td>6</td>
<td>80</td>
<td>149</td>
<td>3.2</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Recovery

Recovery was assessed by adding osteocalcin to samples prior to assay.

<table>
<thead>
<tr>
<th>Sample Concentration (ng/mL)</th>
<th>Osteocalcin added (ng/mL)</th>
<th>Measured (ng/mL)</th>
<th>Recovery (ng/mL)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.2</td>
<td>100</td>
<td>94.2</td>
<td>7.5</td>
<td>92</td>
</tr>
<tr>
<td>9.2</td>
<td>50</td>
<td>97.1</td>
<td>100</td>
<td>100</td>
</tr>
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<td>3.5</td>
<td>97.3</td>
<td>100</td>
<td>100</td>
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<td>9.2</td>
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<td>100</td>
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<tr>
<td>14.5</td>
<td>100</td>
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<td>45.5</td>
<td>91</td>
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<td>3</td>
<td>83.7</td>
<td>45.5</td>
<td>91</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 osteocalcin were assayed in duplicate. 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</th>
<th>Measured Concentration</th>
<th>Variation</th>
</tr>
</thead>
</table>
Method Comparison
The IDS-ISYS N-Mid® Osteocalcin Assay was compared against recognised enzymeimmunoassay for the quantitative determination of osteocalcin, following CLSI EP-9A2, “Method Comparison and Bias Estimation Using Patient Samples”. A total of 157 samples, selected to represent a wide range of osteocalcin concentrations [1.9-175 ng/mL], was assayed by each method. Passing-Bablok regression analysis was performed on the comparative data:

IDS-ISYS = 1.09 + 1.4 (95% CI of the slope and intercept were 1.06 to 1.12, and 1.1 to 1.8 respectively); correlation coefficient (r) = 1.00.

Bibliography

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<table>
<thead>
<tr>
<th>(ng/mL)</th>
<th>(ng/mL)</th>
<th>(ng/mL)</th>
<th>%</th>
</tr>
</thead>
<tbody>
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<td>-0.8</td>
<td>-9%</td>
</tr>
<tr>
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<td>25.7</td>
<td>1.2</td>
<td>5%</td>
</tr>
<tr>
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<td>40.4</td>
<td>0.4</td>
<td>1%</td>
</tr>
<tr>
<td>55.4</td>
<td>55.8</td>
<td>0.4</td>
<td>1%</td>
</tr>
<tr>
<td>70.9</td>
<td>72.0</td>
<td>1.1</td>
<td>2%</td>
</tr>
<tr>
<td>86.3</td>
<td>87.0</td>
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<td>1%</td>
</tr>
<tr>
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<td>-1%</td>
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<td>-2%</td>
</tr>
<tr>
<td>133</td>
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<td>-2%</td>
</tr>
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<tr>
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