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Immunoassay

REF **CMS0101 / CMS0102 / CMS0103 / CMS0104 / CMS0105**

50 tests*1 / 100 tests*1 / 100 tests*2 / 100 tests*5 / 50 tests*2

Osteocalcin CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative detection of osteocalcin in human serum or plasma (EDTA or heparin).

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
EC REP	authorized representative in the European Community		

EC REP

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CE IVD

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Introduction

Osteocalcin, also known as bone gamma-carboxyglutamic acid containing protein, is a non-collagenous protein hormone found in bone and dentin which is dependent on vitamin K. In human, the osteocalcin is encoded by the BGLAP, [1] whose receptor is GPRC6A. [2, 3] It contains 49 amino acids and has a molecular weight of approx. 5800 daltons.

Osteocalcin is secreted solely by osteoblasts and though to play a role in the body's metabolic regulation and is pro-osteocalcin, or bone-building, by nature.[4] It is also implicated in bone mineralization and calcium ion homeostasis. Osteocalcin acts as a hormone in the body, causing β cell in the pancreas to release more insulin, and at the same time directing fat cells to release the hormone adiponectin, which increases sensitivity to insulin.[4] Osteocalcin acts on leydig cells of the testis to stimulate testosterone biosynthesis and therefore affect male fertility.[5]

As osteocalcin is produced by osteoblasts, it is often used as a marker for the bone formation process. It has been observed that higher serum osteocalcin levels are relatively well correlated with increases in bone mineral density (BMD) during treatment with anabolic bone formation drugs for osteoporosis, such as Teriparatide. In many studies, osteocalcin is used as a preliminary biomarker on the effectiveness of a given drug on bone formation. [6]

Measurement Principle

This assay is based upon the one-step sandwich method. The sample, anti-osteocalcin coated microparticles and enzyme labeled anti-osteocalcin are combined. During the incubation, osteocalcin present in the sample is allowed to react simultaneously with the two antibodies, resulting in the osteocalcin being sandwiched between the solid phase and enzyme-linked antibodies. After washing, a complex is generated among the solid phase, osteocalcin in the sample and enzyme-linked antibodies by immunological reactions. The complex catalyzes substrate, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is proportional to the amount of osteocalcin in the sample.

Materials provided

1. Calibrators

6 vials lyophilized Calibrator A through F. The matrix is PBS (phosphate buffered saline) containing BSA (bovine serum albumin). Contains a selection of preservatives.

Reconstitute each lyophilized calibrator with 1.0 mL of distilled water. Allow the reconstituted material to stand for at least 10 minutes. Then invert the calibrator to mix it completely.

2. Reagent pack

Reagent pack provided ready to use.

Σ	50*1	100*1	100*2	100*5	50*2
Microparticles Solution	1.2mL*1	2.3mL*1	2.3mL*2	2.3mL*5	1.2mL*2
Enzyme Conjugate	5.5mL*1	11.0mL*1	11.0mL*2	11.0mL*5	5.5mL*2
Sample Diluent	5.5mL*1	11.0mL*1	11.0mL*2	11.0mL*5	5.5mL*2

- Microparticles Solution

Containing of anti-osteocalcin coated microparticles in PBS (phosphate buffered saline) buffer containing BSA. Contains a selection of preservatives.

- Enzyme Conjugate

Contains of horseradish-peroxidase labeled anti-osteocalcin in Tris-NaCl buffer containing BSA (bovine serum albumin). Contains a selection of preservatives.

- Sample Diluent

Contains of Tris-NaCl buffer containing BSA. Contains a selection of preservatives.

Assay Analyzers on which the kit can be used

- AutoLumo A2000 Plus
- AutoLumo A2000 Plus B
- AutoLumo A1000

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzers which are AutoLumo A2000 Plus, AutoLumo A2000 Plus B and AutoLumo A1000.

Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample tube(s) or cup(s) for sample containing
4. Diluent Universal
5. Chemiluminescent Substrate
6. System Buffer for washing the pipetting needles
7. Wash Buffer used in the washing procedure
8. Distilled water or deionized water

Metrological Traceability of Calibrators

The measurand or analyte in the osteocalcin calibrators is traceable to the manufacturer's working calibrators. Traceability process is based on EN ISO 17511. The assigned values were established using representative samples from this lot of calibrator and are specific to the assay methodologies of the reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this instruction for use.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
4. Handle the potentially contaminated materials safely according to local requirement.
5. CAUTION: This assay contains materials of animal origin. Bovine components originate from countries where BSE has not been reported.
6. Some reagents contain ProClin 300® may cause sensitization by skin contact, which must be avoided to contact with skin. This material and its container must be disposed in a safe way. If swallowed, seek medical advice immediately.
7. Do not smoke, drink, eat or use cosmetics in the working area.
8. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
9. Conduct the assay away from bad ambient conditions. e.g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
10. Do not mix or use components from kits with different batch codes.
11. When storing the calibrators, be certain the vials are securely sealed.
12. Ensure the microparticles are re-suspended before loading it on the analyzer.

13. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).
14. Do not substitute any reagent in this kit from other manufacturers or other lots.
15. When any damage to the protective packaging or any change of analytical performance is observed, do not use the kit.
16. Do not use reagents beyond the labeled expiration date.

Storage

1. Store the kit at 2-8 ℃. Do not freeze. Avoid strong light. When stored as directed, all reagents are stable until the expiration date.
2. Refrigerate the reagent pack at 2-10 ℃ for a minimum of 2 hours prior to use.
3. Store the unsealed reagents pack upright on the analyzer or 2-10℃ for a maximum of 28 days. After 28 days, the reagent pack must be discarded. Once they are removed from the analyzer, store them at 2-10 ℃ in an upright position.
4. Seal and return the remaining calibrators at 2-8℃ immediately after the experiment, under which conditions the stability will be retained for 28 days.

Sample

1. Collect serum samples in accordance with correct medical practices.
2. Samples collected in tubes containing EDTA or heparin have no notable interference to this assay, but samples collected in tubes containing sodium citrate may result in a low measuring result.
3. Do not use heat-inactivated samples and samples with obvious microbial contamination. Do not use sodium azide preservative in samples.
4. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
5. Prior to shipment, it is recommended that samples be removed from the clot, serum separator or red blood cells.
6. Insufficient processing of sample or disruption of the sample during transportation may cause depressed results.
7. Avoid grossly hemolytic, lipemic or turbid samples.
8. Cap and store the samples at 18-25 ℃ for no more than 8 hours, for longer use samples should be capped and stored at 2-8 ℃ up to 7 days. Or freeze the samples that need to be stored or transported for more than 7 days at -20 ℃. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortex or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
9. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy in appearance prior to use to ensure consistency in the results.
10. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
11. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
12. For optimal results, inspect all samples for bubbles. Remove bubbles with a tip prior to analysis. Use a new tip for each sample to prevent cross contamination.

Measurement Procedure

1. Check the consumable materials
- Verify adequate volume of consumable materials is present prior to running the test.
- Refer to the Assay Analyzer's operation manual.

2. Load the kit
- Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the analyzer. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
- Analyzer can read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Refer to the Assay Analyzer's operation manual.

3. Order tests
- Place the sample tubes or cups on the sample rack, 10 μL of samples for each test. But consider the sample container and 150 μL of system dead volumes, which can be refer to the appropriate Assay Analyzer manuals for the minimum sample volume required.
- Load the sample rack and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically conduct test operation. The analyzer perform the following functions:
  - Moves the sample to the set point
  - Loads a reaction vessel into the process path
  - Aspirates and transfers sample into the reaction vessel
  - Adds Microparticles Solution, Enzyme Conjugate and Sample Diluent to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine the quantity of osteocalcin in the sample
  - Discards the used reaction vessel
  - Calculates the result
- Refer to the Assay Analyzer's operation manual.

4. Calibrate the curve
- Analyzer can read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the calibrators into the sample tubes and place the sample tubes on the sample rack. Conduct duplicate detection on the system.
- Load the sample rack and input calibrators' information on the system software interface.
- Select "run" to start the test and generate the calibration curve.
- Once a calibration curve is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - Controls are out of range after repeated measurements
  - A reagent kit and substrate solution with new batch code is used
  - Beyond the expiration date of a calibration curve
  - Important parts of the analyzer are replaced or repaired
- Refer to the Assay Analyzer's operation manual.

5. Dilute the sample

Samples with an osteocalcin value exceeding 300 ng/mL may be diluted with manually. Diluent Universal or low concentration analyte serum is used to dilute the samples. After dilution, multiply the result by the dilution factor.

Measurement Results

The sample test results are determined automatically by the system software. The amount of osteocalcin in the samples is determined from the measured light production by means of the stored calibration data. Refer to the Assay Analyzer's operation manual on reviewing sample results.

Control Procedure

Controls for the various concentration ranges should be run individually when the test is in use, once per reagent kit, and following each calibration.
The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.
Follow the applicable government regulations and local guidelines for quality control.

Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulin, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis. This kind of samples is not suitable to be tested by this assay.
4. Performance of this test has not been established with neonatal samples.
5. Patients who have received mouse monoclonal antibodies for either diagnosis or therapy can develop HAMA (human anti-mouse antibodies). HAMA can produce either falsely high or falsely low values in immunoassays which use mouse monoclonal antibodies. Additional information may be required for diagnosis.
6. This test measures concentrations within the range of 3-300 ng/mL. If osteocalcin concentrations above the measuring range to be expected, it is recommended to dilute samples with Diluent Universal or low concentration analyte serum. The recommended dilution is 1:5 of this test, allowing samples to be up approximately 1800 ng/mL.

Biological Reference Interval

A normal range of 10 ng/mL to 46 ng/mL (central 95% interval) was obtained by testing serum samples from 197 individuals defined as normal by clinician. It is recommended that each laboratory establish its own normal range which may be unique to the population it serves depending upon geographical, patient, dietary, or environmental factors.

Performance Characteristics

1. Measurement Precision

3 samples were assayed in duplicate of 2, twice per day across 20 testing days. Data from this study are summarized in the following table.

Panel Member	n	Mean (ng/mL)	Within-run	Total
			%CV	%CV
1	80	32.18	2.22	2.47
2	80	77.12	3.87	3.89
3	80	145.78	6.00	6.04

*Representative data; results in individual laboratories may vary from these data.

2. Analytical Sensitivity

Limit of Blank: 0.5 ng/mL.
Limit of Detection: 1.5ng/mL.
Limit of Quantitation: 3.0ng/mL with a coefficient of variation of 20%.

3. Analytical Specificity

Cross Reaction：The following substances and concentrations were tested and found no cross reaction with the test.

Substances	Concentration (ng/mL)	Measured Value (ng/mL)
PTH	100	≤0.5
CT	100	≤0.5

Interference: No interference with 500 mg/dL of haemoglobin, 50 mg/dL of Bilirubin, 3000 mg/dL of Triglyceride, 322 IU/mL of Rheumatoid factors.

4. Measurement Accuracy by Correlation

A comparison of the Osteocalcin CLIA Microparticles with a commercially available Osteocalcin assay using clinical samples gave the following correlations (ng/mL):

Correlation Method	Number of Samples	Intercept	Slope	Correlation Coefficient
Linear Regression	40	0.000	0.995	0.988

5. High Dose Hook Effect

A sample spiked with Osteocalcin up to 4500 ng/mL was determined, the concentration result obtained was ≥300 ng/mL.

Literature References

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