en

Immunoassay

REF CML0101 / CML0102 / CML0103 / CML0104 / CML0105

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

IGF-1 CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of Insulin-like Growth Factor I (IGF-I) concentration in human serum and plasma (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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Introduction

Insulin-like growth factor 1 (IGF-1), also called somatomedin C, is a protein that in humans is encoded by the IGF 1 gene [1, 2]. IGF-1 consists of 70 amino acids in a single chain with three intramolecular disulfide bridges. IGF-1 has a molecular weight of 7,649 daltons [3]. It plays an important role in childhood growth and continues to have anabolic effects in adults.

IGF-1 is along with growth hormone (GH) [4], helps promote normal bone and tissue growth and development. IGF-1 mirrors growth hormone excesses and deficiencies, but the level in the blood is stable throughout the day, making it a useful indicator of average growth hormone levels [5]. A synthetic analog of IGF-1, mecasermin, is used for the treatment of growth failure [4]. Plasma IGF-I levels are barely detectable at birth, rise gradually during childhood, peak during mid-puberty until approximately 40 years of age, then decline gradually [6].

Decreased levels of IGF-1 may be seen with a GH deficiency or insensitivity to GH. If this is in a child, the GH deficiency may have already caused short stature and delayed development and may be treated with GH supplementation. Adults will have an age-related decrease in production, but lower than expected levels may reflect a GH deficiency or insensitivity [6]. Elevated levels of IGF-1 usually indicate an increased production of GH. Since GH levels vary throughout the day, IGF-1 levels are a reflection of average GH production, not of the actual amount of GH in the blood at the time that the sample for the IGF-1 measurement was taken. This is accurate up to the point at which the liver's capacity to produce IGF-1 is reached. With severely increased GH production, the IGF-1 level will stabilize at an elevated maximum level. Increased levels of GH and IGF-1 are normal during puberty and pregnancy but otherwise are most frequently due to pituitary tumors (usually benign) [6].

Measurement Principle

This assay is based upon the one-step sandwich method. The diluted sample, anti-IGF-1 coated microparticles and enzyme labeled anti-IGF-1 are added to the reaction vessel. During incubation, IGF-1 present in the sample, the microparticles-coated antibodies and enzyme-labeled antibodies are combined together. After washing, a sandwiched complex is generated among the solid phase, the IGF-1 within the sample, enzyme-linked antibodies and microparticles-coated antibodies by immunological reactions. Chemiluminescent Substrate is then added and catalyzed by this complex, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is proportional to the amount of IGF-1 in the samples.

Materials Provided

1. Calibrators

6 vials each containing 1.0mL of Calibrator A through F. The matrix is PBS (phosphate buffered saline) buffer containing Casein. Contains a selection of preservatives.

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	65mL*1	80mL*1	80mL*2	80mL*5	65mL*2

- Microparticles Solution

Contains of mouse monoclonal anti-IGF-1 coated microparticles in PBS (phosphate buffered saline) buffer containing BSA. Contains a selection of preservatives.

- Enzyme Conjugate

Contains of horseradish-peroxidase labeled mouse anti-rabbit antibody in Tris-Nacl buffer containing BSA (bovine serum albumin). Contains a selection of preservatives.

- Sample Diluent

Contains of citric acid buffer.

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 Wash for washing the pipetting needle
7. Wash Buffer used in washing procedure
8. Distilled or deionized water

Metrological Traceability of Calibrators

The measurand or analyte in these IGF-1 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 and wastes 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 2 months.

Sample

1. Collect serum or plasma samples in accordance with correct medical practices (It is recommended to use the serum samples, citrate and EDTA anticoagulants are not recommended).
2. Do not use heat-inactivated samples. Do not use sodium azide preservative in samples.
3. Do not use samples with obvious microbial contamination.
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 24 hours, for longer use samples should be capped and stored at 2-8 ℃ up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20 ℃. Avoid multiple freeze-thaw cycles, it should be no more than 3 times. 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 etc. 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. Avoid foam formation in all reagents. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
- 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 holder, 10 μL of serum or plasma samples are automatically diluted 1:19 with 190 μL of Sample Diluent and mixed well. But considering 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 holder and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically operates tests. It performs the following functions:
  - Moves the sample to the set point
  - Loads a reaction vessel into the process path
  - Aspirates and transfers 25 μL of diluted sample into the reaction vessel
  - Adds Microparticle Solution and Enzyme Conjugate to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine the quantity of IGF-1 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 or cups and place them on the sample holder. Conduct duplicate detection on the system.
- Load the sample holder and input calibrators' information on the system software interface.
- Select "run" to start the test and generate the calibration curve, calibration is required every 28 days.
- 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 Chemiluminescent Substrate 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 IGF-1 value exceeding 1000 ng/mL may be diluted manually. Diluent Universal 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 IGF-1 in the samples is determined from the measured light production by means of the stored calibration data. Sample test results can be reviewed using the appropriate computer or printed out. Refer to the Assay Analyzer's operation manual on reviewing sample results.

The default unit for this assay is ng/mL.

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 immunoglobulins, 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. Because of pulsatile secretion, samples obtained within the same day from the same patient may fluctuate widely within the reference interval, reflecting physiological variation rather than errors in technique or methodology.
5. This assay was designed and validated for use with human serum from individual patient and donor samples. Pooled samples must not be used since the accuracy of their test results has not been validated.
6. This test measures concentrations within the range of 15-1000 ng/mL. If IGF-1 concentrations above the measuring range to be expected, it is recommended to dilute samples with Diluent Universal. The recommended dilution is 1:4 of this test, allowing samples to be up to approximately 5000 ng /mL.

Biological Reference Interval

The suggested normal range (95% confidence interval) was obtained by testing 884 serum samples. The results are presented in the following table. 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.

The normal range for the person aged 1-20:

Age Range	Reference Interval (ng/mL)
1	56 – 344
2	50 – 300
3	49 – 311
4	53 – 307
5	55 – 305
6	54 – 306
7	57 – 312
8	64 – 358
9	73 – 385
10	88 – 463
11	111 – 549
12	143 – 686
13	183 – 859
14	220 – 972
15	235 – 988
16	226 – 938
17	193 – 754
18	163 – 579
19	143 – 486
20	128 – 464

The normal range for the person aged 21-85:

Age Range	Reference Interval (ng/mL)
21-25	138 – 363
26-30	116 – 334
31-35	115 – 320
36-40	111 – 284
41-45	101 – 270
46-50	94 – 266
51-55	87 – 234
56-60	85 – 230
61-65	75 – 223
66-70	69 – 211
71-75	64 – 188
76-80	59 – 181
81-85	49 – 161

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.

Sample	n	Mean (ng/mL)	Within-run	Total
			%CV	%CV
1	80	149.54	4.55	5.02
2	80	359.17	4.99	5.43
3	80	509.64	5.10	5.31

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

2. Analytical Sensitivity

Limit of Blank: 0.79 ng/mL.
Limit of Detection: 2.20 ng/mL.
Limit of Quantitation: 4.67 ng/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
INS	18000 μIU/mL
GH	400 ng/mL
LH	80000 mIU/mL
TSH	310 mIU/mL
IGF-2	5000 ng/mL

Interference: No interference with 40 mg/dL of Bilirubin, 2000 mg/dL of Triglyceride, 37.5 mg/dL of Hemoglobin.

4. Measurement Accuracy by Correlation

A comparison study was performed where samples were tested using this assay and an IGF-1 reference assay. Data were analyzed and are summarized in the following table.

Correlation Method	Number of Samples	Intercept	Slope	Correlation Coefficient
Linear Regression	23	-1.9362	1.0134	0.9891

Literature References

1. Höppener JW, de Pagter-Holthuizen P, Geurts van Kessel AH, Jansen M, Kittur SD, Antonarakis SE, Lips CJ, Sussenbach JS. 1985. The human gene encoding insulin-like growth factor I is located on chromosome 12. Hum. Genet. 69 (2): 157–60.

2. Jansen M, van Schaik FM, Ricker AT, Bullock B, Woods DE, Gabbay KH, Nussbaum AL, Sussenbach JS, Van den Brande JL. 1983. Sequence of cDNA encoding human insulin-like growth factor I precursor. Nature 306 (5943): 609–11.

3. Rinderknecht E, Humbel RE. 1978. The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin. J Biol Chem 253 (8): 2769–2776.

4. Conti E, Carrozza C, Capoluongo E, Volpe M, Crea F, Zuppi C, Andreotti F. 2005. Insulin-like growth factor-1 as a vascular protective factor. Circulation 110 (15): 2260–5.

5. Trojan LA, Kopinski P, Wei MX, Ly A, Glogowska A, Czarny J, Shevelev A, Przewlocki R, Henin D, Trojan J. 2004. IGF-I: from diagnostic to triple-helix gene therapy of solid tumors. Acta Biochim. Pol. 49(4): 979–90.

6. Winn N, Paul A, Musaró A, Rosenthal N. 2003. Insulin-like growth factor isoforms in skeletal muscle aging, regeneration, and disease. Cold Spring Harb. Symp. Quant. Biol. 67: 507–18.