Snibe Diagnostic

CE

REF 130205001M: 100 tests
130605001M: 50 tests

MAGLUMI® C-Peptide (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of C-Peptide in human serum and plasma using the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer (including Maglumi 600, Maglumi 800, Maglumi 1000, Maglumi 1000 Plus, Maglumi 2000, Maglumi 2000 Plus, Maglumi 4000, Maglumi 4000 Plus, MAGLUMI X8, MAGLUMI X3 and MAGLUMI X6) and Biolumi series Integrated System (including Biolumi CX8).

SUMMARY AND EXPLANATION OF THE TEST

The connecting peptide, or C-peptide, is a short 31-amino-acid polypeptide that connects insulin's A-chain to its B-chain in the proinsulin molecule. C-peptide was first described in 1967 in connection with the discovery of the insulin biosynthesis pathway[1]. It facilitates the efficient assembly, folding, and processing of insulin in the endoplasmic reticulum. Equimolar amounts of C-peptide and insulin are then stored in secretory granules of the pancreatic beta cells and both are eventually released to the portal circulation. Initially, the sole interest in C-peptide was as a marker of insulin secretion and has, as such, been of great value in furthering the understanding of the pathophysiology of type 1 and type2 diabetes[2-4].

The assessment of endogenous C-peptide production in diabetes can be useful in classifying the subtype of diabetes. In a patient with young-onset diabetes, persistent C-peptide production may reflect the honeymoon period of a patient with Type 1 diabetes but also enduring C-peptide can be a feature of other types of diabetes including Type 2 diabetes where levels are typically high. There is extensive evidence that C-peptide can be used to differentiate between the classification of Type 1 diabetes and Type 2 diabetes. It has also been suggested that C-peptide, as a measure of beta cell function, can predict the need for insulin treatment in Type 2 diabetes[5]. In practice, this is not used routinely, the decision on treatment protocols being made on clinical grounds, and the plasma glucose profile and glycated hemoglobin results. However, the C-peptide response to glucagon may be used to assess beta cell function in pancreatic/islet cell transplantation[6].

Measuring C-peptide can help to determine how much of their own natural insulin a person is producing as C-peptide is secreted in equimolar amounts to insulin. C-peptide levels are measured instead of insulin levels because C-peptide can assess a person's own insulin secretion even if they receive insulin injections, and because the liver metabolizes a large and variable amount of insulin secreted into the portal vein but does not metabolize C-peptide, meaning blood C-peptide may be a better measure of portal insulin secretion than insulin itself[7,8]. A very low C-peptide confirms Type 1 diabetes and insulin dependence and is associated with high glucose variability, hyperglycaemia and increased complications. The test may be less helpful close to diagnosis, particularly where a patient is overweight and insulin resistant, as levels close to diagnosis in Type 1 diabetes may be high and overlap with those seen in type 2 diabetes[9].

PRINCIPLE OF THE TEST

The C-Peptide assay is a sandwich chemiluminescence immunoassay.

The sample (or calibrator/control, if applicable), magnetic microbeads coated with anti-C-Peptide monoclonal antibody and ABEI labeled with anti-C-Peptide monoclonal antibody are mixed thoroughly and incubated, formed sandwich of immuno-complexes. After precipitation in a magnetic field, the supernatant is decanted and then a wash cycle is performed. Subsequently, the Starter 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLUs), which is proportional to the concentration of C-Peptide present in the sample (or calibrator/control, if applicable).

KIT COMPONENTS

Material Provided

Components	Contents	100 tests (REF:130205001M)	50 tests (REF:130605001M)
Magnetic Microbeads	Magnetic microbeads coated with anti-C-Peptide monoclonal antibody, containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator Low	C-Peptide antigen, containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator High	C-Peptide antigen, containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
ABEI Label	Anti-C-Peptide monoclonal antibody labeled ABEI, containing BSA, NaN_3 (<0.1%)	7.5 mL	4.0 mL
Internal Quality Control	C-Peptide antigen, containing BSA, NaN_3 (<0.1%).	2.0 mL	2.0 mL
All reagents are provided ready-to-use.			

Accessories Required But Not Provided

MAGLUMI and Biolumi Series:

Reaction Module	REF: 630003
Starter 1+2	REF: 130299004M, 130299027M
Wash Concentrate	REF: 130299005M
Light Check	REF: 130299006M
Reaction Cup	REF: 130105000101

Please order accessories from Shenzhen New Industries Biomedical Engineering Co., Ltd. (SNIBE) or our authorized representatives.

CALIBRATION

Traceability: This method has been standardized against the WHO 1st International Reference Reagent 84/510.

Test of assay specific calibrators allows the RLU values to adjust the assigned master curve. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve(10 calibrations) provided via the reagent Radio Frequency Identification (RFID) CHIP.

Recalibration is recommended if any of the following conditions occurs:

- After each change of lots (Reagent or Starter 1+2).

- Every week and/or each time a new reagent kit is used (recommended).
- After instrument service is required.
- If controls lie outside the expected range.

QUALITY CONTROL

Follow government regulations or accreditation requirements for quality control frequency.
Internal quality control is only applicable with MAGLUMI and Biolumi systems. For instructions for use and target value refer to ***C-Peptide (CLIA) Quality Control Information***. User needs to judge results with their own standards and knowledge.
For detailed information about entering quality control values, refer to the corresponding Analyzer Operating Instructions.
To monitor system performance and chart trends, commercially available quality control materials are required. Treat all quality control samples the same as patient samples. A satisfactory level of performance is achieved when analyte values obtained are within the acceptable Control Range for the system or within your range, as determined by an appropriate internal laboratory quality control scheme. If the quality control results do not fall within the Expected Values or within the laboratory's established values, do not report results. Take the following actions:

- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the instructions for use.
- Rerun the assay with fresh quality control samples.
- If necessary, contact your local technical supporters or distributors for assistance.

SPECIMEN COLLECTION AND PREPARATION

- Sample material: serum and plasma may be used (including serum collected in serum separator tubes). The anticoagulants EDTA-2K, and sodium heparin have been tested and may be used with this assay. Other types of blood collection tube have not been verified. Collect blood aseptically following the universal precautions for venipuncture.
- Ensure that complete clot formation in specimens has taken place prior to centrifugation. Some serum specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time.
- If the specimen is centrifuged before a complete clotting, the presence of fibrin may cause erroneous results. Serum and plasma must be free of fibrin and other particulate matter.
- Do not use hemolyzed or grossly lipemic specimens as well as specimens containing particulate substance or exhibiting obvious microbial contamination. Inspect all specimens for bubbles, and remove bubbles before analysis for optimal results.
- Avoid repeated freezing and thawing. The sample can be frozen and thawed for only two times. Stored samples should be thoroughly mixed prior to use (Vortex mixer). Frozen specimens must be mixed THOROUGHLY after thawing by LOW speed vortexing. Please ask local representative of SNIBE for more derails if you have any doubt.
- Centrifuged specimens with a lipid layer on the top must be transferred to a sample cup or a secondary tube. Care should be taken to transfer only the clarified specimen without the lipemic material.
- All samples (Patient specimens or controls) should be tested within 3 hours when placed on board the MAGLUMI and Biolumi Systems. Refer to the SNIBE service for more detailed discussion of onboard sample storage constraints.
- Specimens removed from the separator, red blood cells or clot may be stored up to 6 hours at 2-8°C, and stored up to 30 days frozen at -20°C or colder.
- Before shipping specimens, it is recommended that specimens be removed from the clot, red blood cells, or separator. When shipped, specimens should be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances. Specimens should be shipped frozen.
- The sample volume required for a single determination of C-Peptide is 20 µL.

WARNING AND PRECAUTIONS FOR USERS

- **IVD**
- For *In Vitro* Diagnostic Use.
- Follow the package insert carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

- **CAUTION**: This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the 29 CFR 1910.1030 Occupational exposure to bloodborne pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- All samples, biological reagents and materials used in the assay should be considered potentially able to transmit infectious agents. They should therefore be disposed in accordance with the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.
- This product contains Sodium Azide. Dispose of contents and containers must be in accordance with all local, regional and national regulations.
- Refer to safety data sheets which are available on request.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not interchange reagent components from different reagents or lots.
- Prior to loading the reagent kit on the system for the first time, the reagent kit requires mixing to re-suspend magnetic microbeads that have settled during shipment.
- For magnetic microbeads mixing instructions, refer to the Preparation of the Reagent section of this package insert.
- To avoid contamination, wear clean gloves when operating with a reagent kit and samples.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts which have no effect on assay efficacy.
- For detailed discussion of handling precautions during system operation, refer to the SNIBE service information.

STORAGE AND STABILITY

- Sealed: Stored at 2-8°C until the expiration date.
- Opened at 2-8°C: Minimum stability is 4 weeks.
- On-board: Minimum stability is 4 weeks.
- To ensure the best kit performance, it is recommended to place opened kits in the refrigerator after the end of the intraday test work. It is still possible to keep on using the kit beyond the opened or on-board period if the controls are found within the expected ranges.
- Keep upright for storage to facilitate later proper resuspension of magnetic microbeads.
- Keep away from sunlight.

TEST PROCEDURE

Preparation of the Reagent

- Resuspension of the magnetic microbeads takes place automatically when the kit is loaded successfully, ensuring the magnetic microbeads

are totally resuspended homogenous prior to use.
- To ensure proper test performance, strictly adhere to the corresponding Analyzer Operating Instructions. Each test parameter is identified via a RFID CHIP on the Reagent kit. For further information please refer to the corresponding Analyzer Operating Instructions.

DILUTION

Sample dilution by analyzer is not available in this reagent kit.
Samples with concentrations above the measuring range can be diluted manually. After manual dilution, multiply the result by the dilution factor. Please choose applicable diluents or ask SNIBE for advice before manual dilution.

High-Dose Hook

No high-dose hook effect was seen for C-Peptide concentrations up to 200 ng/mL.

LIMITATIONS

- A skillful operation and strict adherence to the instructions are necessary to obtain reliable results. Procedural directions must be followed exactly and careful operation must be used to obtain valid results. Any modification of the procedure is likely to alter the results.
- For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or received immunotherapy may contain human anti-mouse antibodies (HAMA), which may result in falsely elevated or decreased values. Moreover, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples as well. Additional clinical or diagnostic information may be required to determine patient status.
- Patients with renal malfunction show elevated C-peptide values.
- Food intake or therapy with β-cell stimulating drugs (e.g. corticosteroids) increase C-peptide secretion.
- Fasting as well as β-cell inhibiting substances such as insulin or α-sympathomimetic drugs decrease C-peptide levels. Patients with untreated. Addison's disease show subnormal C-peptide concentrations.
- For diagnostic purposes, the results should always be assessed and verified in conjunction with the patient's medical history, clinical examination and other findings.

RESULTS

Calculation of Results

The analyzer automatically calculates the C-Peptide concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in ng/mL. For further information please refer to the corresponding Analyzer Operating Instructions.

Interpretation of Results

The expected range for the C-Peptide assay was obtained by testing 154 apparently healthy individuals in China, and gave the following expected value:
0.929-3.73 ng/mL (before meal) (2.5^{th}-97.5^{th} percentiles)
Results may differ between laboratories due to variations in population and test method. It is recommended that each laboratory should establish its own expected ranges.

PERFORMANCE CHARACTERISTICS

Precision

Precision for the C-Peptide assay was determined as described in the CLSI EP5-A2. 3 human serum pools and 2 controls containing different concentration of analyte were assayed in duplicate at two independent runs per day for 20 testing days. The results are summarized in the following table:

Sample	Mean(ng/mL) (N=80)	Within-Run		Between-Run		Total	
		SD(ng/mL)	%CV	SD(ng/mL)	%CV	SD(ng/mL)	%CV
Serum Pool 1	0.312	0.023	7.37	0.018	5.77	0.029	9.29
Serum Pool 2	4.153	0.156	3.76	0.224	5.39	0.273	6.57
Serum Pool 3	15.358	0.349	2.27	0.606	3.95	0.699	4.55
Control 1	3.331	0.141	4.23	0.178	5.34	0.227	6.81
Control 2	9.284	0.286	3.08	0.290	3.12	0.408	4.39

Limit of Blank (LoB)

The LoB for the C-Peptide assay is 0.01 ng/mL.

Limit of Detection (LoD)

The LoD for the C-Peptide assay is 0.02 ng/mL.

Measuring Range

0.01-20 ng/mL (defined by the limit of blank and the maximum of the master curve). Values below the limit of blank are reported as <0.01 ng/mL. Values above the measuring range are reported as >20 ng/mL.

Linearity

The assay is linear between 0.02 ng/mL to 20 ng/mL. Nine equally distributed levels of samples were prepared by blending a serum sample containing C-Peptide 22 ng/mL with a serum sample depleted of C-Peptide (0.0 ng/mL). The Mean sample recovery ranged from 90% to 110%.

Method Comparison

A total of 100 samples in the range of 0.02 and 18.52 ng/mL were tested by the C-Peptide assay (y) and a commercially available immunoassay (x). The data from the resulting linear regressions are summarized as: $y = 0.964x+0.067$, $r^2 = 0.986$.

Analytical Specificity

The specificity of the assay was obtained by adding Insulin (2000 µIU/mL) and Human Growth Hormone (20.0 µg/mL) to serum samples at the indicated concentrations. No interference was found.

Endogenous Interference

Substances up to the following concentrations did not interfere with the assay:
- Bilirubin 50 mg/dL
- Triglyceride 300 mg/dL

- Hemoglobin 2000 mg/dL
- Total Protein 12 g/dL

REFERENCES

1. Steiner D.F.; Cunningham D.; Spigelman L.; Aten B. (1967). "Insulin Biosynthesis: Evidence for a Precursor". Science. 157 (3789): 697–700.
2. Fiedler H. Fundamentals in Laboratory Medicine: Diabetes mellitus and Metabolic Syndrom. Brochure Roche Diagnostics 2001; English Cat. No. 1951777, German Best.-Nr. 1951769.
3. Törn C. C-peptide and Autoimmune Markers in Diabetes. Clin Lab 2003;49:1-10.
4. Meier CH, Ladewig A, Keller U, et al. Clinical Value of the C-Peptide Measurement. Schweiz Rundsch Med Prax 1997;86(34):1289-1295.
5. Hohberg, C., Pfutzner, A., Forst, T., et al. Successful switch from insulin therapy to treatment with pioglitazone in type 2 diabetes patients with residual betacell function: results from the PioSwitch study. Diabetes Obes. Metab. 11, 464–471 (2009).
6. Thomas L. Chapter 3.7: Insulin, C-peptide, proinsulin. In: Thomas L (ed.) Clinical Laboratory Diagnostics, TH-Books, Frankfurt, 1st English edition 1998:149-155, deutsche Auflage 1998:152-158.
7. Clark PM (1999). "Assays for insulin, proinsulin and C-peptide". Ann Clin Biochem. 36: 541–564.
8. Shapiro ET, Tillil H, Rubenstein AH, Polonsky KS (Nov 1988). "Peripheral insulin parallels changes in insulin secretion more closely than C-peptide after bolus intravenous glucose administration". J Clin Endocrinol Metab. 67 (5): 1094–9.
9. Shapiro ET, Tillil H, Rubenstein AH, Polonsky KS (Nov 1988). "Peripheral insulin parallels changes in insulin secretion more closely than C-peptide after bolus intravenous glucose administration". J Clin Endocrinol Metab. 67 (5): 1094–9.

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SYMBOLS EXPLANATIONS

	Consult instructions for use		Manufacturer
2°C 8°C	Temperature limit (Store at 2-8 °C)		Use-by date
Σ	Contains sufficient for		Keep away from sunlight
	This way up	EC REP	Authorized representative in the European Community
IVD	*In vitro* diagnostic medical device	CONTENTS	Kit components
REF	Catalogue number	LOT	Batch code