For in vitro diagnostic use only.

C-peptide (CP) Assay Reagent Kit (CMIA) Package Insert

INTENDED USE

The C-peptide(CP) Assay Reagent Kit(CMIA) is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of C- peptide (CP) in human serum or plasma.

PACKING SIZE

24 Device/Kit,30Device/Kit,48 Device/Kit,60 Device/Kit.

SUMMARY

C-peptide is a single chain 31-amino acid (AA 33-63) connecting (C) polypeptide with a molecular weight of approximately 3021 daltons.
In the process of biosynthesis of insulin the C-peptide is formed as a by-product together with insulin by the proteolytic cleavage of the precursor molecule proinsulin, stored in secretory granules in the Golgi complex of the pancreatic β-cells. Proinsulin in turn was cleaved from preproinsulin.
C-peptide fulfills an important function in the assembly of the two-chain insulin (A- and B-chain) structure and the formation of the two disulfide bonds within the proinsulin molecule. Insulin and C-peptide are secreted in equimolar amounts and released into circulation via the portal vein. As half of the insulin, but almost none of the C-peptide is extracted in the liver, C-peptide has a longer half-life (about 35 minutes) than insulin; 5 to 10 times higher concentration of C-peptide persist in the peripheral circulation, and these levels fluctuate less than insulin.
The liver does not extract C-peptide, which is removed from the circulation by the kidneys and degraded, with a fraction excreted unchanged in the urine. The concentration in urine is about 20-50 fold higher than in serum. C-peptide concentrations are therefore elevated in renal disease.
In the past, C-Peptide has been considered biologically inactive. However, recent studies have demonstrated that it is capable of eliciting molecular and physiological effects suggesting that C-peptide is in fact a bioactive peptide. There is evidence that C-peptide replacement, together with insulin administration, may prevent the development or retard the progression of long-term complications in type 1 diabetes.
Measurements of C-peptide, insulin and glucose are used as an aid in the differential diagnosis of hypoglycemia (factitious hypoglycemia and hypoglycemia caused by hyperinsulinism) to ensure an appropriate management and therapy of the patients. To quantify the endogenous insulin secretion, C-peptide is measured basally, after fasting and after stimulation and suppression tests. Due to high prevalence of endogenous anti-insulin antibodies C-peptide concentrations reflect the endogenous pancreatic insulin secretion more reliably in insulin-treated diabetics than the levels of insulin itself. Measurements of C-peptide may therefore be an aid in the assessment of a residual β-cell function in the early stages of type-1 diabetes mellitus and for the differential diagnosis of latent autoimmune diabetes of adults (LADA) and type-2 diabetes.
C-peptide measurements are also used to assess the success of islet transplantation and for monitoring after pancreatectomy.
Although testing for C-peptide is not requested for the routine monitoring of diabetes, it is a valuable tool for the individual therapeutic decisions which are essential for an optimal long-term metabolic control.
Elevated C-peptide levels may result from increased β-cell activity observed in hyperinsulinism, from renal insufficiency, and obesity.
Correlation was also found between higher C-peptide levels and increasing hyperlipo protein aemia and hypertension.
Decreased C-peptide levels are observed in: starvation, factitious hypoglycemia, hypoinsulinism (NIDDM, IDDM), Addison's disease and after radical pancreatectomy.

PRINCIPLE OF TEST

The CP assay is a two-step immunoassay for the quantitative measurement of CP in human serum or plasma using CMIA technology, with flexible assay protocols.
In the first step, sample and anti-CP coated paramagnetic microparticles are combined. CP present in the sample binds to the anti-CP coated microparticles. After that, ALP-labeled anti-CP conjugate is added to create a reaction mixture in the second step. Following the wash cycle, substrate is added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of CP in the sample and the RLUs detected by the System optics.

REAGENTS

The device is pre-dispensed with buffer needed for single use.
The device is constituted with Buffers described below is the main reagent

Object	Content
Micro-particles Buffer	Anti-CP (mouse, monoclonal) coated Micro-particles in TRIS buffer with protein (bovine) stabilizer. Minimum concentration: 0.1% solid. Preservative: ProClin-300.
Conjugate Buffer	Anti-CP (mouse, monoclonal) alkaline phosphatase (ALP) labeled conjugate in TRIS buffer with protein (bovine) stabilizer. Preservative: ProClin-300.
Wash Buffer	TRIS buffer with surfactant. Preservative: ProClin-300.
Substrate Buffer	AMPPD,Enhancer,Surfactant,ProClin-300.

Reagent Handing
The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.
All information required for correct operation is read in from the respective reagent barcodes.

MATERIALS PROVIDED

·CP Test Device
·Product Insert
·Calibration Solution(optional)
·Control Solution (optional)

MATERIALS REQUIRED BUT NOT PROVIDED

·Analyzer

STORAGE AND STABILITY

▪Store at 2-8℃.
▪Do not freeze.
▪Store the reagent kit upright prior to use.
▪Expiration date: up to the stated expiration date.
Note: The CP Reagent Kit must be stored at 2-8℃ in an upright position and must be used immediately after removal from 2-8℃ storage or the device was opened.

SPECIMEN COLLECTION AND STORAGE

Specimen Types
Validated specimen types to be used with this assay:

Specimen Types	Collection Tubes
Human serum	Serum Serum separator tubes
Human plasma	Sodium heparin Lithium heparin Potassium EDTA Sodium EDTA

Other anticoagulants have not been validated for use with this assay.
The instrument does not provide the capability to verily specimen type. It is the responsibility of the operator to verify that the correct specimen types are used in the assay.
Specimen Conditions
- Do not use specimens with the following conditions:
  heat-inactivated
  pooled
  grossly hemolyzed
  obvious microbial contamination
- For optimal results, serum and plasma specimens should be free of fibrin, red blood cells or other particulate matter.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens especial those from patients receiving anticoagulant or thrombolytic therapy may exhibit increased clotting time. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis
- Follow the tube manufacturer's processing instructions for specimen collection tubes.
- Specimens must be mixed THOROUGHLY after thawing, by LOW speed vortex, and centrifuged prior to use to remove red blood cells or particulate matter to ensure consistency in the results.
- Inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

Specimen Storage

Specimen Type	Storage Temperature	Maximum Storage Time
Serum/Plasma	2-8℃	2 days

- If testing is delayed more than 24 hours, remove serum or plasma from the clot, serum separator or red blood cells.
- If testing is delayed more than 2 days, specimens should be frozen at -10℃ or colder.
- Specimens stored frozen at -10℃ or colder for 1 month showed no performance difference.
- Avoid more than 2 freeze/thaw cycles.

Specimen Shipping
- Before shipping specimens, it is recommended that specimens be removed from the clot, red blood cells, or separator gel.
- When shipping specimens, package and label specimens in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances.
- Specimens may be shipped ambient, at 2-8℃ (wet ice), or frozen (dry ice). Do not exceed the storage time limitations listed above.

INSTRUMENT

The CP Test Device is designed for use on the REALY Analyzer System.

TEST PROCEDURE

Assay Procedure
For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions. Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the digit sequence of numbers. Bring the cooled reagents to approx. 20℃ and place on the reagent disk of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening of the device.
For this test device, the transfer volume of specimens, calibrators or controls into the sample hole is 60 µL. (No less than 60 µL.)
Reagent strips should be left at room temperature between 20 and 25 ℃ for more than 30 minutes before use and kept away from light.
In order to avoid the magnetic beads adsorbed on the side wall and top due to the upside down and side placement of the reagent strip during transportation, the reagent strip should be mixed by shaking and mixing before use. The reagent strip should be mixed upside down for about 30 seconds, and then the reagent strip should be mixed upward for about 30 seconds.The reagent strip was then gently shaken so that the magnetic beads fell completely to the bottom of the strip.
Calibration
Every Test Device has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.
Calibration frequency: Calibration must be performed before using a new lot of device. Renewed calibration is recommended as follows:
▪After 90 days (when using the same reagent lot on the analyzer);
▪ As required: e.g. quality control findings outside the defined limits.
Note: Refer to Instruction of Analyzer for the procedure of calibration.
Quality Control
For quality control, please use Control of REALY or Control Universal.
In addition, other suitable control material can be used. Controls for the various concentration ranges should be run individually at least once every 24 hours 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.

Specimen Dilution Procedures

Specimens with a CP concentration greater than 40ng/mL will be flagged as ">40 ng/mL" and may be diluted using Manual Dilution Procedure. Use the 1:10 dilution is recommended. The operator must enter the dilution factor in the Patient or Control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution.

EXPECTED VALUES

Normal reference value: 1.1-4.4ng/mL.

Conversion factors: ng/mL (μg/L) × 0.33333 = nmol/L
ng/mL × 333.33 = pmol/L
nmol/L × 3.0 = ng/mL
pmol/L× 0.003 = ng/mL

The normal reference value were performed using serum samples from apparently healthy fasting males and females.

Advice each laboratory set up your own normal reference range.

INTERPRETATION OF RESULTS

As interpret the results, the patient's overall clinical situation, including symptoms, medical history and other related data, should be referred to.

LIMITATIONS

- If the CP results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Specimens containing HAMA may produce anomalous values when tested with assay kits such as REALY CP Assay that employ mouse monoclonal antibodies.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous results may be observed. Additional information may be required for diagnosis.
- Although the REALY CP Assay is specifically designed to minimize the effects of HAMA and heterophilic antibodies, assay results that are not consistent with other clinical observations may require additional information for diagnosis.

PERFORMANCE CHARACTERISTICS

Linearity

The linearity of CP Reagent Kit was determined by using CP calibrator to prepare 6 different specimens, measuring all these specimens follow the test instruction and then do linear fitting, the results show that the linear correlation coefficient (r) was not less than 0.9900.

Precision / Reproducibility

Intra-assay coefficient of variation was evaluated on 3 different levels of control serum. Repeatedly measured 20 times, calculating the coefficient of variation.

Intra-assay Precision			
Control	Mean (ng/mL)	SD	CV
Level 1	2.38	0.085	3.57%
Level 2	10.69	0.253	2.37%
Level 3	24.21	0.627	2.59%

Inter-assay coefficient of variation was evaluated on three batches of kits. Repeatedly measured 3 different levels of control serum 30 times, calculating the coefficient of variation.

Inter-assay Precision			
Control	Mean (ng/mL)	SD	CV
Level 1	2.41	0.101	4.19%
Level 2	10.43	0.316	3.03%
Level 3	24.51	0.987	4.03%

Analytical Sensitivity

The analytical sensitivity is defined as the concentration of CP equivalent to the mean RLU of 20 replicates of the zero standard plus two standard deviations corresponding to the concentration from the standard curve. The analytical sensitivity is typically less than 0.1ng/mL.

Analytical Specificity

The specificity of the CP assay system was assessed by measuring the apparent response of the assay to various potentially cross-reactive analytes.

Compound	Concentration	Cross-reactivity
Proinsulin, human	0.10μg/mL	32.5%
Insulin, human	8.66μg/mL	0.005%
Insulin, porcine	7.50μg/mL	ND
Insulin, bovine	7.69μg/mL	ND
Somatomedin	1μg/mL	ND
hGH	10μg/mL	ND
Glucagon	10μg/mL	ND

ND: not detectable.

Interference

The following compounds in both low-level specimen and high-level specimen show no cross-reactivity when tested with the CP Assay Reagent Kit at a concentration below:

Compound	Concentration
Hemoglobin	300 mg/dL
Bilirubin	50 mg/dL
Triglycerides	2000 mg/dL

Method Comparison

The comparison between the CP Assay Reagent Kit (y) and a commercially available CP test kit (x), using clinical samples gave the following correlations (ng/mL):

Linear regression
y=1.0507x - 0.0937
r=0.9855
Number of samples measured: 161
The sample concentrations were between about 0.27 - 36.89 ng/mL.

WARNINGS AND PRECAUTIONS

- For *In Vitro* Diagnostic Use.
- Do not use expired or clearly damaged kits.
- Operating according to the steps described, can make the risk of daily handling patients` samples and blood products into a minimum, however, no matter what the source of the products, handling mode or the previous proof, these potentially infectious substances used shall be in accordance with the unified considerations and Good Laboratory Practice (GLP).
- Proper disinfectant should be used to eliminate pollution.
- Follow local rules and regulations to keep and dispose of these items and containers for these items.
- The ProClin-300 is a potential skin sensitizer. Avoid dumping or splashing this reagent on your skin and clothing. In case of contact with this reagent, wash thoroughly with soap and water.
- Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

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SYMBOLS

Symbol	Meaning	Symbol	Meaning
IVD	In vitro diagnostic medical device		Storage temperature limit
	Manufacturer	EC REP	Authorized representative in the European Community
	Date of Manufacture		Use by date
	Do not reuse		Consult instruction foe use
LOT	Batch code	CE	Meet the requirements of EC Directive 98/79/EC

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