C-PEPTIDE

ENZYME IMMUNOASSAY TEST KIT
Catalog Number: 10802

Enzyme Immunoassay for the Quantitative Determination of Circulating C-Peptide Concentrations in Human Serum (96 tests)

INTENDED USE

The Quantitative Determination of Circulating C-Peptide Concentrations in Human Serum.

SUMMARY AND EXPLANATION OF THE TEST

Diabetes is one of the leading causes of disability and death in the U.S. It affects an estimated 16 million Americans, about one third of them do not even know they have the disease. The causes of diabetes are not precisely known, but both genetic and environmental factors play a significant role. The disease is marked by deficiencies in the body's ability to produce and properly use insulin. The most common forms of diabetes are type 1, in which the body's ability to produce insulin is destroyed, and type 2, in which the body is resistant to insulin even though some amount of insulin may be produced.

In-vitro determination of insulin and C-Peptide levels help in the differential diagnosis of liver disease, acromegaly, Cushing's syndrome, familial glucose intolerance, insulinoma, renal failure, ingestion of accidental oral hypoglycemic drugs or insulin induced factitious hypoglycemia. Both insulin and C-Peptide are produced by enzymatic cleavage of proinsulin. Proinsulin is stored in the secretory granules of pancreatic -cells and is split into a 31 amino acid connecting peptide (C-Peptide; MW 3600) and insulin (MW 6000). C-Peptide is devoid of any biological activity but appears to be necessary to maintain the structural integrity of insulin. Although insulin and C-Peptide are secreted into portal circulation in equimolar concentrations, fasting levels of C-Peptide are 5-10 fold higher than those of insulin owing to the longer half-life of C-Peptide. The liver does not extract C-Peptide however; it is removed from the circulation by degradation in the kidneys with a fraction passing out unchanged in urine. Hence urine C-Peptide levels correlate well with fasting C-Peptide levels in serum. The glucagon stimulated C-Peptide determination is often used for differential diagnosis of insulin-dependent from non-insulin-dependent diabetic patients.

TEST PRINCIPLE

The C-Peptide Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-C-Peptide antibody for solid phase (microtiter wells) immobilization and another anti-C-Peptide antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The standards and test specimen (serum) are added to the C-Peptide antibody coated microtiter wells. Then anti-C-Peptide antibody labeled with horseradish peroxidase (conjugate) is added. If human C-Peptide is present in the specimen, it will combine with the antibody on the well and the enzyme conjugate resulting in the C-Peptide molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 1 hour incubation at room temperature, the wells are washed to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution. The color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of C-Peptide is directly proportional to the color intensity of the test sample.

PRECAUTIONS

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface antigen, HIV 1&2 and HCV antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS.

SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain red-top venipuncture tube without additives. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

C-Peptide is not stable in serum basis. Samples should be used as fresh as possible. Refrigerated at 2-8°C for a maximum period of one day only. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100mL of the specimen is required.

MATERIALS AND COMPONENTS

Materials provided with the test kits:

- Monoclonal anti C-Peptide antibody coated microtiter plate with 96 wells.
- Enzyme conjugate reagent, 12 mL.
- C-Peptide reference standards containing; 0, 0.5, 1.0, 2.0, 5.0, and 10.0 ng/mL, in liquid form (ready to use) or Lyophilized form. **DO NOT FREEZE and THAW**
- Wash Buffer Concentrate, 50X, 15 mL
- TMB Substrate, 12 mL.
- Stop Solution , 12mL.

The standard calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 1st IRR 84/510.

Materials required but not provided:

- Precision pipettes and tips, 0.04~0.2 mL, 1.0 mL,
- Disposable pipette tips.
- Distilled water.
- Vortex mixer.
- Absorbent paper or paper towel.
- Microtiter plate reader.
- Graph paper.
- Microplate Reader with 450nm and 620nm wavelength absorbance capability (The 620nm filter is optional)
- Dispenser(s) for repetitive deliveries of 0.100mL and 0.300mL volumes with a precision of better than 1.5% (optional).
- Adjustable volume (200-1000µL) repeat dispenser.
- Container(s) for mixing of reagents (see below).
- Timer.
- Quality Control Materials.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to Use. Do not induce foaming.
2. Prepare 1x washing buffer. Prepare washing buffer by adding distilled or deionized water to 50x wash concentrate to a final volume of 750mL.
3. If reference standards are lyophilized, reconstitute each standard with 0.5mL distilled water. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted Standards should be sealed and stored at 2-8°C.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense **50**µL of C-Peptide standards, specimens, and controls into the appropriate wells. Gently but thoroughly mix for 10 seconds.
2. Dispense **100**µL of enzyme conjugate reagent into each well. Mix gently for 30 seconds. It is very important to have complete mixing in this step. Incubate at room temperature for 60 minutes.
3. Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 4times with 1 x washing buffer and one times distilled water. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water Droplets.
4. Dispense **100**µL of TMB substrate reagent into each well. Gently mix for 10 seconds. Incubate at room temperature, in the dark, for 20 minutes.
5. Stop the reaction by adding **100**µL of Stop Solution to each well. Gently mix for 10 seconds until the blue color completely changes to yellow.
6. Read the optical density at 450nm with a microtiter plate Reader within 15 minutes.

IMPORTANT NOTE

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

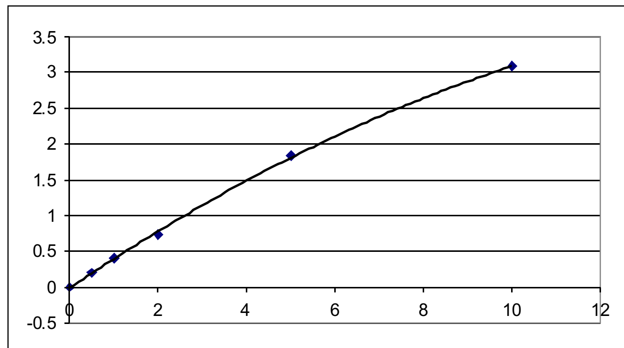
CALCULATION OF RESULTS

Calculate the mean absorbance value for each set of C-peptide reference standards, specimens and controls. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in units per mL on linear graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of C-peptide in units per mL from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density reading at 450nm shown in the Y axis against C-peptide concentrations shown in the X axis.

C-peptide Values (ng/mL)	Absorbance (450nm)
0	0.00
0.5	0.20
1	0.41
2	0.73
5	1.84
10	3.08



LIMITATIONS OF PROCEDURE

1. It is important that the time of reaction in each well is held constant for reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
4. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction.
5. Plate readers measure vertically. Do not touch the bottom of the wells.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Highly lipemeic, hemolysed or grossly contaminated specimen(s) should not be used.
8. Patient samples with C-Peptide concentrations above 10 Ng/mL may be diluted with the zero calibrator and re-assayed. Multiply the value obtained by the dilution factor to obtain the corrected value.
9. Use components from the same lot. No intermixing of Reagents from different batches.

EXPECTED VALUES

C-Peptide values are consistently higher in plasma than in serum; thus, serum is preferred. Compared with fasting values in non-obese non-diabetic individuals, C-Peptide levels are higher in obese non-diabetic subjects and lower in trained athletes.
Each laboratory is advised to establish its own ranges for normal and abnormal populations. These ranges are always dependent upon locale, population, laboratory, technique and specificity of the method.
Based on the clinical data gathered by Diagnostic Automation, Inc. in concordance with the published literature the following ranges have been assigned.

These ranges should be used as guidelines only:
Adult (Normal) 0.7-1.9 ng/mL

SENSITIVITY

The sensitivity (detection limit) was ascertained by determining the variability of the 0 ng/mL serum calibrator and using the 2SD (95% certainty) statistic to calculate the minimum dose. The assay sensitivity was found to be 0.03 ng/mL.

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7/2014

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