CE

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IVD	See external label	2°C-8°C	96 tests	REF 1293-15

Enzyme Immunoassay

C-Peptide

Cat # 1293-15

Test	**C-Peptide ELISA**
Method	**Enzyme Linked Immunosorbent Assay**
Principle	**ELISA: Solid phase enzyme linked immunosorbent assay**
Detection Range	**0-10 ng/mL**
Sample	**25 µl serum**
Specificity	**100%**
Sensitivity	**0.020ng/ml**
Total Time	**~ 135 min**
Shelf Life	**12-14 Months from the manufacturing date**

***** *Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account.*

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 hypoglycemic. Both insulin and C-Peptide are produced by enzymatic cleavage of proinsulin. Proinsulin is stored in the secretory granules of pancreatic β -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.

PRINCIPLE

Immunoenzymometric assay (TYPE 3):

The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (Ab). (enzume conjugated and immobilized), with different and distinct epitope recognition, in excess, and native antigen (Ag). In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal C-peptide antibody.

Upon mixing monoclonal biotinylated antibody, the enzymelabeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab_{(M)} + Ag_{C\text{-}PEP} + {}^{Btn}Ab_{(M)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab_{(M)} - Ag_{C\text{-}PEP} - BtnAb_{(M)}$$

$^{Btn}Ab_{(M)}$ = Biotinylated Monoclonal Ab (Excess Quantity)
$Ag_{C\text{-}PEP}$ = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(M)}$. = Enzyme labeled Monoclonal Ab (Excess Quantity)
$^{Enz}Ab\ (M) - Ag_{C\text{-}PEP}$ = Antigen-Antibodies complex

Ka = Rate Constant of Association
k-a = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:

$^{Enz}Ab_{(M)} - Ag_{C\text{-}PEP} - {}^{Btn}Ab_{(M)} +$ **Streptavidin** $_{c.w} \Rightarrow$ **Immobilized complex**

Streptavidin $_{c.w}$= Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the solid surface

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native free antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

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.

Samples may be refrigerated at 2-8℃ for a maximum period of two (2) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20℃ for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml of the specimen is required.

REAGENTS AND MATERIALS PROVIDED

A. C-Peptide Calibrators – 2.0 ml/vial (Dried) – Icons A – F
Six (6) vials of references for C-Peptide antigen at levels of 0(**A**), 0.2(**B**), 1.0(**C**), 2.0(**D**), 5.0(**E**), and 10.0(**F**) ng/ml. Reconstitute each vial with 2.0ml of distilled or deionized water.
The reconstituted calibrators are stable for 7 days at 2-8℃. In order to store for a longer period of time aliquot the reconstituted calibrators in cryo vials and store at -20℃. **DO NOT FREEZE THAW MORE THAN ONCE**. A preservative has been added.
Note: The calibrator, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 1st IRR 84/510.

B. C-Peptide –Enzyme Reagent - 13ml/vial
One (1) vial containing enzyme labeled affinity purified monoclonal mouse antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8℃.

C. Streptavidin Plate -- 96 wells
One 96-well microplate coated with sheep anti-triiodothyronine serum and packaged in an aluminum bag with a drying agent. Store at 2-8℃.

D. Wash Solution Concentrate -- 20ml
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8℃.

E. Substrate A –7.0 ml/vial
One (1) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8℃.

F. Substrate B -- 7.0ml/vial
One (1) bottle containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8℃.

G. Stop Solution – 8ml/vial
One (1) bottle containing a strong acid (1.0 N HCl) Store at 2-30℃

H. Product Instructions.

REQUIRED BUT NOT PROVIDED

1. Pipette(s) capable of delivering 50µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.350ml volumes with a precision of better than 1.5% (optional).
3. Microplate washer or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Storage container for storage of wash buffer.
10. Distilled or deionized water.
11. Quality Control Materials.

REAGENT PREPARATION

1. **Wash Buffer**
   Dilute contents of Wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store at room temperature (2-30°C) for up to 60 days.
2. **Working Substrate Solution**
   Pour the contents of the vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2-8℃.

Note: Do not use the working substrate if it looks blue.
Do not use reagents that are contaminated or have bacteria growth.

TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 27°C).
*** Test procedure should be performed by a skilled individual or trained professional. ***

1. Format the microplates' wells for calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.050 ml (50µl) of the appropriate calibrators, controls and samples into the assigned wells.
3. Add 0.100 ml (100µl) of the C-Peptide Enzyme Reagent to each well**. It is very important to dispense all reagents close to the bottom of the microwell.**
4. Swirl the microplate gently for 20-30 seconds to mix. Cover with a plastic wrap.
5. Incubate for 120 minutes at room temperature (20-25℃).
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 350µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is used, fill each well to the top by squeezing the container. Avoiding air bubbles. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section).

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

NOTE: Always add reagents in the same order to minimize reaction time differences between wells.

QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated ranges for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

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.

Use components from the same lot. No intermixing of reagents from different batches.

PRECAUTIONS

For In Vitro Diagnostic Use Not for Internal or External Use in Humans or Animals

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.

CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of C-Peptide in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1

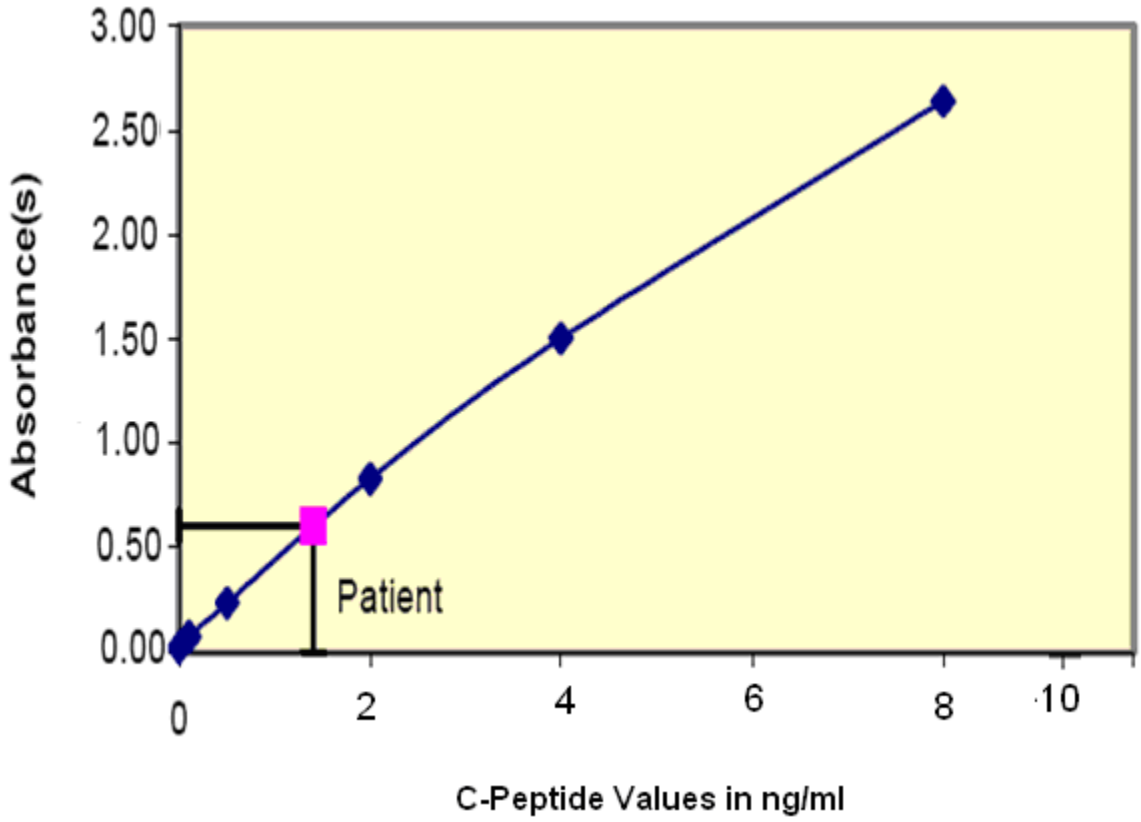
2. Plot the absorbance for each duplicate serum reference versus the corresponding C-Peptide concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of C-Peptide for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.433) intersects the dose response curve at 1.03 ng/ml for the C-Peptide concentration (See Figure 1).

Note: Computer data reduction software designed for DAI (ELISA) assays may also be used for the data reduction.

** The data presented in Example 1 and Figure 1 is for illustration only and should not be used in lieu of a dose response curve prepared with each assay.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	0.022	0.022	0
	B1	0.023		
Cal B	C1	0.097	0.103	0.2
	D1	0.107		
Cal C	E1	0.421	0.429	1
	F1	0.439		
Cal D	G1	0.889	0.901	2
	H1	0.910		
Cal E	A2	1.976	1.971	5
	B2	1.966		
Cal F	C2	2.717	2.643	10
	D2	2.570		
Ctrl 1	E2	0.429	0.433	1.03
	F2	0.437		
Ctrl 2	G2	1.861	1.887	4.64
	H2	1.913		
Patient 1	A3	0.388	0.405	0.82
	B3	0.421		



*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

QC PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 'A' should be < 0.1.
2. The absorbance (OD) of calibrators 'F' should be > 1.3.
3. Four out of six quality control pools should be within the established ranges.

RISK ANALYSIS

A. Assay Performance

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. Highly lipemeic, hemolysed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The 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.
6. Plate readers measure vertically. Do not touch the bottom of the wells.

7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from DAI IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis as required by CE Mark IVD Directive 98/79/EC – for this and other devices, made by DAI can be requested via email from www.rapidtest.com.

B. Interpretations

1. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinanats.
2. For valid test results, adequate controls and other parameters must be within the listed rages and assay requirements.
3. If test kits are altered, such as by mixing part of different ktis, which could produce false test results, or if results are incorrectly interpreted, DAI shall have no liability.
4. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for calibrators fall within 10% of the assigned concentrations.

EXPECTED VALUES

C-Peptide values are consistently higher in plasma than in serum; DAI advises that a serum sample be used for accurate determination. 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.

It is important to keep in mind that any normal range establishment is dependent upon a multiplicity of factors like the specificity of the method, the locale, the population tested and the precision of the method in the hands of technicians. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the technicians using the method with a population indigenous to the area in which the laboratory is located.

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

PERFORMANCE CHARACTERISTICS

A. Precision

The within and between assay precision of the C-Peptide DAI ELISA test system were determined by analyses on three different levels of pool control sera. The number (N) mean value (X), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table1 and Table 2.

TABLE 1

Within Assay Precision (Values in ng/ml)

SAMPLE	N	X	σ	C.V.
Pool 1	20	1.43	0.11	7.7%
Pool 2	20	5.07	0.46	9.0%
Pool 3	20	7.81	0.73	9.3%

TABLE 2
Between Assay Precision* (Values in ng/ml)

SAMPLE	N	X	σ	C.V.
Pool 1	20	1.27	0.12	9.7%
Pool 2	20	5.40	0.54	9.9%
Pool 3	20	8.18	0.50	6.1%

*As measured in ten experiments in duplicate over ten days.

B. Accuracy

The C-Peptide Diagnostic Automation, Inc. ELISA test system was compared with a predicate radioimmunoassay assay. Biological specimens from population (symptomatic and asymptomatic) were used. (The values ranged from 0.2 ng/ml – 11.8 ng/ml). The total number of such specimens was 124. The data obtained is displayed in Table 4.

TABLE 3

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This Method (y)	1.068	y = 0.2079 + 0.8036(x)	0.962
Reference (x)	1.066		

Only slight amounts of bias between the C-Peptide DAI ELISA test system and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

C. Sensitivity

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

D. Specificity

The cross-reactivity of the C-Peptide DAI ELISA system to selected substances was evaluated by adding the interfering substance(s) to a serum matrix at eth following concentration(s). The cross-reactivity was calculated by deriving a ration between dose of interfering substance to dose of C-Peptide needed to produce the same absorbance.

Substance	Cross Reactivity	Concentration
C-Peptide	1.000	-
Proinsulin	0.120	100 ng/ml
Insulin	non-detectable	1.0 mIU/ml
Glucagon	non-detectable	150 ng/ml

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