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C-PEPTIDE ELISA

EU: CE IVD CAN: IVD USA: For Research Use Only. Not for Use in Diagnostic Procedures

REF: CAN-C-P-4380 Version: 7.0
Effective: September 13, 2018

INTENDED USE
For the direct quantitative determination of C-Peptide by an enzyme immunoassay in human serum.

PRINCIPLE OF THE TEST
The principle of the following enzyme immunoassay test follows a typical one-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for C-peptide is immobilized onto the microplate and another monoclonal antibody specific for a different region of C-peptide is conjugated to horse radish peroxidase (HRP). C-peptide from the sample and standards are allowed to bind simultaneously to the plate and to the HRP conjugate. The washing and decanting steps remove any unbound HRP conjugate. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed by the enzymatic reaction is directly proportional to the concentration of C-peptide in the sample.

A set of standards is used to plot a standard curve from which the amount of C-peptide in patient samples and controls can be directly read.

CLINICAL APPLICATIONS
Human C-peptide of insulin is an amino acid chain of 3000 molecular weight, joining the A and B chains of insulin. It is secreted from the granules in the islet beta cells at equimolar concentrations with insulin. It is however, not as rapidly degraded by the liver as in the case of insulin, and therefore is more stable in the blood. These characteristics make the determination of C-peptide an advantageous test as an indicator to quantify insulin. It is therefore used in evaluating hypoglycemia and insulnoma.

PROCEDURAL CAUTIONS AND WARNINGS
1. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
2. Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
5. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
6. A calibrator curve must be established for every run.
7. The controls should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the controls do not reflect established ranges.
9. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
10. The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
11. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
13. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
14. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

LIMITATIONS
1. All the reagents within the kit are calibrated for the direct determination of C-peptide in human serum. The kit is not calibrated for the determination of C-peptide in saliva, plasma or other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only calibrator A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
5. The results obtained with this kit should never be used as the sole basis for clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.
6. Some individuals may have antibodies to mouse protein that can possibly interfere in this assay. Therefore, the results from any patients who have received preparation of mouse antibodies for diagnosis or therapy should be interpreted with caution.

SAFETY CAUTIONS AND WARNINGS
POTENTIAL BIOHAZARDOUS MATERIAL
Human serum that may be used in the preparation of the standards and controls has been tested and found to be non-reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. No test method however, can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS
Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE
Approximately 0.2 mL of serum is required per duplicate determination. Collect 4–5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT
This assay is a direct system; no specimen pretreatment is necessary.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED
1. Precision pipettes to dispense 50, 150 and 300 µL
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10)

REAGENTS PROVIDED
1. Mouse Anti-C-Peptide Antibody-Coated Break-Apart Well Microplate — Ready To Use
- Contents: One 96-well (12x8) monoclonal antibody-coated microplate in a resealable pouch with desiccant.
- Storage: Refrigerate at 2–8°C
- Stability: 12 months or as indicated on label.

2. Mouse Anti-C-Peptide Antibody-Horseradish Peroxidase (HRP) Conjugate Concentrate — Requires Preparation X100
- Contents: Anti-C-peptide monoclonal antibody-HRP conjugate in a protein-based buffer with a non-mercury preservative.
- Volume: 300 µL/vial
- Storage: Refrigerate at 2–8°C
- Stability: 12 months or as indicated on label.
- Preparation: Dilute 1:100 in assay buffer before use (eg. 20 µL of concentrate in 2 mL of assay buffer). If the whole plate is to be used dilute 120 µL of concentrate in 12 mL of assay buffer. Discard any that is left over.

3. C-Peptide Calibrators — Ready To Use
- Contents: Six vials containing C-peptide in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of C-peptide. Calibrated against World Health Organization (WHO) IS 84/510.

* Listed below are approximate concentrations, please refer to bottle labels for exact concentrations.

Calibrator	Concentration	Volume/Vial
Calibrator A	0 ng/mL	2.0 mL
Calibrator B	0.3 ng/mL	0.6 mL
Calibrator C	0.8 ng/mL	0.6 mL
Calibrator D	2 ng/mL	0.6 mL
Calibrator E	8 ng/mL	0.6 mL
Calibrator F	16 ng/mL	0.6 mL

- Storage: Calibrators should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.
- Stability: 12 months or as indicated on label in unopened vials stored frozen.

4. Controls — Ready To Use
- Contents: Two vials containing C-peptide in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of C-peptide. Refer to vial labels for the acceptable range.
- Volume: 0.6 mL/vial
- Storage: Controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.
- Stability: 12 months or as indicated on label in unopened vials stored frozen.

5. Wash Buffer Concentrate — Requires Preparation X10
- Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
- Volume: 50 mL/bottle
- Storage: Refrigerate at 2–8°C
- Stability: 12 months or as indicated on label.
- Preparation: Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water

6. Assay Buffer — Ready To Use
- Contents: One bottle containing a protein-based buffer with a non-mercury preservative.
- Volume: 15 mL/bottle
- Storage: Refrigerate at 2–8°C
- Stability: 12 months or as indicated on label.

7. TMB Substrate — Ready To Use
- Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
- Volume: 16 mL/bottle
- Storage: Refrigerate at 2–8°C
- Stability: 12 months or as indicated on label.

8. Stopping Solution — Ready To Use
- Contents: One bottle containing 1M sulfuric acid.
- Volume: 6 mL/bottle
- Storage: Refrigerate at 2–8°C
- Stability: 12 months or as indicated on label.

ASSAY PROCEDURE

Specimen Pretreatment: **None**.

All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Prepare working solutions of the anti-C-peptide-HRP conjugate and wash buffer.
2. Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette 50 µL of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
4. Pipette 50 µL of the conjugate working solution into each well. (We recommend using a multichannel pipette.)
5. Incubate on a plate shaker (approximately 200 rpm) for 90 minutes at room temperature.
6. Wash the wells 3 times with 300 µL of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended.)
7. Pipette 150 µL of TMB substrate into each well at timed intervals.
8. Incubate on a plate shaker for 15–20 minutes at room temperature (or until calibrator F attains dark blue colour for desired OD).
9. Pipette 50 µL of stopping solution into each well at the same timed intervals as in step 7.
10. Read the plate on a microplate reader at 450 nm within 20 minutes after addition of the stopping solution.

* If the OD exceeds the upper limit of detection or if a 450 nm filter is unavailable, a 405 or 415 nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.

CALCULATIONS

1. Calculate the mean optical density of each calibrator duplicate.
2. Calculate the mean optical density of each unknown duplicate.
3. Subtract the mean absorbance value of the “0” calibrator from the mean absorbance values of the calibrators, controls and serum samples.
4. Draw a calibrator curve on log-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
5. Read the values of the unknowns directly off the calibrator curve.
6. If a sample reads more than 16 ng/mL then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

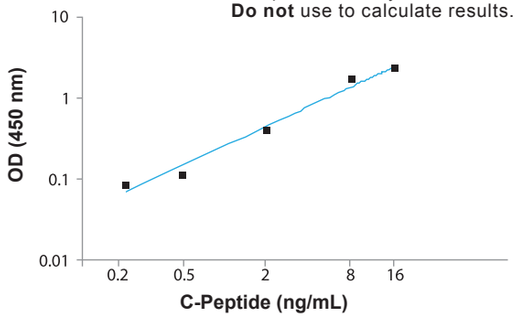
TYPICAL TABULATED DATA

Sample data only. **Do not** use to calculate results.

Calibrator	OD 1	OD 2	Mean OD	Value (ng/mL)
A	0.068	0.069	0.067	0
B	0.089	0.086	0.087	0.2
C	0.111	0.112	0.112	0.5
D	0.412	0.383	0.398	2
E	1.704	1.648	1.676	8
F	2.454	2.307	2.381	16
Unknown	0.292	0.311	0.302	1.6

TYPICAL CALIBRATOR CURVE

Sample curve only.
Do not use to calculate results.



PERFORMANCE CHARACTERISTICS

SENSITIVITY

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator A (based on 10 replicate analyses) plus 2 SD. Therefore, the sensitivity of the DBC Direct C-Peptide ELISA kit is **0.2 ng/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The specificity of the Direct C-Peptide ELISA kit was determined by measuring the apparent C-peptide values of the following compounds:

Substance	Concentration Range	Apparent C-Peptide Value (ng/mL)
Insulin Calibrated against WHO 1st IS 66/304	20–10,000 µIU/mL	Not Detected

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	1.00	0.04	3.5
2	4.14	0.09	2.2
3	12.02	0.55	4.6

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.97	0.03	2.7
2	4.00	0.11	2.7
3	12.30	0.62	5.0

RECOVERY

Spiked samples were prepared by adding defined amounts of C-peptide to three patient serum samples. The results (in ng/mL) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1 Unspiked	0.00	-	-
+ 1.0	0.91	1.00	91.0
+ 6.0	5.44	6.00	91.7
+ 8.0	7.82	8.00	97.8
2 Unspiked	0.64	-	-
+ 1.0	1.51	1.64	92.1
+ 2.0	2.27	2.64	86.0
+ 8.0	7.30	8.64	84.5
3 Unspiked	3.10	-	-
+ 0.25	3.10	3.35	92.5
+ 2.0	4.89	5.10	95.9
+ 6.0	9.47	9.10	104.1

LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in ng/mL) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1	5.00	-	-
1:2	2.35	2.50	94.0
1:4	1.10	1.25	88.0
1:8	0.51	0.63	81.0
2	6.76	-	-
1:2	3.08	3.38	91.1
1:4	1.42	1.69	84.0
1:8	0.71	0.85	83.5
3	13.36	-	-
1:2	6.02	6.68	90.1
1:4	3.09	3.34	92.5
1:8	1.56	1.67	93.4

EXPECTED VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	N	Mean (ng/mL)	Abs. Range (ng/mL)
Males	26	0.89	0.24–1.98
Females	46	1.13	0.15–5.37

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SYMBOLS

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LOT Lot number
X # Dilute 1: # Before use