PERFORMANCE CHARACTERISTICS

A) Internal Evaluation:

1. Accuracy: In an internal study **ELECTRA™ C-Peptide** was evaluated against commercially available licensed kit with 90 random clinical samples & **ELECTRA™ C-Peptide** has demonstrated 95% clinical correlation with the commercially available licensed kit.
2. Precision: **ELECTRA™ C-Peptide** was evaluated with licensed external Quality controls for Precision Studies & following is the data:

Controls	No. of testings	Mean Control values with ELECTRA™ C-Peptide	Coefficient of Variation (CV)
Level 1	10	0.462	8.52
Level 2	10	3.96	7.25
Level 3	10	5.32	6.47

B) External Evaluation:

ELECTRA™ C-Peptide CLIA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **ELECTRA™ C-Peptide** CLIA has demonstrated 95% correlation with the reference method.

*Data file: Zephyr Biomedicals (A Division of Tulip Diagnostics Pvt. Ltd).

Important Note:

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended to use the multiple channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of standards & samples is not mandatory but may provide information on reproducibility & application errors.

LIMITATIONS OF THE ASSAY

1. As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. The activity of the enzyme used is temperature-dependent and the RLU values may vary. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the RLU values. Corresponding variations apply also to the incubation times. However, the Standards are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.
3. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
4. Insufficient washing (e.g., less than 5 wash cycles, too small wash buffer volumes, or shortened reaction times) can lead to incorrect results.

BIBLIOGRAPHY

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SYMBOL KEYS

Temperature Limitation	Consult Instructions for use	Date of Manufacture	LOT Batch Number / Lot Number
Manufacturer	IVD *In vitro* Diagnostic Medical Device	This side up	Σ Contains sufficient for <n> tests
Use by	REF Catalogue Number	Do not reuse	

Manufactured by:
Zephyr Biomedicals
A Division of Tulip Diagnostics (P) Ltd.
M 46-47, Phase III B, Verna Industrial Estate, Verna, Goa- 403 722, INDIA.
Regd. Office: Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex P.O., Goa - 403 202, INDIA.

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0121/VER-01

electra™
C-Peptide

Chemiluminescence Assay for the Quantitative Determination of Circulating C-Peptide in Human Serum.
FOR IN VITRO DIAGNOSTIC USE ONLY
Store at 2°C to 8°C

INTENDED USE

ELECTRA™ C-Peptide CLIA test is intended for the quantitative determination of Circulating C-Peptide in human serum. For In Vitro Diagnostic Use only.

INTRODUCTION

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 plays significant roles. 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.

PRINCIPLE

ELECTRA™ C-Peptide CLIA Quantitative CLIA assay is for use on **ELECTRA** analyzers. **ELECTRA™ C-Peptide CLIA** works on the principle of chemiluminescence wherein light is produced by a chemical reaction from a substance as it returns from an electronically excited state to the ground state. When catalysed by HRP, the oxidation of luminol by hydrogen peroxide produces an electronically excited form of 3-aminophthalate which on relaxation emits light with maximum intensity at λ=425nm.

The **ELECTRA™ C-Peptide CLIA** test 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 short incubation the wells are washed again and bound enzyme is detected by adding the chemiluminescent substrate and Luminescence is measured in RLU. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of C-Peptide in the sample. By reference to a series of C-Peptide standards assayed in the same way, the concentration of C-Peptide in the unknown sample is quantified.

MATERIALS & COMPONENTS

Materials provided with the test kits:

- Coated Microwells: Microwells coated with monoclonal anti-C-Peptide antibody.
- Enzyme Conjugate Reagent. Ready to use.
- Substrate A: Chemiluminescent substrate containing enhanced luminol solution.
- Substrate B: Chemiluminescent substrate containing stabilized peroxide solution.
- C-Peptide Standard set of 6 standards labeled as A to F in liquid form. Ready to use. For standard Concentrations refer vial label.
- Wash Buffer Concentrate (20X).

Materials required but not provided:

- Precision pipettes: 10-100µl, 20-200µl, 100-1000µl
- Disposable pipette tips
- Distilled water
- Disposable Gloves
- **ELECTRA™ Analyzer**

electra™ Chemiluminescence assay
electra™ Chemiluminescence assay
electra™ Chemiluminescence assay

STORAGE AND STABILITY

1. **ELECTRA™ C-Peptide** kit is stable at 2-8°C up to the expiry date printed on the label.
2. Coated microwells should be used within one month upon opening the pouch provided that once opened, the pouch must be resealed to protect from moisture. If the colour of the dessicant has changed from blue to white at the time of opening the pouch, another coated microwells pouch should be used.
3. Diluted Wash Buffer is stable upto one week when stored at 2-8°C.
4. Working Substrate (A+B) must be used immediately.

SPECIMEN COLLECTION

1. Only fasting morning fresh serum samples to be used for testing.
2. Samples should be assayed as fresh as possible, If the samples cannot be assayed on the same day when collected, then the samples may be stored at -20°C for up to 30 days.
3. Collect blood specimen by venipuncture according to the standard procedure.
4. Only serum should be used.
5. Avoid grossly hemolytic, lipemic or turbid samples.
6. Preferably use fresh samples. However, specimens can be stored up to 48 hours at 2-8°C, for short duration.
7. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
8. Do not heat inactivate before use.
9. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
10. Specimen should be free from particulate matter and microbial contamination.

PRECAUTIONS

1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not eat, drink or smoke in the area where testing is done.
4. Use protective clothing and wear gloves when handling samples.
5. Use absorbent sheet to cover the working area.
6. Immediately clean up any spills with sodium hypochlorite.
7. All specimens and standards should be considered potentially infectious and discarded appropriately.
8. Neutralize acid containing waste before adding hypochlorite.
9. Do not use kit after the expiry date.
10. Do not mix components of one kit with another.
11. Always use new tip for each specimen and reagent.
12. Do not allow liquid from one well to mix with other wells.
13. Do not let the strips dry in between the steps.

REAGENT PREPARATION

- 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.
- Dilute Wash Buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.
- If reference standards are lyophilized, reconstitute each standard with 0.5 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be sealed and stored at 2-8°C.
- Prepare a Working Substrate by Mixing Substrate A and Substrate B in equal volume (1:1 ratio) before addition to the micro-wells.

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
Substrate-A µl	250	450	650	850	1050	1250	1450	1650	1850	2050	2250	2450
Substrate-B µl	250	450	650	850	1050	1250	1450	1650	1850	2050	2250	2450

TEST PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense **50**µl of Standards and Serums into the appropriate wells.
2. Dispense **100**µl of Enzyme Conjugate Reagent into each well. Incubate at room temperature(18-25°C) for **30 mins**.
3. Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with Wash Buffer (1X). Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
4. Add **50µl** of working Substrate (A+B) in all the micro-wells. Keep away from direct light while adding the substrate.
5. Cover the **ELECTRA™** microplate and incubate for **10** minutes at room temperature (**18-25°C**) in dark.
6. Read the **ELECTRA™** micro-plate exactly at **10** minutes in **ELECTRA™ Analyzer.** If **ELECTRA™** micro-plate is not read between 10-15 minutes the test results should be considered as invalid.

Add **50** µl Standards/Samples into the respective Microwell

⬇

Add **100** µl Enzyme Conjugate in each well

⬇

Apply plate sealer and incubate for **30** minutes at **18-25°C**

⬇

Wash Microwells **5 times** with 350 µl of diluted Wash Buffer

⬇

Add **50** µl Substrate in each well

⬇

Incubate for **10** Minutes at **18-25°C in dark**

⬇

Read results on **ELECTRA™** Analyzer exactly at the end of **10** minutes

CALCULATION OF RESULTS

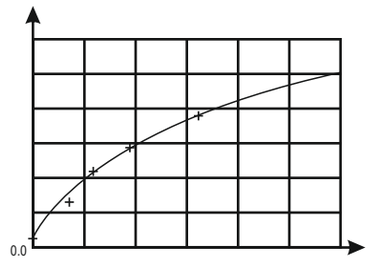
Construct a Standard curve by plotting the mean RLU obtained from each reference standard against its concentration in ng/ml on the graph paper, with RLU values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the RLU values for each specimen to determine the corresponding concentration of C-Peptide in ng/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 RLU's shown in the Y axis against C-Peptide concentrations in the X axis.

Suggest: Use 4-Parameter Standard curve to calculate sample values.

C-Peptide Values (ng/ml)	RLU's
A	60
B	6699
C	73411
D	308318
E	1078593
F	2793622



This Standard curve is for the purpose of illustration only, and should not be used to calculate samples. Each user should obtain his or her own Standard curve and data.

Expected Ranges of 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.

Adult (Normal) 0.7 – 3.2 ng/ml

The minimum detectable concentration of C-Peptide by this assay is estimated to be 0.05 ng/ml

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