PERFORMANCE CHARACTERISTICS

A) Internal Evaluation:

1. Accuracy: In an internal study **ELECTRA™ Insulin** was evaluated against commercially available licensed kit with 90 random clinical samples & **ELECTRA™ Insulin** has demonstrated 100% clinical correlation with the commercially available licensed kit.
2. Precision: **ELECTRA™ Insulin** was evaluated with licensed external Quality controls for Precision Studies & following is the data:

Controls	No. of testings	Mean Control values with ELECTRA™ Insulin	Coefficient of Variation (CV)
Level 1	10	9.70	5.25
Level 2	10	44.51	4.89
Level 3	10	147.38	5.51

B) External Evaluation:

ELECTRA™ Insulin CLIA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **ELECTRA™ Insulin** has demonstrated 100% 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 RLU values.

BIBLIOGRAPHY

(1) Eastham, R.D.: *Biochemical Values in Clinical Medicine*, 7th Ed. Bristol. England. John Wright & Sons, Ltd, 1985. (2) Gerbitz, V.K.D., Pancreatische B-zellen Peptide: *Kinetic and Konzentration von Proinsulin insulin and Insulinin Plasma and Urin Probleme der Mezmethoden Klinische und Literaturubersicht.* J. Clin. Chem. Biochem, 18: 313-326, 1980. (3) Boehm TM, Lebovitz HE, *Statistical analysis of Glucose and insulin responses to intravenous tolbutamide: evalustion of hypoglycemic and hyperinsulinemic states:* Diabetes Care. (1979) 479-490. (4) National Committee for Clinical Laboratory Standards. *Procedures for the collection of diagnostic blood specimens by venipuncture:* approved standards. 4th Ed. NCCLS Document H3-A4, Wayne, PA: 1998. (5) Turkington RW, Estkowkski A, Link M. *Secretion of insulin or connecting peptide; a predictor of insulin dependence of obese diabetics.* Archives of Internal Med. 1982:142: 1102-1105. (6) Sacks BD: *Carbohydrates* In Burtis, C.A. and Ashwood, AR (Eds) Tietz Textbook of Clinical Chemistry. 2nd Ed. Philadelphia. W.B. Saunders Co. 1994. (7) Kahn CR, Rosenthal AS, *Immunologic reactions to insulin, insulin allergy, insulin resistance and autoimmune insulin syndrome*. Diabetes Care 1979; 2. 283-295.

SYMBOL KEYS

Temperature Limitation	Consult Instructions for use	Date of Manufacture	Batch Number / Lot Number
Manufacturer	*In vitro* Diagnostic Medical Device	This side up	Contains sufficient for <n> tests
Use by	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.

An ISO 13485 Certified Company

0321/VER-01

electra™ INSL

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

INTENDED USE

ELECTRA™ Insulin CLIA test is intended for the quantitative determination of Human Insulin in human serum. For In Vitro Diagnostic Use only.

INTRODUCTION

Insulin is the principle hormone responsible for the control of glucose metabolism. It is synthesized in the -cells of the islets of Langerhans as a precursor - proinsulin, which is processed to form Insulin. Both are secreted in equimolar amounts into the portal circulation. The mature insulin molecule comprises two polypeptide chains, the A chain and B chain (21 and 30 amino acids respectively). The two chains are linked together by two inter-chain disulphide bridges. There is also an intra-chain disulphide bridge in the A chain. Secretion of insulin is mainly controlled by plasma glucose concentration, and the hormone has a number of important metabolic actions. 1st principle function is to control the uptake and utilization of glucose in peripheral tissues via the glucose transporter. This and other hypoglycemic activities, such as the inhibition of hepatic gluconeogenesis and glycogenolysis are counteracted by the hyperglycemic hormones including glycogen, epinephrine (adrenaline), growth hormone and cortisol.
Insulin concentrations are severely reduced in insulin-dependent diabetes mellitus (IDDM) and some other conditions such as hypopituitarism. Insulin levels are raised in non-insulin-dependent diabetes mellitus (NIDDM), obesity, insulinoma and some endocrine dysfunctions such as Cushion's syndrome and acromegaly.

PRINCIPLE

ELECTRA™ Insulin Quantitative CLIA assay is for use on **ELECTRA** analyzers. **ELECTRA™ Insulin 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™ Insulin CLIA** test system utilizes one anti-Insulin antibody for solid phase (microtiter wells) immobilization and another anti-Insulin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The standards and test specimen (serum) are added to the Insulin antibody coated microtiter wells. Then anti-Insulin antibody labeled with horseradish peroxidase (conjugate) is added. If human Insulin is present in the specimen, it will combine with the antibody on the well and the enzyme conjugate resulting in the Insulin 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 Insulin in the sample. By reference to a series of Insulin standards assayed in the same way, the concentration of Insulin in the unknown sample is quantified.

MATERIALS & COMPONENTS

Materials provided with the test kits:

- Coated Microwells: Microwells coated with Mouse monoclonal Anti-Insulin antibody.
- Insulin Enzyme Conjugate. Ready to use.
- Substrate A: Chemiluminescent substrate containing enhanced luminol solution.
- Substrate B: Chemiluminescent substrate containing stabilized peroxide solution.
- Insulin Standard set of 6 standards labeled as A to F in lyophillized 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**

STORAGE AND STABILITY

1. **ELECTRA™ Insulin** 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. Collect blood specimen by venipuncture according to the standard procedure.
2. Only serum should be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However, specimens can be stored up to 48 hours at 2-8°C, for short duration.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.
7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
8. 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.

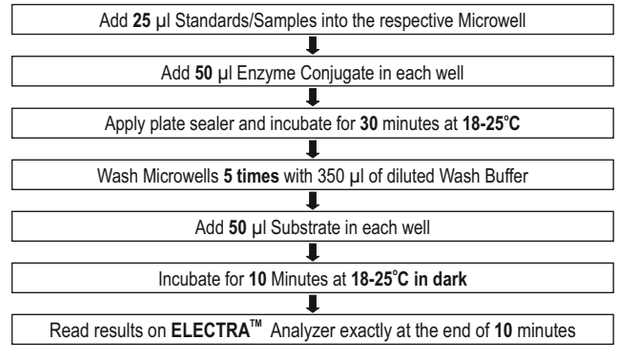
REAGENT PREPARATION

- 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 **25**µl of Standards and Serums into the appropriate wells.
2. Dispense **50**µl of Enzyme Conjugate into each well. Incubate at room temperature (18-25°C) for **30 minutes.**
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 the 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.



CALCULATION OF RESULTS

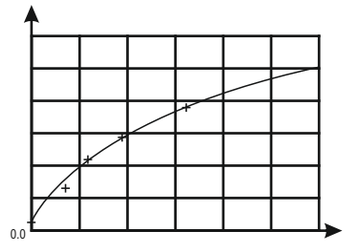
Construct a Standard curve by plotting the mean RLU obtained from each reference standard against its concentration in µIU/ml on the graph paper, with RLU values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the RLU values for each specimen to determine the corresponding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

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

Insulin Values (µIU/ml)	RLU's
A	465
B	133449
C	1195627
D	1880309
E	2874528
F	4130330



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

Insulin values are consistently higher in plasma than in serum; thus, serum is preferred. Compared with fasting values in non-obese non diabetic individuals, insulin 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 local population, laboratory, technique and specificity of the method. Based on the clinical data gathered the following ranges have been assigned.

These ranges should be used as guidelines only:

Children < 12 yrs	< 10 µIU/ml
Adult (Normal)	0.7 – 9.0 µIU/ml
Diabetic (Type II)	0.7 – 25 µIU/ml

The minimum detectable concentration of Insulin by this assay is estimated to be 2.0 µIU/ml.