PERFORMANCE CHARACTERISTICS

A) Internal Evaluation:

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

Controls	No. of testings	Mean Control values with ELECTRA™ IgE	Coefficient of Variation (CV)
Level 1	10	275.35	5.25
Level 2	10	110.29	5.18
Level 3	10	138.79	5.82

B) External Evaluation:

ELECTRA™ IgE CLIA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **ELECTRA™ IgE** 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.

An ISO 13485 Certified Company

0321/VER-01

electra™ IgE

Chemiluminescence Assay for the Quantitative Determination of Immunoglobulin E (IgE) in Human Serum.
FOR IN VITRO DIAGNOSTIC USE ONLY
Store at 2°C to 8°C

INTENDED USE

ELECTRA™ IgE CLIA test is intended for the quantitative determination of Immunoglobulin E (IgE) in human serum. For In Vitro Diagnostic Use only.

INTRODUCTION

IgE is also known as the reagenic antibody. In general, elevated levels of IgE indicate an increased probability of an IgE-mediated hypersensitivity, responsible for allergic reactions. Parasitic infestations such as hookworm and certain clinical disorders including aspergillosis, have also been demonstrated to cause high levels of IgE. Decreased levels of IgE are found in cases of hypogammaglobulinemia, autoimmune diseases, ulcerative colitis, hepatitis, cancer and malaria. Cord blood or serum IgE levels may have prognostic value in assessing the risk of future allergic conditions in children.
The IgE serum concentration in a patient is dependent on both the extent of the allergic reaction and the number of different allergens to which he is sensitized. Nonallergic normal individuals have IgE concentrations that vary widely and increase steadily during childhood, reaching their highest levels at age 15 to 20, and there after remaining constant until about age 60, when they slowly decline. Patients with atopic allergic diseases such as atopic asthma, atopic dermatitis, and hay fever have been shown to exhibit increased total immunoglobulin E (IgE) levels in blood. The IgE Quantitative Enzyme Immunoassay provides a rapid, sensitive, and reliable assay for total serum IgE.

PRINCIPLE

ELECTRA™ IgE Quantitative CLIA assay is for use on **ELECTRA** analyzers. **ELECTRA™ IgE 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™ IgE CLIA** system utilizes one anti-IgE antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-IgE antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test specimen (serum) is added to the IgE antibody coated microtiter wells and incubated with the Zero Buffer. If human IgE is present in the specimen, it will combine with the antibody on the well. The well is then washed to remove any residual test specimen, and IgE antibody labeled with horseradish peroxidase (conjugate) is added. The conjugate will bind immunologically to the IgE on the well, resulting in the IgE molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a incubation, the wells are washed with Wash Buffer to remove unbound labeled antibodies. A solution of chemiluminescent substrate is then added 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 unlabeled IgE in the sample. By reference to a series of IgE standards assayed in the same way, the concentration of IgE in the unknown sample is quantified.

MATERIALS & COMPONENTS

Materials provided with the test kits:

- Coated Microwells: Microwells coated with Anti-IgE antibody.
- Zero Buffer. Ready to use.
- Enzyme Conjugate Reagent. Ready to use.
- Substrate A: Chemiluminescent substrate containing enhanced luminol solution.
- Substrate B: Chemiluminescent substrate containing stabilized peroxide solution.
- IgE 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**

STORAGE AND STABILITY

1. **ELECTRA™ IgE** 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.
- 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 **20µl** of Standards and Serums into the appropriate wells.
2. Dispense **100µl** of Zero Buffer into each well and thoroughly mix for 10 seconds.
3. Incubate at 18-25°C for **15 mins**.
4. 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.
5. Dispense **150µl** of Enzyme Conjugate Reagent into each well. Gently mix for 5 seconds.
6. Incubate at 18-25°C for **15 mins**.
7. 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.
8. Add **50µl** of working Substrate (A+B) in all the micro-wells. Keep away from direct light while adding the substrate.
9. Cover the **ELECTRA™** microplate and incubate for **10** minutes at room temperature (**18-25°C**) in dark.
10. 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 **20** µl Standards/Samples into the respective Microwell

↓

Add **100** µl Zero Buffer in each well

↓

Apply plate sealer and incubate for **15** minutes at **18-25°C**

↓

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

↓

Add **150** µl Enzyme Conjugate in each well

↓

Apply plate sealer and incubate for **15** 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

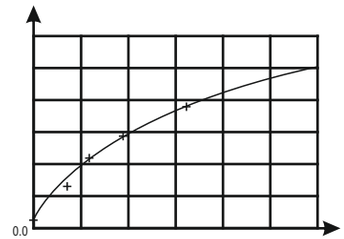
Construct a Standard curve by plotting the mean RLU obtained from each reference standard against its concentration in IU/ml on the graph paper, with RLU values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the RLU values for each specimen to determine the corresponding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

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

IgE Values (IU/ml)	RLU's
A	2319
B	159849
C	910045
D	1785755
E	3888503
F	5159720



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 VALUES AND SENSITIVITY

The above reference range is for guideline only. As the spread of IgE values is extremely wide in subjects with and without known allergic diseases it is recommended that each laboratory must establish its own normal ranges based on patient population.

The suggested reference values for IgE:

Age	IgE Reference Range
< 1 year	1.4 - 53 IU/ml
1-4 years	0.4 - 352 IU/ml
5-10 years	0.5 - 393 IU/ml
11-15 years	1.9 - 170 IU/ml
> 15 years	2.0 - 306 IU/ml

The minimal detectable concentration of IgE by this assay is estimated to be 5 IU/ml.