PERFORMANCE CHARACTERISTICS

A) Internal Evaluation:

1. Accuracy: In an internal study **Electra™ Anti-dsDNA** was evaluated against commercially available licensed kit with 90 random clinical samples & **Electra™ Anti-dsDNA** has demonstrated 100% clinical correlation with the commercially available licensed kit.

B) External Evaluation:

Electra™ Anti-dsDNA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **Electra™ Anti-dsDNA** 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 RLU readings.
2. It is recommended to use the multi channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of controls, calibrator & 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.
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 3 wash cycles, too small wash buffer volumes, or shortened reaction times) can lead to incorrect RLU values.

BIBLIOGRAPHY

(1) White RH, Robbins DL. West J Med. 147: 210-213, 1987. (2) Hardin JA. Arthritis Rheum. 29(4): 457-460, 1986. (3) Condemi JJ. JAMA. 258(20): 2920-2929, 1987. (4) Tan, EM, Cohen AS, Fries JF, et. al. Arthritis Rheum. 25(11): 1271-1277, 1982. (5) Tan EM, Schur PH, Carr RI, Kunkel HG. J Clin Inves. 45(11): 1732-1740, 1966. (6) Koffler D. Ann Rev Med 25: 149-164, 1974. (7) Emlen W, Pisetsky D, Taylor R. Arthritis Rheum. 29: 1417, 1986. (8) Pincus T, Schur PH, et. al. New Engl J Med. 281: 701-705, 1969. (9) Minter MF, Stollar BD, Agnello V. Arthritis Rheum. 22: 959-968, 1979. (10) Emlen W, Jarusiripipat P, Burdick G. J Immunol Methods. 132: 91-101, 1990. (11) Notman DD, Kurata N, Tan EM. Annal of Inter Med. 83: 464-469, 1975. (12) Locker JD, Medof ME, et al. J Immunol. 118: 694, 1977. (13) Smeenk RJT, Brinkman K, et. al. J Immunol. 140: 3786-3792, 1988. (14) Tan EM. Adv in Immunol. 44: 93-151, 1989.

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

0120/VER-01

electra™

Anti-dsDNA

Chemiluminescence Assay for the Qualitative Determination of Anti double-stranded DNA (dsDNA) Antibody in Human serum.
FOR IN VITRO DIAGNOSTIC USE ONLY
Store at 2°C to 8°C

INTENDED USE

ELECTRA™ Anti-dsDNA CLIA test is intended for the qualitative determination of dsDNA antibodies in human serum. For in vitro diagnostic use only.

INTRODUCTION

Antinuclear antibodies (ANAs) directed against a variety of macromolecules occur in extraordinarily high frequency in systemic rheumatic diseases. Many rheumatic diseases are characterized by the presence of one or more of these Antinuclear antibodies. Therefore, the identification of the specific antibody is useful in the detection and diagnosis of the disease. Anti-dsDNA is present in 50% to 70% of patients with SLE. Circulating DNA/anti-DNA immune complexes are considered to play a part in the pathogenesis of SLE. The presence of anti-dsDNA is one of the diagnostic criteria for SLE. IgG antibodies to dsDNA are considered clinically most useful for the diagnosis and management of SLE. Antibodies to single stranded DNA (ssDNA) and IgM antibodies to dsDNA are found in a number of other connective diseases, liver diseases, as well as in some normal individuals. Accurate detection of anti-dsDNA is important in the diagnosis and management of SLE. The tests for anti-dsDNA have demonstrated greater sensitivity than standard IFA and RIA tests allowing for improved detection of low titer antibodies to dsDNA.

PRINCIPLE

ELECTRA™ Anti-dsDNA Qualitative CLIA assay is for use on **ELECTRA** analyzers. **ELECTRA™ Anti-dsDNA 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.

ELECTRA™ Anti-dsDNA test uses Purified dsDNA is bound to microwells. The DNA retains its antigenicity and remains double stranded. Antibodies to dsDNA, if present in diluted serum, bind in the microwells. Washing of the microwells removes unbound serum antibodies. Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically binds to the bound patient antibodies forming a "conjugate - anti-dsDNA - dsDNA" sandwich. Washing of the microwells removes unbound conjugate. A solution of chemiluminescent substrate is then added and Luminescence is measured in RLU and is propotional to the amount of IgG specific antibody in the sample. The results are read by a microwell luminometer compared in a parallel manner with calibrator and controls.

MATERIALS & COMPONENTS

Materials provided with the test kits:

- Coated Microwells: Microwells coated with dsDNA antigen.
- Sample diluent. Ready to use.
- Anti-dsDNA Negative Control. Ready to use.
- Anti-dsDNA Positive Control. Ready to Use.
- dsDNA HRPO Enzyme Conjugate. Ready to use.
- Anti-dsDNA Calibrator. Ready to use.
- Substrate A: Chemiluminescent substrate containing enhanced luminol solution.
- Substrate B: Chemiluminescent substrate containing stabilized peroxide solution.
- 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™ Anti-dsDNA** kit is stable at 2-8°C up to the expiry date printed on the label.
2. Coated micro-wells should be used within one month of opening the pouch. Once opened, the pouch must be sealed

properly to protect from moisture. In case the desiccant pouch changes color from blue to white, the strips should not 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

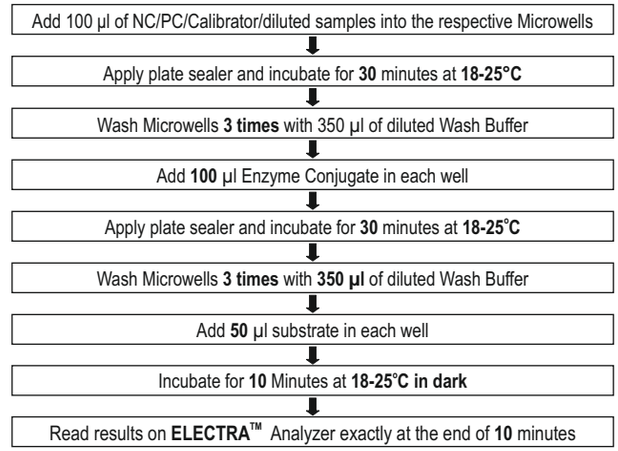
1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not eat, drink or smoke in the area where testing is done.
4. Use protective clothing and wear gloves when handling samples.
5. Use absorbent sheet to cover the working area.
6. Immediately clean up any spills with sodium hypochlorite.
7. All specimens, calibrator, Negative Control and Positive Control 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.

TEST PROCEDURE

1. Patient serum should be diluted 1:100 times before use. (ie Dilute 5 µl of patient's sample in 500 µl of sample diluent). **Important Note: Negative Control, Positive Control and Anti-dsDNA Calibrator have been already prediluted and are ready for use. Please DO NOT dilute again.**
2. Secure the desired number of coated wells in the holder.
3. Dispense **100 µl** of negative control, positive control, Anti-dsDNA calibrator & diluted specimens into the appropriate wells. Gently mix for **10 seconds** & incubate for **30 minutes** at room temperature (**18-25°C**). (Do not incubate diluted sera in wells for more than 40 minutes.)
4. Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the micro titer plate 3 times with 1X wash buffer. Strike the microtiter plate sharply onto the absorbent paper or paper towels to remove all residual water droplets.
5. Dispense **100µl** of Enzyme Conjugate reagent into each well. Gently mix for 10 seconds & incubate for **30 minutes** at room temperature (**18-25°C**).
6. Remove the contents and wash the plate as described in step 4 above.
7. Add **50 µl** of working Substrate (A+B) in all the micro-wells. Keep away from direct light while adding the substrate.
8. Cover the **ELECTRA**™ microplate and incubate for **10** minutes at room temperature (**18-25°C**) in dark.
7. 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.



RUN CRITERIA

The anti-dsDNA Index of the Negative control and Positive control should be in the range as stated on the labels. If any of these criteria are not met, the results are invalid and the test should be repeated.

CALCULATION OF RESULTS

1. To obtain Cut off Value (COV): Multiply the RLU of the Calibrator by Factor (f) (which is lot specific & will be printed on the label of the calibrator vial).
2. Calculate the anti-ds DNA Index of each determination by dividing the RLU values of each sample by obtained RLU value of Cut off.

For example:
If Factor (f) value on label = 0.3

This factor (f) is a variable. It is specific for a lot manufactured and printed on label of Calibrator.

Obtained Calibrator RLU = 3630267
Cut-off RLU = 3630267 x 0.3 = 1089080
Patient sample RLU = 1399372
anti-ds DNA Index = 1399372/1089080 = 1.28 (Positive result)

Patient sample RLU = 186733
anti-ds DNA Index = 186733/1089080 = 0.17 (Negative result)

INTERPRETATION OF RESULTS

Negative: Index of 0.90 or less.
Equivocal: Index of 0.91 - 1.40 are equivocal. Sample should be retested.
Low Positive: Index of 1.41 - 2.0.
Positive: Index of 2.1 - 3.5.
Strong Positive: Index of 3.51 or greater.

EXPECTED RANGES AND SENSITIVITY

The negative range was determined from serum samples of 84 confirmed negative normal blood donors which were assayed by the anti-dsDNA test. If the cut-off factor was dertermined by mean value + 4 standard deviation (M + 4SD), nearly all the samples show negative results.
The following are the frequency of anti-dsDNA antibodies found in autoimmune diseases: SLE - 40%; MCTD - 0%; Sjogren's Syndrome - 0%; Drug Induced Lupus - 0%; Progressive Systemic Sclerosis - 0%; Dermatomyositis/Polymyositis - 0%.