4. **ELECTRA™ Tp. Ab. CLIA** was evaluated using serial dilution of 5 RPR Syphilis Positive Research Panels against other ***T. pallidum*** EIA.

## REMARKS

1. **ELECTRA™ Tp. Ab. CLIA** kit alone cannot be used as sole criteria for diagnosis of syphilis infection even if the sample is repeatedly reactive or as high RLU.
2. As with all immunoassays, the **ELECTRA™ Tp. Ab. CLIA** may yield non-specific reactions due to other causes, particularly when testing in low prevalence populations. A repeatedly reactive specimen should be tested further with supplemental confirmatory tests.
3. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
4. A negative result does not indicate that an individual is absolutely free of syphilis infection.
5. It has been observed that most VDRL/RPR-positive samples show positive with **ELECTRA™ Tp. Ab. CLIA.** However since **ELECTRA™ Tp. Ab. CLIA** detects treponemal and regain antibodies, its results should not be compared with non-treponemal tests like VDRL, RPR etc.
6. Testing of pooled samples is not recommended.

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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:

**Qualpro Diagnostics**

A Division of Tulip Diagnostics (P) Ltd.

88/89, Phase II C, 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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1220/VER-02

# electra™ Tp. Ab.

**Chemiluminescence Assay for the detection of antibodies to *Treponema pallidum* in human serum or plasma.**

**FOR IN VITRO DIAGNOSTIC USE ONLY**

## INTENDED USE

**ELECTRA™ Tp. Ab. CLIA** is intended to be used for the detection of total antibodies (i.e. IgG, IgM, IgA etc) to *Treponema pallidum* in human serum or plasma.

## SUMMARY


Syphilis is a sexually transmitted (venereal) disease caused by the spirochete *Treponema pallidum.* The disease can also be transmitted congenitally thereby attaining importance in antenatal screening. After infection the host forms non-treponemal anti-lipoidal antibodies (reagins) to the lipoidal material released from the damaged host cells as well as treponema specific antibodies. **ELECTRA™ Tp. Ab. CLIA** is a microwell Chemiluminescence assay (CLIA) which employs recombinant 47Kd and 17Kd antigens to detect agglutinating sera specific for *Treponema pallidum.*


## PRINCIPLE

The **ELECTRA™ Tp. Ab. CLIA** assay is for use on **ELECTRA™ SA** and **ELECTRA™ FA** analyzers. **ELECTRA™ Tp. Ab. 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™ Tp. Ab. CLIA** micro-well strips are coated with recombinant 47Kd and 17Kd antigens. The same antigens are conjugated to HRP. Samples along with positive & negative controls are pipetted into the coated wells and incubated simultaneously with antigen HRP conjugate. The wells are washed extensively. After washing wells to remove the unbound enzyme, chemiluminescent substrate is added. The bound enzyme converts substrate to a reaction product that emits a photon of light.

Chemiluminescence is measured in Relative Light Units (RLU) that is typically proportionate to the amount of analyte present in the sample. The presence or absence of analyte in the sample is determined by comparing the sample RLU with Cut-off which is calculated by **ELECTRA™ SA** and **ELECTRA™ FA** analyzers and expressed as Electra cutoff Index (ECI). ECI is equivalent to S/CO ratio which is calculated by using sample RLU & calculated Cutoff of the specific testing batch. Samples with ECI values greater than or equal to 1.00 are considered reactive and samples with ECI values less than 1.00 are considered nonreactive. A grey zone of ± 10% is recommended.

## KIT COMPONENTS

**ELECTRA™ Tp. Ab. CLIA** has following components:

1. Coated micro-wells: Micro-wells (3 x 8 wells) are coated with recombinant 47Kd and 17Kd antigens. Ready to use. 96 Wells: (3x8) x 4 pouches, 192 Wells: (3x8) x 8 pouches.
2. Positive control: Inactivated and stabilized human serum reactive for *Treponema Pallidum* antibodies with preservatives. Produces a positive reaction.
3. Negative control: Inactivated and stabilized human serum non-reactive for *Treponema Pallidum.*
4. Enzyme Conjugate: *Treponema pallidum* antigens-HRP conjugate (25X). To be diluted 25 times with conjugate diluent.
5. Conjugate diluent: Buffered solution containing stabilizing proteins and preservatives.
6. Substrate A: Chemiluminescent substrate containing enhanced luminol solution.
7. Substrate B: Chemiluminescent substrate containing stabilized peroxide solution.
8. Wash buffer: Buffer containing surfactants (20 X). To be diluted 20 times with distilled or deionized water.
9. Microwell holder.
10. Instruction for use.
11. Plate sealer.
12. CLIA protocol sheet.

| | | |
|---|---|---|
| REF | 407040096 | 407040192 |
| Σ | 96 Tests | 192 Tests |

## STORAGE AND STABILITY

1. **ELECTRA™ Tp. Ab. CLIA** 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 conjugate must be used immediately.
4. Diluted wash buffer should preferably be used within the same day. However excess wash buffer may be stored at up 2-8°C for one week.
5. Working Substrate (A+B) must be used immediately.

## MATERIAL REQUIRED BUT NOT PROVIDED

1. Manual or automatic pipettor.
2. Pipettor tips.
3. Incubator.
4. Micro-well washer
5. **ELECTRA™ SA** or **ELECTRA™ FA**.
6. Reagent grade water.
7. Disposable gloves
8. Timer/ Stop Watch.

## SAMPLE COLLECTION

1. No prior preparation of the patient is required.
2. Collect blood specimen by venipuncture according to the standard procedure.
3. Serum or plasma can be used.
4. Specimen should be free of particulate matter and microbial contamination.
5. Use of fresh sample is preferred. However, specimen can be stored refrigerated for upto three days. For long storage, samples should be frozen at 2°C or below.
6. Specimen should be brought to room temperature prior to testing.
7. Do not heat inactivate before use.

## PRECAUTIONS

1. Do not pipette any material by mouth.
2. Do not eat, drink or smoke in the area where testing is done.
3. Use protective clothing and wear gloves when handling samples.
4. Immediately clean up any spills with sodium hypochlorite.
5. Dispose off all the reagents and material used as if they contain infectious agent.
6. Neutralize acid containing waste before adding hypochlorite.
7. Do not use the kit after the expiry date.
8. Do not mix the components of one kit with those from another.
9. Always use a new tip for each specimen and reagent.
10. Do not allow liquid from one well to mix with other wells.
11. Do not let the strips dry in between the steps.
12. Do not expose working substrate to direct light.

## REAGENT PREPARATION

1. Dilute wash buffer 20 times (for example add 5 ml concentrated buffer to 95 ml distilled or deionized water).
2. Dilute enzyme conjugate with Conjugate diluent according to the requirement as shown below. Prepare a fresh dilution for each assay.

| No. of Strips | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Enzyme Conjugate (µl) | 40 | 80 | 120 | 160 | 200 | 240 | 280 | 320 | 360 | 400 | 440 | 480 |
| Conjugate Diluent (µl) | 960 | 1920 | 2880 | 3840 | 4800 | 5750 | 6720 | 7680 | 8640 | 9600 | 10560 | 11520 |

3. 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. Bring all the reagents and specimen to room temperature before use.
2. Take out required number of strips and immediately close the pouch.
3. Well A1 must be used as blank.
4. Add 100 µl Diluted conjugate in each well except well A1
5. Add 50 µl each of NC in wells B1 & C1 & PC in wells D1 & E1 respectively.
6. Add 50 µl of samples to the remaining wells.
7. Apply plate sealer and incubate for 45 minutes at 37°C.
8. Wash each well using a semi-automated micro-plate washer (Preferably Lisa Wash models) with diluted wash buffer for 6 wash cycles giving 30 secs soak time for each wash cycle and Blot dry.
9. Add 50 µl of working Substrate (A+B) in all the micro-wells. Keep away from direct light while adding the substrate.
10. Cover the Electra microplate and incubate for 10 mins at room temperature (18-25°C) in dark.
11. Read the Electra micro-plate exactly at 10 mins in **ELECTRA™ SA** or **ELECTRA™ FA.** If Electra micro-plate is not read between 10- 15 mins, the test results should be considered as invalid.

## TEST VALIDATION CRITERIA

1. The individual RLU of negative controls should be less than 200000 for **ELECTRA™ SA** and **ELECTRA™ FA.**
2. The individual RLU of positive controls should be more than 7500000 for **ELECTRA™ SA** and **ELECTRA™ FA.**

## CALCULATIONS

The cutoff value is calculated by the software of **ELECTRA™ SA** and **ELECTRA™ FA** using the mean of negative control RLU and a factor.
Factor for **ELECTRA™ SA and ELECTRA™ FA** is 400000

## SAMPLE DATA

For **ELECTRA™ SA** and **ELECTRA™ FA:**
**Considering the Cutoff for this batch is 400527**

| Well | RLU | ECI | Interpretation |
|---|---|---|---|
| Blank | 80 | | |
| NC | 541 | | |
| NC | 513 | | |
| PC | 14401045 | | |
| PC | 14562704 | | |
| Sample 1 | 3780160 | 9.43 | Reactive |
| Sample 2 | 9936 | 0.02 | Non-reactive |
| Sample 3 | 1487376 | 3.71 | Reactive |
| Sample 4 | 209727 | 0.52 | Non-reactive |

## INTERPRETATION OF RESULTS

1. Results are interpreted in E.C.I. This is determined by dividing the RLU of the sample by the Cutoff value calculated for that specific run.
2. Samples with E.C.I values greater than or equal to 1.00 are considered Reactive and samples with E.C.I values less than 1.00 are considered Non-reactive.
3. Samples that are initially reactive in **ELECTRA™ Tp. Ab. CLIA** should be retested in duplicate. Repeated reactivity is highly predictive Syphilis infection.

## PERFORMANCE CHARACTERISTICS

1. 449 samples out of which four ***T. pallidum*** positive & 445 ***T. pallidum*** negative samples were tested with **ELECTRA™ Tp. Ab. CLIA and with** compared with commercially available 3rd generation ***T. pallidum*** EIA.

| Specimen Data | Total | ELECTRA™ Tp. Ab. CLIA | *Other T. pallidum* EIA |
|---|---|---|---|
| **Total Specimens** | 449 | 449 | 449 |
| **Syphilis Reactive** | 4 | 4 | 4 |
| **Syphilis Non-Reactive** | 445 | 445 | 445 |

2. **ELECTRA™ Tp. Ab. CLIA** was also evaluated with 105 samples from RPR Syphilis Positive Research Panels (Panels obtained from SLR research, Carlsbad, CA).
3. **ELECTRA™ Tp. Ab. CLIA** was evaluated using In House syphilis Positive Panels (P.P/SY/E-09, & P.P/S/E-10) for sensitivity using serial dilution against other ***T. pallidum*** EIA.