

antibodies levels as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide only a guide.

Detection Limit

The analytical sensitivity (lower detection limit, 0 ± 2 SD) was established to be 2.3 U/ml.

PERFORMANCE CHARACTERISTICS

A) Internal Evaluation:

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

B) External Evaluation:

Electra™ Anti-CCP CLIA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **Electra™ Anti-CCP** has demonstrated 97% correlation with the reference method.

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

Important Note:

(1). The **Electra™ Anti-CCP** assay is a temperature sensitive assay. The best temperature condition for this assay is from 18°C to 22°C. (2). The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated RLU readings. (3). 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. (4). Duplication of Controls, 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 3 wash cycles, too small wash buffer volumes, or shortened reaction times) can lead to incorrect RLU values.

BIBLIOGRAPHY

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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.

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Certified Company

0120/VER-01

electra™
●●●●●●●●●● **Anti-CCP**

Chemiluminescence Assay for the quantitative measurement of Anti-Cyclic Citrullinated Peptides (CCP) present in human serum.

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

ELECTRA™ Anti-CCP CLIA is intended for the quantitative determination of IgG class autoantibodies against cyclic citrullinated peptides present in human serum. For In Vitro Diagnostic Use only.

INTRODUCTION

Rheumatoid arthritis (RA) is an inflammatory rheumatic disorder with a worldwide prevalence of about 0.5-1%. The serum of RA patients contains a variety of antibodies directed against self-antigens. The most widely known of these autoantibodies is the rheumatoid factor (RF) antibody directed against the constant domain of IgG molecules. Although the RF test has good sensitivity for RA, it is not very specific for the disease as it can also be detected in the serum of patients with other rheumatic or inflammatory diseases and even in a substantial percentage of the healthy (elderly) population. The RF antibodies are sensitive but not very specific markers; In contrast, Anti-CCPs are characterized by a specificity of over 90% in patients affected by RA and are detectable in a very early asymptomatic stage in the approximately 70% of RA patients whereas only 2% of the control subjects resulted positive. Therefore, the presence of Anti-CCP antibodies can be used in the diagnosis of RA, particularly in the case of erosive arthritis, in childhood in the case of juvenile RA. The Anti-CCP antibody test, together with the determination of RF, increases the ratio of sensitivity/specificity. The simultaneous positive result of a sample to RF and CCP has a positive predictive value of about 100%. The advantage of CCP antibodies is a higher sensitivity and specificity for the diagnosis of rheumatoid arthritis in comparison to the rheumatoid factors alone. Anti-CCP is often found at a very early state of the disease and it has a high predictive value for development of the disease.

PRINCIPLE

ELECTRA™ Anti-CCP Quantitative CLIA assay is for use on **ELECTRA** analyzers. **ELECTRA™ Anti-CCP 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 $\lambda=425\text{nm}$.

The **ELECTRA™ Anti-CCP CLIA**, purified cyclic citrullinated peptides (CCP) is coated on the surface of microwells. Diluted patient serum is added to wells and the specific antibody, if present, will bind to the antigen coated on the surface of the reaction well. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off, solution of chemiluminescent substrate is then added and Luminescence is measured in RLU. The light generated RLU is proportional to the amount of anti-CCP IgG antibodies in the sample. The concentration of the anti-CCP IgG antibodies in the sample is calculated through a standard curve.

MATERIALS & COMPONENTS

Materials provided with the test kits:

- Coated Microwells: Microwells coated with Cyclical citrullinated peptides.
- Anti-CCP Sample Diluent.
- Anti-CCP Enzyme Conjugate. Ready to use.
- Anti-CCP Negative Control.
- Anti-CCP Positive Control.
- Substrate A: Chemiluminescent substrate containing enhanced luminol solution.
- Substrate B: Chemiluminescent substrate containing stabilized peroxide solution.
- Anti-CCP 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: 10-100 μl , 20-200 μl , 100-1000 μl
- Disposable pipette tips
- Distilled water
- Disposable Gloves
- **ELECTRA™ Analyzer**

STORAGE AND STABILITY

1. **ELECTRA™ Anti-CCP** 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.

electra™ Chemiluminescence assay

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- Diluted wash buffer is stable up to one week at 2-8°C.
- Working Substrate (A+B) must be used immediately.

SPECIMEN COLLECTION

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

PRECAUTIONS

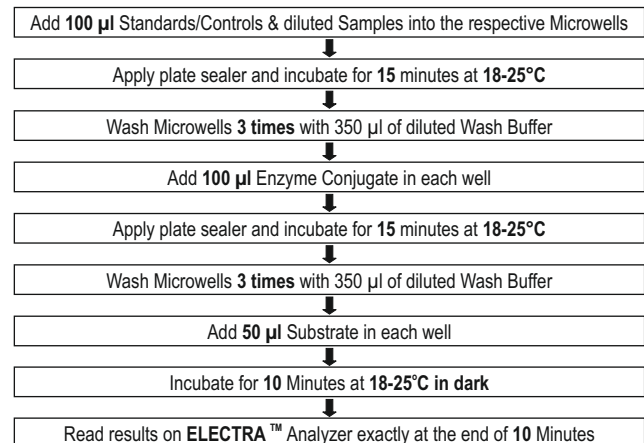
- Bring all reagents and specimen to room temperature before use.
- Do not pipette any material by mouth.
- Do not eat, drink or smoke in the area where testing is done.
- Use protective clothing and wear gloves when handling samples.
- Use absorbent sheet to cover the working area.
- Immediately clean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

Preparation for Assay

- 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

- Patient serum should be diluted 1:100 times before use. (Sample dilution: Add 5 µl of the patient sample to 500 µl of sample diluent). Mix well.
Important Note: The Anti-CCP standards, Negative Control and Positive Control have already been prediluted and are ready for use. Please DO NOT dilute again.
- Secure the desired number of coated wells in the holder.
- Dispense **100 µl** of Standards, Controls and diluted Serum into the appropriate wells. Dispense **100 µl** sample diluent in A1 well as Blank. Tap the holder to remove air bubbles from the liquid and mix well. Incubate at room temperature (18-25°C) for **15 mins**.
- Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 3 times with wash buffer (1X). Strike the microtiter plate sharply onto the absorbent paper or paper towels to remove all residual water droplets.
- Dispense **100 µl** of Enzyme Conjugate into each well. Incubate at room temperature (18-25°C) for **15 mins**.
- Remove the incubation mixture by emptying the plate (repeat step 4).
- Add **50 µl** of working Substrate (A+B) in all the micro-wells. Keep away from direct light while adding the substrate.
- Cover the **ELECTRA™** microplate and incubate for **10 minutes** at room temperature (**18-25°C**) in dark.
- 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.



CALCULATIONS

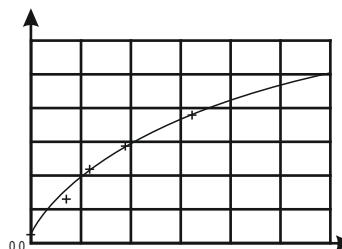
Construct a standard curve by plotting the RLU obtained from each reference standards against its Concentrations in U/ml on the graph paper, with RLU values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the RLU values for each specimen to determine the corresponding concentration of Anti-CCP in U/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 Anti-CCP concentrations shown in the X axis.

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

Anti-CCP Values (U/ml)	RLU's
A	38742
B	165060
C	265753
D	689201
E	1730500
F	3787523



This Standard curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain their own Standard curve and data.

Quality Control

The test run may be considered valid provided the following criteria are met:

- If the RLU value of the standard 6 is lower than 10000, the test is not valid and must be repeated.
- The concentration of controls should be in the range stated on the labels.
- The samples having an RLU value higher the Standard 6 (800 U/mL) should be subsequently diluted and the concentration of Anti CCP antibodies should be calculated applying the dilution factor.

Interpretation

In a normal range study with samples from 183 healthy blood donors the following ranges have been established as followings:

Cut-off: 10 U/ml
 Negative: < 10 U/ml
 Positive: ≥ 10 U/ml

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum anti-CCP