Anti-Cyclic Citrullinated Peptides (Anti-CCP)

ENZYME IMMUNOASSAY TEST KIT

Enzyme Linked Immunosorbent Assay (ELISA) for Quantitative Determination of IgG class autoantibodies against cyclic citrullinated peptides in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

Anti-CCP sandwich ELISA test is intended for the quantitative measurement 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 stage of the disease and it has a high predictive value for development of the disease.

PRINCIPLE OF THE ASSAY

Anti-CCP sandwich ELISA test which uses Purified cyclic citrullinated peptides (CCP) is coated on the surface of microwells. Patient serum is added to wells, and the specific antibody, if present, will bind to the antigen coated on the surface of the micro well. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off and TMB Substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated 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 AND 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. Ready to use.
- Anti-CCP Positive Control. Ready to use.
- TMB Substrate.
- Stop 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).
- Pack Insert
- Plate Sealer
- Protocol Sheet
- Microwell Holder

Materials required but not provided

- Precision pipettes: 10-100µl, 20-200µl, 100-1000µl
- Disposable pipette tips
- Distilled water
- Disposable Gloves
- ELISA reader
- ELISA washer

STORAGE AND STABILITY

1. **Anti-CCP** kit is stable at 2-8°C upto 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 pink at the time of opening the pouch, another coated microwells pouch should be used.
3. Diluted Wash Buffer is stable for upto one week when stored at 2-8°C.

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

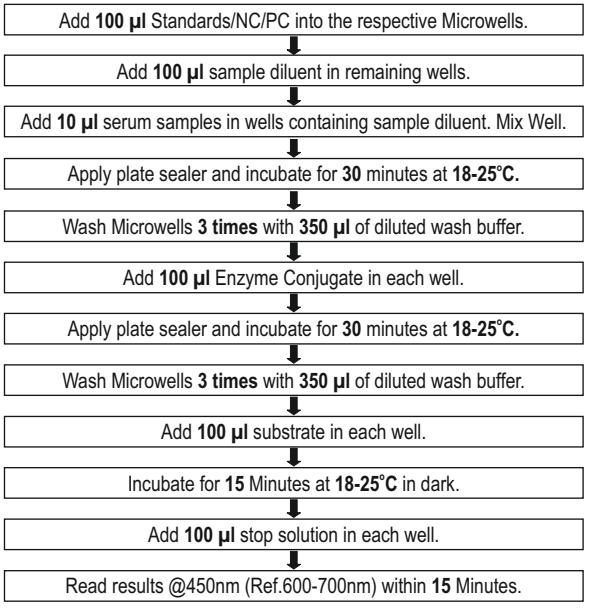
1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not eat, drink or smoke in the area where testing is done.
4. Use protective clothing and wear gloves when handling samples.
5. Use absorbent sheet to cover the working area.
6. Immediately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

1. 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.
2. Dilute Wash Buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.

TEST PROCEDURE

1. Bring all the reagents and patient samples to room temperature.
2. Secure the desired number of coated wells in the holder.
3. Dispense **100µl** Standards/NC/PC in appropriate wells.
4. Dispense **100µl** of sample diluent in remaining wells.
5. **Add 10µl of samples into appropriate wells containing sample diluent.** Tap the holder to remove air bubbles from the liquid and mix well. Incubate at room temperature (18-25°C) for **30 minutes**.
6. After incubation, empty the microtitre wells and wash the plate **3 times** with **350µl** of diluted wash buffer. Strike the microtitre plate sharply onto the absorbent paper towel to remove all residual droplets.
7. Dispense **100µl** of Enzyme Conjugate into each well. Incubate at room temperature (18-25°C) for **30 minutes**.
8. Remove the incubation mixture by emptying the plate (repeat step 6).
9. Dispense **100µl** of TMB substrate into each well. Incubate at room temperature (18-25°C), in the dark (without shaking) for **15 minutes**.
10. Stop the reaction by adding **100µl** of Stop Solution to each well. Gently mix for 10 seconds until the blue color completely changes to yellow.
11. Read the optical density at 450/630 nm with a microtiter plate reader within **15 minutes**.



CALCULATION OF RESULTS

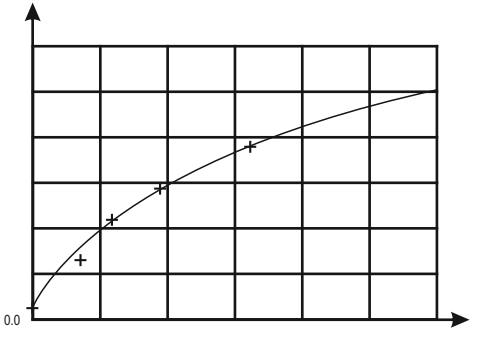
Construct a Standard curve by plotting the absorbance obtained from each reference standard against its concentrations in U/ml on the graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the absorbance values for each specimen to determine 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 optical density reading at 450nm (ref 600-700nm) 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)	Absorbance (450nm)
A	0.026
B	0.125
C	0.434
D	1.025
E	1.716
F	2.082



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.

Quality Control

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

1. The O.D. value of the reagent blank against air from a microwell reader should be less than 0.150.
2. If the O.D. value of the standard 6 is lower than 1.0, the test is not valid and must be repeated.
3. The concentration of controls should be in the range stated on the labels.
4. The samples having an OD value higher than Standard 6 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 with this ELISA assay:

Cut-off: 25 U/ml
Negative: < 25 U/ml
Positive: ≥ 25 U/ml

Detection Limit

The analytical sensitivity (lower detection limit, 0 ±2 SD) was established to be 1.9 U/ml.

PERFORMANCE CHARACTERISTICS

A) Internal Evaluation:

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

B) External Evaluation:

Anti-CCP ELISA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **Anti-CCP** ELISA has demonstrated 100% correlation with the reference method.
*Data file: Orchid Biomedicals (P) Ltd.

IMPORTANT NOTE

1. The Anti-CCP assay is a temperature sensitive assay. The best temperature condition for this assay is from 18°C to 25°C.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance 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 Standards, Controls & 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 OD values may vary. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the OD 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 OD values.

BIBLIOGRAPHY

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SYMBOL KEYS

Temperature Limitation		Consult Instructions for use	
Manufacturer		IVD	*In vitro* Diagnostic Medical Device
Use by		REF	Catalogue Number
Date of Manufacture		LOT	Batch Number / Lot Number
This side up		Σ	Contains sufficient for <n> tests
Do not reuse			

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