TurbiMAX Anti-CCP

Latex Enhanced Turbidimetric Immuno Assay (LETIA)

ORDERING INFORMATION

Ref./Cat. No.	Pack Size	Presentation
AVCCP-25	25 ml	Two Liquid Reagents with
AVCCP-50	50 ml	Calibrator

INTENDED USE:

TurbiMAX Anti-CCP is an in-vitro diagnostic kit for the quantitative determination of Anti Cyclic Citrullinated Peptide (CCP) Antibodies in Human Serum.

PRODUCT FEATURES

1. Latex Enhanced Turbidimetric Immuno Assay (LETIA)
2. Two Liquid Reagents (R1 & R2)
3. Available with 4 Level Calibrator format
4. Linearity : 300 U/mL
5. Measuring wavelength 546 nm. (540-630)
6. 30 +300 Seconds Fixed Time Assay.
7. Results correlate with Immuo Assays

CLINICAL SIGNIFICANCE:

Avecon's Anti-CCP Kit is a Latex Enhanced Turbidimetric Immuno Assay for the quantitative determination of the IgG class of auto antibodies specific to cyclic citrullinated peptide (CCP) in human serum on clinical chemistry analyzer platforms. Detection of anti-CCP antibodies is used as an aid in the diagnosis of Rheumatoid Arthritis (RA) and should be used in conjunction with other clinical information. Autoantibody levels represent one parameter in a multicriterion diagnostic process, encompassing both clinical and laboratory-based assessments

Rheumatoid Arthritis (RA) is a common, systemic autoimmune disease affecting 0.5-1% of the population. It is characterized by chronic inflammation of the synovium, which commonly leads to progressive joint destruction and in most cases to disability and reduction of quality of life.Evidence gained over the last few years suggests that aggressive therapy given early in the disease has the greatest therapeutic potential.

The serum of RA patients contains a variety of antibodies directed against self-antigens. The most widely known of these auto antibodies is the rheumatoid factor (RF) antibody directed against the constant domain of IgG molecules. The presence of RF is one of the American College of Rheumatology's (ACR) criteria for the classification of RA.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. on the contrary, 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 test also appears, to be useful in differentiating the collagen pathologies with concomitant arthritis from the RA. The Anti-CCP antibodies test has an important prognostic value in the monitoring of articular radiologically detectable damage. The kit's quantitative determination is useful in the control and verification of the effects of pharmacological therapy. The Anti-CCP antibody test, together with the determination of RF, increases the ratio of sensitivity / specificity. 20% of the RAs are RF-negative and 15/20% of the RAs are positive only to RF. The simultaneous positive result of a sample to RF and CCP has a positive predictive value of about 100%. The levels of anti-CCP antibodies are not necessarily correlated to the evolutionary stage of the illness. The advantage of the anti-CCP antibodies is that they are detectable in the patient sera up to 10 years prior to the appearance of symptoms. In addition, in cases of early arthritis a positive test result, according to some studies is related to the development of bone erosive lesions of the articulations.

PRINCIPLE:

The determination of the anti-CCP antibodies is based on the turbidimetric specific reaction which occurs between the antibodies present in the serum of patients affected by RA and highly purified synthetic Cyclic Citrullinated Peptide coated on the surface of latex microparticles. Reaction occurs under optimal pH conditions and in the presence of a polymeric enhancer. The turbidity of the immuno-complex is proportional to the concentration of the analyte in the examined sample.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the labels when stored at 2-8ºC and the contaminations is prevented during their use. Do not freeze the latex and diluent.

KIT COMPONENTS

1. Buffer Reagent R1
2. Turbi Latex Reagent R2
3. Anti CCP Calibrators (4) : Concentration as stated on the label

COMPOSITION

Reagent -1

Good's buffer, accelerator, Bovine Serum Albumin (BSA), sodium azide < 0.1%, Detergents and stabilizers.

Reagent-2

Good's buffer, Synthetic Citrullinated peptide coated on microparticles surface, sodium azide < 0.1%, detergents and stabilizers.

REAGENT RECONSTITUTION & STABILITY

Reagent are liquid stable no need for reconstitution.

When the reagent is stored properly at 2-8°C & the contamination avoided, it is stable up to the expiry date mention on the label & kit box.

MATERIAL REQUIRED BUT NOT PROVIDED

Laboratory Instrumentation, Spectrophotometer UV/VIS with thermostatic cuvette holder or clinical chemistry analyzer: semi auto, calibrated micropipettes, glass or high quality polystyrene cuvettes, test tube/rack, heating bath controls, saline.

REAGENT DETERIORATION

Discard reagent if blank reagent absorbance exceeds 1.2 at 546 nm Against distilled water.

WARNING & PRECAUTIONS

- Reagent may contain some non reactive and preservative components. It is recommended to handle carefully, avoiding contact with skin and ingestion.
- Specimen should be considered infectious and handled appropriately.
- Contamination by soap or glycerol will affect this assay.
- Perform the test according to the general " Good Laboratory Practice" GLP

SPECIMEN COLLECTION AND HANDLING:

Serum: Use unhemolyzed fresh serum collected by standard venipuncture techniques. Ensure complete clot formation has taken place prior to centrifugation. When processing samples, separate serum from blood cells or gel according to the specimen collection tube manufacturer's instructions. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may take longer to complete their clotting processes. Fibrin clots may subsequently form in these sera and the clots could cause erroneous test results.

Fresh Serum tested within 6 hours of collection would give authentic results.

Anti CCP Stability in Fresh Serum Samples 24 Hours at 2-8°C.
Frozen samples must not be used for testing

SYSTEM PARAMETERS:

Calibration Method	**Multi Point -Linear- Spline**
Reaction Type (Mode)	Fixed Time
Reaction Direction	Increasing
Wavelength:	546 nms (540 - 630)
Flow Cell Temp.	37°C
Delay Time	30 Seconds
Measuring Time	300 Seconds
Blank	Distilled Water Blank
Reagent Volume	300 µl (R1) + 100 µl (R2)
Sample Volume	20 µl
Calibrator Concentrations	(On the Vials Lot Specific)
Linearity	300 U/mL

TEST PROCEDURE

Reagent	Calibrator	Sample/Control
Anti CCP R1	300 µl	300 µl
Calibrators 1,2,3,4	20 µl	----
Serum Sample	—	20 µl
Mix and incubate for 5 Minutes at 37 °C		
Anti CCP R2	100 µl	100 µl

1) Read absorbance A1 after 30 Seconds. (Delay)
2) Incubate and Read the absorbance A2 after 300 Seconds (Measuring)
3) Calculate the absorbance differences ΔA=A2–A1 for each point of the calibration curve, controls and all unknown samples.
4) The concentration of Anti-CCP in the unknown sample can be calculated from ΔA=A2–A1
5) Using a 3rd order polynomial mathematical model where abscissa (X) is the ΔA=A2–A1 and ordinate (Y) is the concentration of Anti CCP or plotting the values of ΔA=A2–A1 obtained for every concentration level of the calibrator against the Anti CCP concentration and interpolating the individual ΔA=A2–A1 of every sample in the calibration curve.

Calculations with Calibrators/ Calibration Curve/ Result Interpretation:

CALCULATION:

The concentration of Anti-CCP in unknown samples is derived from a calibration curve using an appropriate mathematical models such as Multi Point / Linear/Spline. The calibration curve is obtained with 5 Level calibrators . Stability of calibration: 4 weeks

EXPECTED VALUE

Negative: Less than 70.0 U/mL
Weak Positive: 70-100 U/mL
Strong Positive: Above 100.0 U/mL

QUALITY CONTROL & CALIBRATION:

Use the following control materials to verify test accuracy:
Anti-CCP Control Set 2 x 0.5 mL Liquid human based control set.
2 levels human based liquid control with A-CCP values. For use, follow the instructions contained in the kit. Controls are ready to use.

PERFORMANCE CHARACTERISTICS

1. Linearity

Linearity : 300 U/ml

2. Sensitivity/ Limit of Detection (LOD)

The lower limit of detection is 2 U/ml

3. Interferences

No significant interference was observed from Bilirubin up to 20 mg/dl (Both conjugated and unconjugated Bilirubin) Hemoglobin up to 50 mg/dl,Lipemia as Triglycerides up to 2000 mg/dl, Ascorbic acid up to 50 mg/dl.

4. Precision:

Intra-Assay

N=20	Mean (U/mL)	SD (U/mL)	CV%
Control serum 1	25.8	0.5	1.93
Control serum 2	65.2	0.61	0.93
Control serum 3	98.3	1.2	1.22
Inter-Assay			
Control serum 1	25.9	0.51	1.96
Control serum 2	65.7	0.71	1.08
Control serum 3	98.7	1.3	1.31

5. Method Comparison:

A comparison of the TurbiMAX Anti-CCP (y) with a commercial obtainable assay (x) gave the following result : y = 1.113 x – 0.278; r = 0.990

LIMITATIONS

Measuring range: 2-300 U/ml. Determine samples having higher concentrations manually dilute with 0.9% NaCl or distilled/deionized water (e.g. 1 + 1). Multiply the result by the appropriate dilution factor (e.g. 2).

WASTE DISPOSAL

Reagents must be disposed off in accordance with local regulations.

REFERENCE

1. Bizzaro N. et al. Diagnostic Accuracy of the anti-citrulline antibody assay for rheumatoid arthritis. Clin Chem 47:6, 1089-1093,2001
2 Schellekens G. et al. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. Arthritis Rheum 43:155-163 (2000)
3. Baeten D. et al. Specific presence of intracellular citrullinated proteins in rheumatoid arthritis synovium. Arthritis Rheum 44:2255-2262 (2001)
4 del Val del Amo N, Ibanez Bosch R, Fito Manteca C, et al. Anti-cyclic citrullinated peptide antibody in rheumatoid arthritis: relation with disease aggressiveness. Clin Exp Rheumatol. 2006;24(3):281-6
5 Samanci N, Ozdem S, Akbas H, et al. Diagnostic value and clinical significance of anti-CCP in patients with advancedrheumatoid arthritis. J Natl Med Assoc. 2005;97(8):1120-6
6 Matsui T, Shimada K, Ozawa N, et al. Diagnostic utility of anti-cyclic citrullinated peptide antibodies for very early rheumatoid arthritis. J Rheumatol. 2006;33(12):2390-7

Symbols Used on Pack

REF	Catalogue Number		Warning/Caution
LOT	Batch No.	IVD	In vitro diagnostic device
	Manufacturing Date		Storage Limit
	Expiry Date		Consult instruction for use
	Manufacturer		Keep away from sunlight
	Keep Dry		Do not use if package is damaged
	Contains sufficient no. of test		

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