Pariksha Biotech
A game changer in IVD

Anti CCP Estimation Kit

High-Q Anti CCP (5 th Gen)

Latex Enhanced Turbidimetric Immuno Assay (LETIA)

HIGH®
Command on Quality

Intended Use:
Kit for the quantitative determination of Anti Cyclic Citrullinated Peptide (CCP) Antibodies in Human Serum

Clinical Significance:
Pariksha'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

Summary and Explanation of Test:
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.

Reagents:
The components of the kit, stored at 2-8 °C in unopened vials, are stable up to the expiry date indicated on the package.

Specimen collection and handling:
Serum: Use nonhemolyzed fresh serum collected by standard venipuncture techniques. Ensure complete clot formation has taken place prior to centrifugation. **When processing samples, separate serum immediately from blood cells or gel after centrifugation . Do not keep the serum with RBC for long time** . 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 separated from RBC and 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

Assay Procedure: Multi Point Calibration with 4 Level Calibrators

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

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 4 Level calibrators . Stability of calibration: 4 weeks

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Components of the kit and concentration of reactive ingredients:
Reagent- 1 and Reagent- 2 are ready to use.

Reagent -1
Good's buffer, accelerator, Bovine Albumin, 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.

Calibration:
For the calibration, use the following material:

Anti CCP Cal Set 4**x 0.5 mL** Liquid human based calibrator set. For use, follow the instructions contained in the kit. Calibrators are ready to use.

Quality Control:

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.

Reference Range:
The Anti-CCP test is one of several RA blood tests that are used in the diagnosis and management of the disease.
The Anti-CCP test is generally considered one of the more reliable tests for RA. Ninety percent of people who have an elevated Anti CCP have RA. However, it's important to know that Anti CCP can also be elevated for other reasons, such as other autoimmune diseases. As well, some people who have RA don't have an elevated Anti CCP. Generally, the range of values and their implications are as follows:

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

Other RA blood tests include the CRP and ESR, both inflammation markers. When elevated, these indicate inflammation in the body, such as what happens it in cases of active RA, but also if you're brewing a sinus infection. The Rheumatoid Factor is usually positive if you have RA, but up to 30% of people with RA are negative for rheumatoid factor. This is called having seronegative RA.

Keep in mind that interpreting blood tests is a tricky business and should not be done in isolation of a physical exam. Often, taking a look at your joints and hearing your medical history will tell an experienced rheumatologist more about whether you have RA than a blood test. Your best bet is to talk to your doctor about the specifics of your case.

Literature:

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

ORDERING INFORMATION

Ref./Cat.	Pack Size	Presentation
P-ACCP-25	25 ml	(18.75 ml R1 + 6.25 ml R2 with 4 Calibrators)
P-ACCP-50	2 x 25 ml	(2 x 18.75 ml R1 + 2 x 6.25 ml R2 with 4 Calibrators)

Product Features

- Latex Enhanced Turbidimetric Immuno Assay (LETIA)
- Multi Point / Linear / Fixed Time Assay
- Two Liquid Reagents (R1 & R2)
- Available with 4 Level Calibrator format
- Linearity : 300 U/mL
- Measuring wavelength 546 nm. (540-630)
- 5 Minutes Fixed Time Assay.
- Results correlate with Immuo Assays

Symbols used with IVD devices

	Date of manufacture		Manufactured by
IVD	In vitro diagnostic device		Keep away from sunlight
	Do not freeze		This way up
	Use by (yyyy-mm-dd or mm/yyyy)	R	Reagent
CAL	Calibrator Material	LOT	Batch code
2°C 8°C	Temperature limitation (store at)	CONTROL	Control
	Consult instructions for use		Keep dry Keep away from rain
REF	Catalog Number		

eIFU Indicator

Pariksha's world inside
SCAN TO EXPLORE MORE

Manufactured in India by :
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AN ISO 13485 Certified Company

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