Rheumatoid Arthritis

ichroma™ Anti-CCP Plus

INTENDED USE

ichroma™ Anti-CCP Plus is a fluorescence Immunoassay (FIA) for the qualitative or semi-quantitative determination of human IgG autoantibodies to cyclic citrullinated peptides (CCP) in human whole blood/serum/plasma. It is useful as an aid in the diagnosis of rheumatoid arthritis (RA) in combination with other clinical and laboratory findings.

For *in vitro* diagnostic use only.

INTRODUCTION

Rheumatoid Arthritis (RA) is a common, systemic autoimmune disease affecting 0.5-1.0% of the world population. RA is characterized by chronic inflammation of the synovium which can lead to progressive joint destruction, disability and mortality.[1] As joint damage is irreversible, early therapeutic intervention is of paramount importance for the prognosis of patients.[2,3]

The diagnosis of rheumatological disease are the medical history, clinical findings (including imaging techniques) and serological laboratory tests. Serological diagnostic testing is of growing importance in the early detection and differentiation of RA. The most frequent serological diagnostic testing is the measurement of rheumatoid factor (RF).[4] The RF antibody is present in about 75% of RA patients, but its specificity is limited, as it is often present in healthy individuals and patients with other rheumatic or inflammatory diseases, autoimmune diseases or chronic infections.[5]

More recently, new specific autoantibodies to citrullinated proteins antigens (ACPAs) have made a crucial contribution to the diagnosis of RA.[6] Although many assays are available to test for ACPAs to specific antigens, for the clinical management of RA, most ACPA testing is performed using a synthetic cyclic citrullinated protein (CCP) as the antigen to detect ACPAs. An anti-CCP assay is capable to detect the autoantibodies against citrullinated proteins which have a relatively high sensitivity (reportedly between 50-75%) for rheumatoid arthritis and extremely high specificity (about 90%) for RA.[7] Its high specificity is why the anti-CCP test has become an important part of the diagnostic process for RA.

PRINCIPLE

The test uses a sandwich immunodetection method; the antigens in the detector bind to antibodies in the sample, forming antigen-antibody complexes, and migrate onto nitrocellulose matrix to be captured by the immobilized-streptavidin on test strip.

More antibodies in the sample will form more antigen-antibody complexes which lead to stronger fluorescence signal by detector antigens, which is processed by instrument for ichroma tests to show Anti-CCP concentration in the sample.

COMPONENTS

ichroma™ Anti-CCP Plus consists of 'Cartridges', 'Detector tubes', 'Detector diluent', 'Capillary tubes', 'ID chip' and 'Instruction for use'.

- The cartridge contains the membrane called a test strip, which has streptavidin at the test line, and chicken IgY at the control line. All cartridges are individually sealed in an aluminum foil pouch containing a desiccant in a box.
- The detector tube has a granule containing anti-human Immunoglobulin G-fluorescence conjugate, anti-chicken IgY-fluorescence conjugate, CCP-biotin conjugate, bovine serum albumin (BSA) as a stabilizer, sucrose, bromophenol blue in phosphate buffered saline. All detector tubes are packed in a pouch.
- The detector diluent contains sodium azide as a preservative, bovine serum albumin (BSA) as a stabilizer, tween 20 in phosphate buffered saline, and it is pre-dispensed in a vial. The detector diluent is packed in a box.

WARNING AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow instructions and procedures described in this 'Instruction for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge, detector tube, detector diluent and ID Chip) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, as either of which might yield incorrect test result(s).
- Do not reuse cartridges or detector. A cartridge should be used for testing one sample only. A detector tube should be used for processing of one sample only.
- The cartridge should remain sealed in its original pouch until just before use. Do not use the cartridge, if its pouch is damaged or has already been opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with local regulations. Sample with severe hemolysis and/or hyperlipidemia must not be used.
- Allow cartridge, detector tube, detector diluent and sample to be at room temperature for approximately 30 minutes before use.
- The instrument for ichroma™ tests may generate slight vibration during use.
- Used cartridges, detector tubes, detector diluent, capillary tubes and pipette tips should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- An exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.
- **ichroma™ Anti-CCP Plus** will provide accurate and reliable results subject to the below conditions.
  - **ichroma™ Anti-CCP Plus** should be used only in conjunction with the instrument for ichroma™ tests.
  - Have to use recommended anticoagulant sample.

Recommended anticoagulant

Na_2 EDTA, K_2 EDTA, Sodium citrate

STORAGE AND STABILITY

Storage condition			
Component	Storage Temperature	Shelf life	Note
Cartridge	4 - 30 °C	20 months	Disposable
Detector tube	2 - 8 °C	20 months	Disposable
Detector diluent	2 - 8 °C	20 months	Unopened
	2 - 8 °C	20 months	Opened

- After the cartridge pouch is opened, the test should be performed immediately.

LIMITATION OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result(s) due to the non-responsiveness of the antigen to the antibodies which is most common if the epitope is masked by some unknown components, so therefore not being able to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may also cause false negative result as it makes antigen unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician including clinical symptoms and other relevant test results.

MATERIALS SUPPLIED

REF CFPC-97

Components of **ichroma™ Anti-CCP Plus**

- Cartridge Box:
  - Cartridge 25
  - 5 µL Capillary tube 25
  - ID Chip 1
  - Instruction for Use 1
- Buffer box
  - Detector tube 25
  - Detector diluent 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **ichroma™ Anti-CCP Plus**.

Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ II** REF FPRR021
- **i-chamber** REF FPRR009
- **Boditech Anti-CCP Plus Control** REF CFPO-288

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ Anti-CCP Plus** is human whole blood/serum/plasma.

- It is recommended to test the sample within 24 hours after collection.
- The serum or plasma should be separated from the clot by centrifugation within 3 hours after the collection of whole blood.
- The serum, plasma and whole blood may be stored for up to a month at 2-8 °C prior to being tested. If testing will be delayed more than a month, samples should be frozen at -20 °C.
- The freezing storage of sample up to 3 months does not affect the quality of results. However, the whole blood sample should not be kept in a freezer in any case.
- Once the sample was frozen, it should be used one time only for test, because repeated freezing and thawing can result in the change of test values.
- Fingertip blood sample should be collected as follows:
  - Position the hand with the palm facing upwards. Blood should be normally drawn from the middle or ring finger of the non-dominant hand. Apply intermittent pressure towards its tip.
  - Wipe the fingertip clean with an alcohol pad.
  - Allow the finger to dry completely because blood will not form a drop if the puncture site is moist and because the residual alcohol at the fingertip may dilute the blood sample and affect the test result.
  - Hold the finger and puncture the fingertip by firmly pressing a new sterile lancet against it at an off-center position.
  - Wipe away the first drop of blood with a sterile gauze pad or cotton ball.
  - Massage the finger towards its tip to form a new drop of blood. Blood will flow easily if the finger is held lower than the elbow.
  - Hold the handle of a capillary tube and touch the mouth of the capillary to the drop of blood.
  - Let the blood fill the capillary tube completely.
  - It may be sometimes necessary to massage the finger again for an additional drop of blood for filling the capillary tube.

TEST SETUP

- Check the contents of **ichroma™ Anti-CCP Plus**: Sealed Cartridges, Detector tubes, Detector diluent, Capillary tubes, ID Chip and Instruction for use.
- Ensure that the lot number of the cartridge matches that of the buffer box as well as an ID Chip.
- If the sealed cartridge, the detector tube and the detector diluent have been stored in a refrigerator, place them on a clean and flat surface at room temperature for at least 30 minutes before testing.
- Turn on the instrument for ichroma™ tests.
  (Please refer to the 'Instrument for ichroma™ Tests Operation Manual' for complete information and operating instructions.)

CAUTION

- To minimize erroneous test results, we suggest that the ambient temperature of the cartridge should be 25 °C during the reaction time after loading sample mixture to the cartridge.
- To maintain the ambient temperature to 25 °C, you can use various devices such as an i-Chamber or an incubator and so on.

TEST PROCEDURE

1) Transfer 150 µL of detector diluent using a pipette to a detector tube.
2) Transfer 5 µL of sample (Human whole blood/serum/plasma/control) to the detector tube.
   * If you use a capillary tube (5 µL), put it into the detector tube after collecting whole blood sample.
3) Close the lid of the detector tube and mix the sample thoroughly by shaking it about 20 times.
4) Pipette out 75 µL of a sample mixture and load it into the sample well on the cartridge.
5) Insert and leave the sample mixture-loaded cartridge in the i-Chamber or incubator (25 °C) for 12 minutes.
6) To scan the sample mixture-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow is marked on the cartridge especially for this purpose.

   ⚠ *Scan the sample mixture-loaded cartridge immediately when the incubation time is over. If not, it will cause inaccurate test result.*
7) Tap the 'Start' button on the instrument for ichroma™ tests to start the scanning process.
8) The instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
9) Read the test result on the display screen of the instrument for ichroma™ tests.

Please refer to the 'Instrument for ichroma™ Tests Operation Manual' for complete information and operating instructions.

INTERPRETATION OF TEST RESULT

- The instrument for ichroma™ tests calculates the test result automatically and displays anti-CCP concentration and anti-CCP state of the test sample.

Test result [U/mL]	Display [U/mL]
< 5.0	< 3.5 or value, (Neg)
5.0 ≤, < 300	Value, (Pos)

- Cut-off : 5.0 U/mL
- Working range : 3.5 - 300.0 U/mL

QUALITY CONTROL

- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- The control tests should be performed immediately after opening a new test lot to ensure the test performance is not altered.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are not provided with **ichroma™ Anti-CCP Plus**. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance.
  (Please refer to the instruction for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical sensitivity**
  - Limit of Blank (LoB) 2.32 U/mL
  - Limit of Detection (LoD) 3.49 U/mL
- **Analytical specificity**
  - Cross-reactivity
    Biomolecules such as below the ones in the table were added to the test sample(s) at concentrations much higher than their normal physiological levels in the blood. **ichroma™ Anti-CCP Plus** test results did not show any significant cross-reactivity with these biomolecules.

Material	
α-SSA	α-Jo-1
α-SSB	α-Scl-70
α-Sm	α-Ribo-P
α-RNP	anti-nulcear antibody(ANA)
α-ds-DNA	

  - Interference
    Interference materials such as below the ones in the table were added to the test sample(s) the same as the below concentrations. **ichroma™ Anti-CCP Plus** test results did not show any significant interference with these materials.

Interference material	Concentration
Hemoglobin	500 mg/dL
Bilirubin	0.2 mg/mL
Triglyceride	2,000 mg/dL
Rheumatoid factor	78 IU/mL
Human serum albumin	12 g/dL

- **Precision**
  - Between lot
    One person tested three different lots of **ichroma™ Anti-CCP Plus**, ten times at each concentration of the control standard.
  - Between person
    Three different people tested **ichroma™ Anti-CCP Plus**; ten times at each concentration of the control standard.
  - Between day
    One person tested **ichroma™ Anti-CCP Plus** for five days; five times at each concentration of the control standard.
  - Between site
    One person tested **ichroma™ Anti-CCP Plus** at three different sites; five times at each concentration of the

control standard.

Anti-CCP	Between lot		Between person		Between day		Between site	
[U/mL]	AVG	CV(%)	AVG	CV(%)	AVG	CV(%)	AVG	CV(%)
6.25	6.22	5.0	6.21	5.9	6.25	6.6	6.13	5.2
30.00	29.61	5.7	30.39	5.3	29.98	5.7	29.50	6.2
100.00	99.42	6.6	99.27	6.5	100.64	5.6	98.55	6.9

■ **Accuracy**

The accuracy was determined by 3 different lots testing six times each human serum.

Anti-CCP [U/mL]	Lot 1	Lot 2	Lot 3	Mean	CV (%)	Bias (%)
4.15	3.83	3.86	3.87	3.86	2.5	-7.1
5.31	5.05	4.88	4.91	4.95	3.3	-6.9
16.66	15.21	15.32	15.17	15.23	1.8	-8.6
19.79	18.53	18.07	18.38	18.33	4.0	-7.4
67.02	67.16	67.67	66.55	67.13	2.2	0.2

■ **Comparability**

Total (N=216)		ichroma™ Anti-CCP Plus	
		Positive	Negative
Axis-shield FCCP600	Positive	109	7
	Negative	4	96
Positive agreement rate (≥5 U/mL) (%)		93.9	
Negative agreement rate (< 5 U/mL) (%)		96.0	
Total (%)		94.9	

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Note: Please refer to the table below to identify various symbols

Σ	Sufficient for <n> tests
	Read instruction for use
	Use by Date
LOT	Batch code
REF	Catalog number
	Caution
	Manufacturer
EC REP	Authorized representative of the European Community
IVD	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
CE	This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices

For technical assistance; please contact:
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