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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 other immobilized-streptavidin on a 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' and 'detector diluent.

- 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, and they are further packaged 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 'Instructions 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 tubes. 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 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.
- If test components and/or sample are stored in refrigerator, then 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 tube and pipette tips should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.

- The detector tube and the detector diluent contain sodium azide (NaN_3), and they may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure. Avoid contact with skin, eyes, and clothing. In case of contact, rinse immediately with running water.
- No Biotin interference was observed in **ichroma™ Anti-CCP Plus** when biotin concentration in the sample was below 2 ng/mL. If a patient has been taking biotin at dosage of more than 0.03 mg a day, it is recommended to test again 24 hours after discontinuation of biotin intake.
- **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.

Recommended anticoagulant
Na_2 EDTA, K_2 EDTA, Sodium citrate

- **The capillary tube should be used when the following conditions are met.**
  - The capillary tube provided with the kit is recommended to obtain correct test result.
  - Whole blood should be immediately tested after collection.
  - Excess whole blood around the capillary tube should be wiped off.
  - In order to avoid cross-contamination, please do not re-use capillary tube for multiple samples.

STORAGE AND STABILITY

Storage condition			
Component	Storage Temperature	Shelf life	Note
Cartridge	2 - 30 °C	20 months	Disposable
Detector tube	2 - 8 °C	20 months	Disposable
Detector diluent	2 - 30 °C	20 months	Unopened
	2 - 30 °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 in conjunction with 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
- Detector tube 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
  - **ichroma™ III** **REF** FPRR037
  - **ichroma™ M2** **REF** FPRR031
- **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 samples (serum, plasma) should be separated from the clot by centrifugation within 3 hours after the collection of whole blood.
- The samples (whole blood, serum, plasma) 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 (serum, plasma) should be frozen at -20 °C.
- The samples (serum, plasma) stored frozen at -20 °C for 3 months showed no performance difference.
- However, the whole blood sample should not be kept in a freezer in any case.
- As a repeated freeze-thaw cycle may affect the test result, do not refreeze previously frozen samples.
- Whole blood sample may be used to collect according to below:
  ① Wear disposable gloves and protective equipment for safety.
  ② Open the zipper bag which has capillary tubes.
  ③ Take out the capillary tube and check for damage or contamination.
  ④ Hold the handle of the capillary tube and touch the surface of blood with the capillary tube.

⑤ Fill it with blood completely.
(Make sure that no air bubbles are present in the capillary bubble. Do not get blood on the surface of the capillary tube. If the blood gets on the surface of the capillary tube, remove it gently with gauze.)

TEST SETUP

- Check the contents of **ichroma™ Anti-CCP Plus**: Sealed cartridges, detector tubes, a detector diluent, capillary tubes, ID chip and instructions for use.
- Ensure that the lot number of the cartridge matches that of the detector tube, the detector diluent 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.
- Insert the ID chip into the 'ID chip port'.

※ **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

▸ **ichroma™ II, ichroma™ M2**

Multi test mode / Read now mode

1) Take 150 µL of detector diluent using a pipette and dispense it to the detector tube containing a granule. When the granule form is completely dissolved in the tube, it becomes detection buffer.
(The detection buffer must be used immediately. Do not exceed 30 seconds.)
2) Take 5 µL of sample (Human whole blood/ serum/plasma/control) using a pipette and dispense it to the detector tube. Close the lid of the detector tube and mix the sample thoroughly by shaking it about 20 times.
(The sample mixture must be used immediately. Do not exceed 30 seconds.)
* If you use capillary tube (5 µL), put it into the detector tube after collecting whole blood sample.
3) Take 75 µL of the sample mixture and dispense it into the sample well of the cartridge.
4) Insert the sample-loaded cartridge into the slot of the i-Chamber or an incubator (25 °C).
5) Leave the sample-loaded cartridge in the i-Chamber or an incubator for 12 minutes.

⚠ *Scan the sample mixture-loaded cartridge immediately when the incubation time is over. If not, it will cause inaccurate test result.*

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.
7) Press 'Select' or tap the 'Start' button on the instrument for ichroma™ tests to start the scanning process.
(ichroma™ M2 is tested automatically after inserting.)
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.

▸ **ichroma™ III**

1) The test procedure is same with the 'ichroma™ test procedure 1) - 3)'.
2) Insert the sample-loaded cartridge into the holder of the instrument for ichroma™ III. 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.
3) Tap the 'Start' button on the instrument for ichroma™ III to start scanning process.
4) The ichroma™ III will start scanning the sample-loaded cartridge immediately.
5) Read the test result on the display screen of ichroma™ III.

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 in terms of U/mL.

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.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are provided on demand 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 instructions 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 listed in the following 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
  Interferents listed in the following tables were added to the test sample at the concentration mentioned below. **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**, 10 times at each concentration of the control standard.
- Between person
  Three different people tested **ichroma™ Anti-CCP Plus**; 10 times at each concentration of the control standard.
- Between day
  One person tested **ichroma™ Anti-CCP Plus** for 5 days; 5 times at each concentration of the control standard.
- Between site
  One person tested **ichroma™ Anti-CCP Plus** at 3 different sites; 5 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 confirmed by 3 different lots of **ichroma™ Anti-CCP Plus**. The tests were repeated 6 times at each concentration of the 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
Comparator A	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

	Sufficient for <n> tests
	Read instruction for use
	Use by Date
	Batch code
	Catalog number
	Caution
	Manufacturer
	Authorized representative of the European Community
	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
	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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