BIO TECHNOLOGY boditech

Rheumatoid Arthritis

ichroma™ Anti-CCP

INTENDED USE

ichroma™ Anti-CCP 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

A synthetic cyclic citrullinated peptide (CCP) is immobilized on a porous membrane. After a sample is added to the sample port, detection buffer, consisting of fluorescent conjugated polyclonal antibody to human IgG, is loaded to the buffer port. The more anti-CCP antibodies in sample forms the more the peptide antigen/anti-CCP antibodies complex and leads to stronger intensity of fluorescence signal on detector anti-human IgG, which is processed by instrument for ichroma™ tests to show anti-CCP level in sample.

COMPONENTS

ichroma™ Anti-CCP consists of 'Cartridges', 'Detection Buffer Tubes', 'Sample collectors' and an 'ID chip'.

- The cartridge contains a test strip, the membrane which has synthetic cyclic citrullinated peptide (CCP) at the test line, while chicken IgY at the control line.
- Each cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed cartridges are packed in a box which also contains an ID chip and 25 sample collectors.
- The detection buffer contains anti-human Immunoglobulin G-fluorescence conjugate, anti-chicken IgY-fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide in phosphate buffered saline (PBS) as a preservative.
- The detection buffer is dispensed in a tube. 25 detection buffer tubes are packaged in a box and further packed in a Styrofoam box with ice-pack for the shipment.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Carefully follow the 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, ID chip and detection buffer) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield misleading of test result(s).
- Do not reuse. A detection buffer tube should be used for processing one sample only. So should a cartridge.
- The cartridge should remain sealed in its original pouch before use. Do not use the cartridge, if is damaged or already opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with the regulations. Sample with severe hemolytic and hyperlipidemia cannot be used and should be recollected.
- Just before use, allow the cartridge, detection buffer and sample to be at room temperature for approximately 30 minutes.
- **ichroma™ Anti-CCP** as well as the instrument for ichroma™ tests should be used away from vibration and/or magnetic field. During normal usage, it can be noted that instrument for ichroma™ tests may produce minor vibration.
- Used detection buffer tubes, pipette tips and cartridges 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** will provide accurate and reliable results subject to the following conditions.
  - Use **ichroma™ Anti-CCP** should be used only in conjunction with instrument for ichroma™ tests.
  - Any anticoagulants other than **EDTA, sodium citrate** should be avoided.

STORAGE AND STABILITY

- The cartridge is stable for 20 months (while sealed in an aluminum foil pouch) if stored at 4-30 °C.
- The detection buffer dispensed in a tube is stable for 20 months if stored at 2-8 °C.
- 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 peptide/detector antibody.
- The test may yield false negative result. The non-responsiveness of the antigen to the antibodies is most common where the epitope is masked by some unknown components, so as not to be detected or captured by the antibodies. The instability or degradation of the antibody with time and/or temperature may cause the false negative as it makes antibody 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-51

Components of **ichroma™ Anti-CCP**

- Cartridge Box:
  - Cartridges 25
  - Sample collectors 25
  - ID Chip 1
  - Instruction For Use 1
- Box containing Detection Buffer Tubes
  - Detection Buffer Tubes 25

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **ichroma™ Anti-CCP**. Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ Reader** REF FR203
  - **ichroma™ II** REF FPRR021
- **ichroma™ Printer** REF FPRR007
- **Boditech Anti-CCP Control** REF CFPO-125

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ Anti-CCP** 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. If longer storage is required, e.g. if the test could not be performed within 24 hours, serum or plasma should be immediately frozen below -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.

TEST SETUP

- Check the contents of **ichroma™ Anti-CCP**: Sealed Cartridge, Detection Buffer Tubes, Sample collectors and ID Chip.
- Ensure that the lot number of the cartridge matches that of the ID chip as well as the detection buffer.
- Keep the sealed cartridge (if stored in refrigerator) and the detection buffer tube at room temperature for at least 30 minutes just prior to the test. Place the cartridge on a clean, dust-free and flat surface.
- Turn on the instrument for ichroma™ tests.
- Insert the ID Chip into the ID chip port of the instrument for ichroma™ tests**.**
- Press the 'Select' button 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) Make a puncture on the top of the detection buffer tube by inserting an empty sample collector.
2) Load 5 µL (Human serum / plasma / control) or 10 µL (Human whole blood) of sample using a pipette into the sample well on the cartridge. (Sample well is a square shape.)
3) Assemble the sample collector and the detection buffer tube into one and shake the 2 times or more.
4) Remove the cap off the top of assembled tube. Discard one drops of detection buffer onto the paper towel before loading.
5) Load only 3 drops of the detection buffer onto the detection buffer (DB) well of the cartridge. (DB well is a round shape.)
6) Insert and leave the sample/DB-loaded cartridge in the i-Chamber or incubator (25 °C) for 12 minutes.
   ⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inexact test result.*
7) To scan the sample-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 has been marked on the cartridge especially for this purpose.
8) Press 'Select' button on the instrument for ichroma™ tests to start the scanning process.
9) Instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
10) Read the test result on the display screen of the instrument for ichroma™ tests.

INTERPRETATION OF TEST RESULT

- Instrument for ichroma™ tests displays anti-CCP concentration and anti-CCP state of the test sample.

Test result [U/mL]	Display	
	Ichroma™ Reader	ichroma™ II
< 5.0	Negative, <5.0 U/mL	< 5.0 U/mL (Neg)
5.0 ≤, <200	Positive, Value	Value, (Pos)

- The cut-off : 5.0 U/mL
- Working range : 5.0-200.0 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**. 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)	0.76 U/mL
Limit of detection	(LoD)	1.67 U/mL

- **Analytical specificity**
  - Interference

There was no significant interference from these materials with the **ichroma™ Anti-CCP** test measurements.

Interference material	Standard material Conc. (U/mL)		
	6.25	30	100
	Interference rate (%)		
Hemoglobin	2.2	4.4	0.7
Bilirubin	6.1	4.3	2.0
Triglyceride	1.7	1.9	1.0
Rheumatoid factor	9.2	9.0	8.8
Human serum albumin	7.5	7.5	4.6

- Cross-reactivity
  There was no significant cross-reactivity from these materials with the **ichroma™ Anti-CCP** test measurements.

cross reactivity materials	Standard material Conc. (U/mL)	
	50	100
	Recovery (%)	
anti-SSA	94.9	99.5
anti-SSB	97.0	101.3
anti-Scl70	97.0	103.1
anti-Jo-1	103.6	109.8
anti-RNP	97.0	99.5
anti-Sm	93.5	95.7
anti-dsDNA	99.8	100.7
anti-Ribo-P	98.4	100.4
ANA (anti-nuclear antibody)	98.8	100.4

- **Precision**

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

Anti-CCP [U/mL]	Between lot		Between person		Between day		Between site	
	AVG	CV(%)	AVG	CV(%)	AVG	CV(%)	AVG	CV(%)
6.25	6.64	5.0	6.73	7.9	7.05	6.0	6.90	8.2
25.00	31.77	1.8	31.88	7.7	32.27	7.4	31.95	8.6
100.00	106.30	5.0	107.70	7.8	109.67	4.8	111.86	6.7

- **Accuracy**
  The accuracy was confirmed by 3 different lots testing six times each different concentrations.

Anti-CCP [U/mL]	Lot 1	Lot 2	Lot 3	Mean	CV (%)	Bias (%)
4.13	3.79	3.79	3.81	3.80	3.81	-7.9
5	4.66	4.67	4.64	4.65	2.99	-6.9
16	15.23	15.55	15.37	15.39	3.04	-3.8
19	18.37	17.97	18.28	18.20	3.36	-4.2
65	66.81	67.42	66.41	66.88	2.04	2.9

- **Comparability**

Total (N=216)		n	ichroma™ Reader		ichroma™ II	
			Positive	Negative	Positive	Negative
Axis-shield FCCP600	Positive	116	99	17	100	16
	Negative	100	2	98	1	99
Total sample		216	216		216	
Positive (≥5 U/mL) Agreement			85%		86%	
Negative (< 5 U/mL) Agreement			98%		99%	
Total			92%		93%	

REFERENCES

1. Birch JT Jr, Bhattacharya S. Emerging trends in diagnosis and treatment of rheumatoid arthritis. Prim Care. 2010;**37**:779–92.
2. Landewe RB. The benefits of early treatment in rheumatoid arthritis: confounding by indication, and the issue of timing. *Arthritis Rheum* 2003;**48**(1):1-5.
3. Lard LR, Visser H, Speyer I, *et al.* Early versus delayed treatment in patients with recent-onset rheumatoid arthritis: comparison of two cohorts who received different treatment strategies. *Am J Med* 2001;**111**:446-51.
4. Mageed RA. The RF antigen. In Manual of Biological Markers of Disease Edited by van Venrooij WJ & Maini RN Dordrecht: Kluwer Academic Publishers; 1996. pp. 1–27.
5. van Boekel MA, Vossenaar ER, van den Hoogen FH, van Venrooij WJ. Autoantibody systems in rheumatoid arthritis: specificity, sensitivity and diagnostic value. Arthritis Res2002;**4**:87–93
6. Systematic review: accuracy of anti-citrullinated Peptide antibodies for diagnosing rheumatoid arthritis.Whiting PF, Smidt N, Sterne JA, Harbord R, Burton A, Burke M, Beynon R, Ben-Shlomo Y, Axford J, Dieppe P Ann Intern Med. 2010 Apr 6; **152**(7):456-64; W155-66.
7. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. Schellekens GA, Visser H, de Jong BA, van den Hoogen FH, Hazes JM, Breedveld FC, van Venrooij WJ Arthritis Rheum. 2000 Jan; 43(1):155-63.

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

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