CCPoint®

CCPoint 20 US

EURO DIAGNOSTICA

CCPoint®

A visually read, qualitative rapid lateral flow test
for the detection of Rheumatoid Arthritis specific autoantibodies
(anti-CCP)
(Cat. No. CCPoint 20 US)
For use by trained laboratory professionals
This test is not intended for use in point-of-care settings

Doc. no. E-23-0202-02
March, 2014

INTENDED USE

The Euro Diagnostica CCPoint® test is a visually read, qualitative rapid lateral flow test for the detection of IgG antibodies to Cyclic Citrullinated Peptides (CCP) in human serum or plasma. The results of the test are to be used as an aid to the diagnosis of Rheumatoid Arthritis (RA), in conjunction with other laboratory and clinical findings. For use by trained laboratory professionals. For in vitro diagnostic use.

SUMMARY AND EXPLANATION

Rheumatoid Arthritis (RA) is one of the most common systemic autoimmune diseases. The aetiology of the disease, which affects up to 1-2% of the world population, is unknown. The diagnosis of RA depends primarily on clinical manifestation of the disease. A serological test routinely used is the determination of rheumatoid factors (RF) in serum. RF are antibodies directed to the Fc part of antibodies of the human IgG class. However, these antibodies are also present in relatively high percentages in other autoimmune diseases, infections and in up to 15% of healthy individuals.

Antibodies of a more specific nature have also been found in sera of RA patients (see (1) for an overview). A number of cyclic synthetic peptides have been described specifically recognized by autoantibodies in sera from RA patients (2). These peptides have been used in an EIA for the detection of RA-specific autoantibodies (2). Clinical evaluation studies showed that the EIA was positive in a significant number of well-defined RA patient sera with a high specificity against disease controls (2-7). A diagnostic and prognostic value for the measurement of antibodies reactive to Cyclic Citrullinated Peptides (anti-CCP) was found in relation to joint involvement and radiological damage in early RA (6, 8-13). Anti-CCP antibodies can be detected years before the development of clinical symptoms (13). The CCPoint® test offered by Euro Diagnostica is a fast lateral flow immunoassay for the detection of anti-CCP antibodies in human serum or plasma. The test is based on highly purified synthetic peptides containing citrulline residues and is a valuable addition to the diagnosis of RA. The sensitivity and specificity of the assay equals the Immunoscan RA anti-CCP ELISA (Euro Diagnostica).

PRINCIPLE OF THE CCPoint® TEST

The CCPoint® test is a colloidal gold based lateral flow immunoassay. Reactive cyclic citrullinated peptides are immobilised as a discrete line on a porous membrane located in the test zone.
The detection reagent, consisting of colloidal gold particles conjugated to anti-human IgG, is deposited within the device onto the conjugate pad.
In the assay procedure, a sample of serum or plasma is added to the sample port. A blood cell separation membrane transfers the sample fluid onto the porous membrane. After a short incubation, running buffer is added to the buffer port. This buffer mobilizes the colloidal gold particles from the conjugate pad. The gold particles and the sample move by capillary force across the membrane.

If the sample contains anti-CCP antibodies they will bind to the peptide-antigens and a red line will appear in the test zone (marked T). If the sample does not contain any anti-CCP antibodies no line will appear. With any sample a red control line should appear in the control zone (marked C). The control ensures that the coated colloidal gold is still active.

MATERIALS AND REAGENTS PROVIDED

Contents of CCPoint® test kit:
- 20 Foil pouches containing the CCPoint® test device
- 2 Droplet bottles, each containing 3 mL of running buffer.
- 1 CCPoint® Instructions for Use

MATERIALS/EQUIPMENT REQUIRED BUT NOT PROVIDED

- Disposable gloves
- Timer
- Precision pipettes to dispense 10 µL serum or plasma

HANDLING AND STORAGE

- Store the kit at 2-25° C, in a dry place.
- If stored in a refrigerator, allow the test device and buffer bottle to reach room temperature (18-25° C) before opening and testing.
- Keep the test device in its foil pouch until use.
- Do not use expired reagents.
- Do not mix different lot numbers.
- Do not freeze the kit.
- Kit contents are stable until the expiration date printed on the outer box.

PRECAUTIONS

- For in vitro diagnostic use only.
- Optimal results will be obtained by strict adherence to this protocol.
- Do not use components past the expiration date and do not mix components from different lots.
- Each test device can be used only once.
- Do not use a CCPoint® test if its foil pouch is damaged.
- When performing the test, wear disposable gloves throughout the test procedure.
- Observe universal precautions and other appropriate laboratory and safety practices when collecting, handling and disposing of patient blood. All blood samples and used materials (including disposable gloves) should be considered as potentially hazardous and handled in the same manner as an infectious agent. Proper handling and disposal methods should be established according to local, state and federal regulations.

SAMPLE COLLECTION

The assay is recommended for human serum, EDTA, heparin or citrate plasma samples.
Do not use grossly haemolysed or turbid serum/plasma samples.

ASSAY PROCEDURE

1. Preparations
Collect the materials required: test device in foil pouch, buffer, timer, precision pipette and gloves. For serum or plasma samples follow standard laboratory procedures.

2. Test device
When the CCPoint® test has been stored in a refrigerator, allow the test to reach room temperature (18-25° C) before use.

DO NOT open the foil pouch until you are ready to perform the test!
Open the foil pouch and place the test device horizontally on a clean and level surface.

DO NOT open the flip-up section of the device before running the test!

3. Adding the sample
The serum/plasma (10 µL) should be applied onto the blood separation membrane of the sample port. Do not allow the disposable pipette tip to come in contact with the membrane.

After application of the sample, **wait 30 seconds.**

Lift the flip-up section and immediately add 5 drops of running buffer into the buffer port.
To avoid contamination, do not allow the tip of the running buffer vial to touch the device.

DO NOT CLOSE THE LID WHILE RUNNING THE TEST!

Keeping the lid open, allow the test to run for 10 minutes.

4. Interpretation of the test results

Read the results after 10 minutes under good lighting conditions.
To ensure assay validity, a procedural control line is incorporated in the device and is labelled 'C'. If the control line does not appear in this region by the assay completion, the test result is invalid and the sample should be retested with a new test device.

When the sample is positive for anti-CCP antibodies, a second line (the test line, labelled by 'T') will be visible.

POSITIVE: 2 lines appear; one at the control line (C) and one at the test line (T)

NEGATIVE: 1 line appears at the control line (C)

INVALID: 1 line appears at the test line (T) only

INVALID: No lines appear

Positive (two lines)
The appearance of both the test line (T) and the control line (C) in the result window, regardless of relative or absolute intensity should be interpreted as positive. The test should be interpreted as positive even if the test line is lighter in colour than the control line, incomplete over the width of the test strip, or uneven in colour. A positive result indicates the presence of anti-CCP antibodies in the sample.

Negative (one line)
The appearance of only the control line (C) in the result window indicates that the test is negative. A negative result should be interpreted as presumptive negative for the presence of anti-CCP antibodies in the sample.

Invalid
A test with a non-reactive control line (C), regardless of any other reactivity is considered invalid.
The sample should be retested in another test device.

PROCEDURAL LIMITATIONS

Persons performing this test should do so only after carefully reading and understanding this package insert. Strict adherence to the protocol is necessary in order to obtain reliable test results.

The CCPoint® test is designed to detect anti-CCP antibodies in serum or plasma. Diluted solutions of these specimens and/or the use of other body fluids may not yield correct results.

A negative test result does not preclude the possibility that the patient is in a phase of developing RA.

A positive result must be used in conjunction with clinical evaluation and other diagnostic procedures. The values obtained from this assay are intended to be an aid to diagnosis only.

Each physician must interpret the results in conjunction with the patient's history, physical findings and other diagnostic procedures.

Elevated anti-CCP antibodies may be seen in individuals with no evidence of clinical disease. Also, some individuals with RA may have undetectable antibodies.

PERFORMANCE CHARACTERISTICS

Table 1a. Percent agreement of the CCPoint® assay compared to alternative anti-CCP ELISA. A total of 1062 frozen retrospective sera were assayed, 606 of which were from RA patients and 456 samples were apparently healthy blood donors. The following table summarises the results.

		Alternative ELISA		
		Positive	**Negative**	**Total**
CCPoint assay	Positive	444	4	448
	Negative	2	612	614
	Total	446	616	1062

Positive Percent Agreement:	444/446	= 99.6%	95% CI = 98.4 - 99.9%
Negative Percent Agreement:	612/616	= 99.4%	95% CI = 98.3 - 99.8%
Overall Percent Agreement:	1056/1062	= 99.4%	95% CI = 98.8 - 99.8%

Table 1b. Percent agreement of the CCPoint® assay compared to alternative anti-CCP ELISA. Samples extracted from table 1a in the range 15-1600 U/mL with the ELISA.

		Alternative ELISA		
		Positive	**Negative**	**Total**
CCPoint assay	Positive	377	4	381
	Negative	2	16	18
	Total	379	20	399

Positive Percent Agreement:	377/379	= 99.5%	95% CI = 98.1 - 99.9%
Negative Percent Agreement:	16/20	= 80.0%	95% CI = 56.3 - 94.3%
Overall Percent Agreement:	393/399	= 98.5%	95% CI = 96.8 - 99.4%

Table 2. Clinical sensitivity and specificity. A total of 1815 frozen retrospective sera with clinical characterisation were assayed. The following table summarizes the results.

	n	**negative**	**positive**
Healthy controls (blood donors)	456	452	4
RA	596	158	438
Infectious diseases	108	105	3
Inflammatory diseases (nonRA)	576	572	4
Routine samples (nonRA)	79	77	2
TOTAL	1815	1364	451

(Data on file)

Clinical sensitivity

RA	438/596	= 73.5%	95% CI = 69.9 - 77.0%

Clinical specificity

Healthy controls (blood donors)	452/456	= 99.1%	95% CI = 97.8 - 99.8%
Infectious diseases	105/108	= 97.2%	95% CI = 92.1 - 99.4%
Inflammatory diseases (nonRA)	572/576	= 99.3%	95% CI = 98.2 - 99.8%
Routine samples (nonRA)	77/79	= 97.4%	95% CI = 91.2 - 99.7%
Combined non-RA groups	1206/1219	= 98.9%	95% CI = 98.2 - 99.4%

The 95% confidence interval (CI) was calculated using the exact method.

Accuracy

Inter-assay performance of the CCPoint assay was evaluated using negative, low positive and high positive samples for antibodies against anti-CCP. Six different samples tested eight times each, by three different persons. All results obtained were 100% in agreement with the expected results.

Batch-to-batch performance of the CCPoint assay was evaluated using negative, low positive and high positive samples for antibodies against anti-CCP. Six different samples tested eight times each, with three different batches. All results obtained were 100% in agreement with the expected results.

Interference study

Three low positive samples were spiked to the following concentrations in serum samples; Bilirubin F at 18.8 mg/dL, Bilirubin C at 20 mg/dL, Haemoglobin at 453 mg/dL, Chyle at 23.6 U/dL and Rheumatoid Factor at 55 IU/mL. The data indicates that the assayed concentrations do not affect the accuracy of the test.

To assess potential cross-reactivity of CCP IgG antigen with other autoantibodies, a total of 498 samples of different aetiology were assayed. Samples from patients diagnosed with Crohn's disease, Colitis ulcerosa, SLE, Sjögren's syndrome, Osteoarthritis, Scleroderma, Multiple sclerosis, MCTD, Inflammatory bowel disease, Polymyositis/Dermatomyositis, nonRA autoimmune patients and samples reacting with MPO-ANCA, PR3-ANCA and ds-DNA. Data indicates that the assayed autoantibodies show no significant cross-reactivity.

LITERATURE

1. Van Boekel, M., Vossenaar, E., Van den Hoogen, F., Van Venrooij, W. Autoantibody systems in Rheumatoid Arthritis: specificity, sensitivity and diagnostic value. Arthritis Res. 4, 87-93 (2002).

2. Schellekens, G., De Jong, B., Van den Hoogen, F., Van de Putte, L.,Van Venrooij, W. Citrulline is an essential constituent of antigenic determinants recognized by Rheumatoid Arthritis-specific autoantibodies. J. Clin. Invest. 101, 273-281 (1998).

3. Van Jaarsveld, C., Ter Borg, E., Jacobs, J., Schellekens, G., Gmelig-Meyling, F., Van Booma-Frankfort, C., De Jong, B., Van Venrooij, W.J., Bijlsma, J. The prognostic value of the antiperinuclear factor, anti-citrullinated peptide antibodies and rheumatoid factor in early Rheumatoid Arthritis. Clin. Exp. Rheumatol. 17, 689-697 (1999).

4. Schellekens, G., Visser, H., De Jong, B., Van den Hoogen, F., Hazes, J., Breedveld, F., Van Venrooij, W. The diagnostic properties of Rheumatoid Arthritis antibodies recognizing a cyclic citrullinated peptide. Arthritis Rheum. 43, 155-163 (2000).

5. Bizzaro, N., Mazzanti, G., Tonutti, E., Villalta, D., Tozzoli, R. Diagnostic accuracy of the anti-citrulline antibody assay for Rheumatoid Arthritis. Clinical Chemistry 47, 1089-1093 (2001).

6. Visser, H., Le Cessie, S., Vos, K., Breedveld, F., Hazes, J. How to diagnose Rheumatoid Arthritis early? A prediction model for persistent (erosive) arthritis. Arthritis Rheum. 46, 357-365 (2002).

7. Van Venrooij, W., Hazes, J., Visser, H. Anti-citrullinated protein/peptide antibody and its role in the diagnosis and prognosis of early Rheumatoid Arthritis. Neth. J. Med. 60, 383-388 (2002).

8. Van Venrooij, W.J., Zendma, A.J.W., Pruijn, G.J.M. Autoantibodies to citrullinated antigens in (early) rheumatoid arthritis. Autoimmunity Rev. 6, 37-41 (2006).

9. Meyer, O., Labarre, C., Dougados, M., Goupille, Ph., Cantagrel, A., Dubois, A., Nicaise-Roland, P., Sibilia, J., Combe, B. Anticitrullinated protein/peptide antibody assays in early Rheumatoid Arthritis for predicting five year radiographic damage. Ann. Rheum. Dis 62, 120-126 (2003).

10. Rantapää-Dahlqvist, S., de Jong, B., Berglin, E., Hallmans, G., Wadell, G., Stenlund, H., Sundin, U., Van Venrooij, W. Antibodies against citrullinated peptide and IgA rheumatoid factor predict the development of Rheumatoid Arthritis. Arthritis Rheum. 48, 2741-2749 (2003).

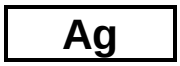
11. Forslind, K., Ahlmén, M., Eberhardt, K., Hafström, I., Svensson, B. Prediction of radiological outcome in early RA in clinical practice: role of antibodies to citrullinated peptides (anti-CCP). Ann. Rheum. Dis. 63, 1090-1095 (2004).

12. Kastbom, A., Strandberg, G., Lindroos, A., Skogh, T., Anti-CCP antibody test predicts the disease course during 3 years in early rheumatoid arthritis (the Swedish TIRA project). Ann. Rheum. Dis. 63, 1085-1089 (2004).

13. van Gaalen, F., Linn-Rasker, S., Van Venrooij, W., de Jong, B., Breedveld, F., Verweij, C., Toes, R., Huizinga, T. Autoantibodies to cyclic citrullinated peptides predict progression to Rheumatoid Arthritis in patients with undifferentiated arthritis. Arthritis Rheum. 50, 709-715 (2004).

APPENDIX

Symbols used on labels

	Lot number.
	Catalogue number.
	Use by.
	Temperature limitation.
	Read instructions for use.
	In vitro diagnostic use.
	Manufacturer.
	Number of tests.
	Antigen (test device).
	Buffer.
	Do not reuse.

Euro Diagnostica AB
P.O. Box 50117, SE-202 11 Malmö, Sweden
Phone: +46 40 53 76 00, Fax: +46 40 43 22 88
E-mail: info@eurodiagnostica.com
www.eurodiagnostica.com