



DIASSTAT™
Anti-CCP

IVD

Σ
96

REF FCCP200

For professional use only



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INTENDED USE

The DIASTAT™ Anti-CCP test is a semi-quantitative/qualitative enzyme-linked immunosorbent assay (ELISA) for the detection of the IgG class of autoantibodies specific to cyclic citrullinated peptide (CCP) in human serum or plasma (EDTA, lithium heparin or sodium citrate). It is intended to aid in the diagnosis of Rheumatoid Arthritis (RA) and is not definitive in isolation. Autoantibody levels represent one parameter in a multicriterion diagnostic process, encompassing both clinical and laboratory-based assessments.

INTRODUCTION




Rheumatoid arthritis (RA) is a systemic autoimmune disease that is multi-functional in origin and is characterised by the inflammation of the membrane lining joints. The disease spreads from small to large joints, with the greatest damage in the early phase¹. The diagnosis of RA is primarily based on clinical, radiological and immunological features. The most frequent serological test is the measurement of rheumatoid factor (RF). The presence of RF is one of the American College of Rheumatology's criteria for the classification of RA. The IgM class is the most common and is found in 60-80% of RA patients. RF is not specific for RA, as it is often present in healthy individuals and patients with other autoimmune diseases and chronic infections². It is reported that up to 30% of Systemic Lupus Erythematosus (SLE) patients with no evidence of RA are RF positive³. Despite its low specificity, a positive RF is considered an important predictor of outcome in RA. For several years, it has been recognised that antibodies to anti-perinuclear factor (APF) and keratin (AKA) are highly specific for RA. Antibodies to APF and AKA have been detected by indirect immunofluorescence using buccal epithelium or rat oesophagus⁴. The lack of availability of suitable buccal cell donors has limited the use of APF as a routine laboratory test. Recently the antigen of both these antibodies has been identified as epidermal filaggrin, an intermediate filament-associated protein involved in the cornification of the epidermis^{5,6}. Profilaggrin, which is present in the keratohyaline granules of human buccal mucosa cells, is proteolytically cleaved into filaggrin subunits during cell differentiation. At this stage, the protein is dephosphorylated and some arginine residues are converted to citrulline by the enzyme peptidylarginine deiminase (PAD)⁷. In 1998, Schellekens and colleagues reported that autoantibodies reactive with linear synthetic peptides containing the unusual amino acid citrulline were present in 76% of RA sera with a specificity for RA of 96%. The antibodies in patients with RA that recognised the citrulline containing epitopes were predominantly of the IgG class and of relatively high affinity⁸. In a subsequent paper, Schellekens and colleagues reported that an ELISA test based on cyclic citrullinated peptide (CCP) showed superior performance characteristics to one based on the linear version in the detection of antibodies to RA⁹. Very recently, it has been reported that, in principle, most citrullinated proteins/peptides are recognised by autoantibodies in RA sera although with differing sensitivities and specificities¹⁰. These findings suggest an important role for citrullinated antigens in the diagnosis of RA.


The DIASTAT™ Anti-CCP assay is an ELISA based on the detection of autoantibodies in human serum or plasma towards a synthetic cyclic peptide containing modified arginine residues. The test provides an additional tool in the diagnosis of patients with RA.

PRINCIPLE OF THE ASSAY

The wells of the microtitre strips are coated with a highly purified synthetic cyclic peptide containing modified arginine residues. During the first incubation, specific autoantibodies in diluted serum or plasma bind to the antigen-coated surface. The wells are then washed to remove unbound components. In the second incubation, the Conjugate, an enzyme-labelled monoclonal antibody to human IgG, binds any surface-bound autoantibodies. After further washing, specific autoantibodies are traced by incubation with the Substrate. Addition of Stop Solution terminates the reaction, resulting in a coloured end-product. The amount of Conjugate bound is measured in absorbance units. In the qualitative protocol, the amount of Conjugate bound by the sample is compared with that bound by the Reference Control. In the semi-quantitative protocol, the concentration of anti-CCP autoantibody can be estimated by interpolation from a dose-response curve based on Calibrators.

KIT COMPONENTS

CONJ	1 x 15 mL	Alkaline phosphatase-labelled murine monoclonal antibody to human IgG, Tris buffer, protein stabiliser, < 0.1% (w/v) sodium azide. Ready-to-use.	
SUBS	1 x 15 mL	Mg ²⁺ , phenolphthalein monophosphate (PMP), buffer solution. Ready-to-use. Do not expose to light during storage. N.B. IRRITANT.	
SOLN STOP	1 x 15 mL	Sodium hydroxide, EDTA, carbonate buffer (pH > 10). Ready-to-use. N.B. IRRITANT.	
BUF WASH 16x	2 x 25 mL	Borate buffer, 0.8% (w/v) sodium azide. Dilute before use. N.B. HARMFUL.	

MTP 8 x 12	8 x 12 well microtitre strips	Coated with synthetic citrullinated peptide, in a resealable foil pack with desiccant.
DIL 5x	1 x 25 mL	Phosphate buffer, protein stabiliser, 0.5% (w/v) sodium azide. Dilute before use. N.B. HARMFUL. 
CAL 1 - CAL 5	5 x 1.0 mL	Human plasma, buffer, < 0.1% (w/v) sodium azide. 0, 2, 8, 30, 100 U/mL. Ready-to-use.
CONTROL REF	1 x 1.5 mL	Human plasma, buffer, < 0.1% (w/v) sodium azide. Ready-to-use.
CONTROL +	1 x 0.2 mL	Human plasma, < 0.1% (w/v) sodium azide. Dilute 1:100 with diluted Sample Diluent before use, as for samples.
CONTROL -	1 x 0.1 mL	

S T O R A G E O F R E A G E N T S

Opened Kit Stability

A kit was opened and reused on three occasions over a three month period with no adverse effect on kit performance.

Handling and Procedural Notes

1. Store kit components at 2-8 °C and use until the expiry date on the labels. Do not use expired reagents.
2. Do not mix different lot numbers.
3. Do not freeze kits.
4. Wash Buffer Concentrate, Sample Diluent Concentrate and Positive and Negative Controls must be diluted before use. All other reagents are ready-to-use.
5. Diluted Wash Buffer and diluted Sample Diluent are stable at 2-8 °C for up to 6 months if microbial contamination is avoided.
6. Replace surplus microtitre strips in the foil pack with the desiccant at 2-8 °C, until required.
7. Do not expose Substrate to light during storage.
8. Avoid contamination of reagents. Use a new disposable pipette tip for each reagent or sample manipulation.

Indications of Deterioration

The Substrate should be pale yellow in colour. Pink colouring indicates contamination and the reagent must be discarded. Turbidity or precipitation in any component indicates deterioration and the component should be discarded.

Sample Collection and Storage


The assay is recommended for serum or plasma (EDTA, lithium heparin, sodium citrate) samples; do not use grossly haemolysed or turbid samples. Thoroughly mix thawed samples before assay and avoid repeated freeze/thawing. Do not heat-inactivate samples, this may yield false positive results.

Samples may be stored undiluted at 2-8 °C for four weeks; for longer storage store at -20 °C. Samples diluted at 1:100 in diluted Sample Diluent must be used within the same day of dilution.

W A R N I N G S A N D P R E C A U T I O N S

For in vitro diagnostic use only.

Safety Precautions

1. Adhere strictly to the instructions in this booklet, particularly for handling and storage conditions.
2.  Calibrators and Controls contain human plasma tested by FDA-cleared assays for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HIV-1/HIV-2, and anti-HCV and found to be non-reactive/negative. As no known test offers complete assurance that infectious agents are absent, Calibrators and Controls should be considered potentially infectious and handled with the same precautions as any other potentially biohazardous material. The Clinical and Laboratory Standards Institute (CLSI) approved guidelines "Protection of Laboratory Workers from Occupationally Acquired Infections" (M29-A3 –Third Edition)¹¹, describes how these materials should be handled in accordance with Good Laboratory Practice.
3. Do not pipette by mouth.
4. Do not smoke, eat, drink or apply cosmetics in areas where kits and samples are handled.
5. Any skin complaints, cuts, abrasions and other skin lesions should be suitably protected.


- The Calibrators, Controls, Conjugate, Sample Diluent Concentrate and Wash Buffer Concentrate contain sodium azide which can react with lead and copper plumbing to form highly explosive metal azides. On disposal, drain with large quantities of water to prevent azide build-up.
- The Stop Solution contains sodium hydroxide. Avoid contact with skin, eyes and mucous membranes. Spillage should be mopped up with copious amounts of water. If contact with skin or eyes occurs, irrigate with water and seek medical attention immediately.
- Material safety data sheets for all hazardous components contained in this kit are available on request from Axis-Shield Diagnostics.

 **STOP SOLUTION**
Irritant


R36/38: Irritating to eyes and skin.
 S23: Do not breathe fumes.
 S25: Avoid contact with eyes.
 S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
 S29/35: Do not empty into drains; dispose of this material and its container in a safe way.
 S36/37/39: Wear suitable protective clothing, gloves and eye/face protection.

 **WASH BUFFER CONCENTRATE (16X)**
Harmful

R22: Harmful if swallowed.
 R32: Contact with acids liberates very toxic gas.
 R52/53: Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
 S23: Do not breathe fumes.
 S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
 S28: After contact with skin, wash immediately with plenty of water.
 S29/35: Do not empty into drains; dispose of this material and its container in a safe way.
 S36/37/39: Wear suitable protective clothing, gloves and eye/face protection.
 S46: If swallowed, seek medical advice immediately and show this container or label.
 S60: This material and its container must be disposed of as hazardous waste.
 S61: Avoid release to the environment. Refer to special instructions / safety data sheets.

 **SUBSTRATE**
Irritant

R36: Irritating to eyes.
 S23: Do not breathe fumes.
 S25: Avoid contact with eyes.
 S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
 S29/35: Do not empty into drains; dispose of this material and its container in a safe way.
 S36/37/39: Wear suitable protective clothing, gloves and eye/face protection.

 **SAMPLE DILUENT CONCENTRATE (5X)**
Harmful

R22: Harmful if swallowed.
 R32: Contact with acids liberates very toxic gas.
 R36: Irritating to eyes.
 R52/53: Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
 S23: Do not breathe fumes.
 S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
 S28: After contact with skin, wash immediately with plenty of water.
 S29/35: Do not empty into drains; dispose of this material and its container in a safe way.
 S36/37/39: Wear suitable protective clothing, gloves and eye/face protection.
 S46: If swallowed, seek medical advice immediately and show this container or label.
 S60: This material and its container must be disposed of as hazardous waste.
 S61: Avoid release to the environment. Refer to special instructions / safety data sheets.

P R E P A R A T I O N

Materials/Equipment Required but not Provided

- 96 well plate/strip reader with 550 nm filter (540-565 nm is acceptable).
- Precision pipettes to dispense 10 μ L, 100 μ L, 1 mL. Automatic pipette to dispense 100 μ L. Automatic pipette to dispense 200 μ L for manual washing, automatic plate washer optional.
- Glass/plastic measuring cylinders: 1x100 mL, 1x400 mL.
- 1 mL volume containers.
- Distilled/deionised water.
- Paper towels.
- Timer for 30 and 60 minute intervals.

Preparation for the Assay

Allow all kit components, including the microtitre strips, to warm up to 18-25 °C for 30-60 minutes before use. Mix reagents by gentle inversion.

Do not dilute the Reference Control.

Dilute the following reagents and mix thoroughly.

Reagent	Volume	Add
Wash Buffer Concentrate	1 vial	375 mL distilled/deionised water
Sample Diluent Concentrate	1 vial	100 mL distilled/deionised water
Positive and Negative Controls/samples	10 µL	1 mL diluted Sample Diluent

Calculate the number of microtitre strips required for the current assay, and retain these in the microtitre strip holder. Return surplus strips to the resealable foil pack with the desiccant and store at 2-8 °C until required. Ensure that all strips are securely held within the microtitre strip holder. Users may wish to number each strip along the top edge to aid identification. Retain the microtitre strip holder for further use.

ASSAY PROTOCOL

Qualitative protocol: run Reference Control, Positive and Negative Controls, and samples.

Semi-Quantitative protocol: run Calibrators (1-5), Positive and Negative Controls, and samples.

1. Reference wells for identification.
2. Pipette 100 µL Reference Control/Calibrators in duplicate, pre-diluted (1:100) Positive and Negative Controls, and pre-diluted (1:100) patient samples into appropriate wells. Remember to change pipette tips between additions. This step should not exceed 15 minutes for any one set of Calibrators /Controls/samples.
3. Incubate 60 ± 10 minutes at 18-25 °C.
4. Decant strip contents by quick inversion over a sink suitable for the disposal of biological materials, bearing in mind the potential infective hazard of the samples. Blot inverted strips well with paper towels.
5. Wash wells **three times** with a minimum of 200 µL diluted Wash Buffer. **Decant and blot after each wash step.**
6. Add 100 µL Conjugate to each well.
7. Incubate 30 ± 5 minutes at 18-25 °C.
8. Repeat steps 4 and 5.
9. Add 100 µL Substrate to each well.
10. Incubate 30 ± 5 minutes at 18-25 °C. **Do not decant.**
11. Add 100 µL Stop Solution to each well, in the same order and rate as the Substrate. Tap wells gently to mix.
12. Read strips within 24 hours at 550 nm (540-565 nm).

CALCULATION AND INTERPRETATION OF RESULTS

Consider each assay separately when calculating and interpreting results.

Qualitative Protocol

Calculate the absorbance value (optical density) ratio for the Positive and Negative Controls, and for each sample.

$$\text{Absorbance Ratio} = \frac{\text{Sample or Control Absorbance Value}}{\text{mean Reference Control Absorbance Value}}$$

Users should calculate a cut-off between positive and negative samples that is specific to their patient populations. Results from the patient populations used in the Axis-Shield clinical trial suggest the following cut-off:

<u>Absorbance Ratio</u>	<u>Result Interpretation</u>
< 0.95	Negative
≥ 0.95 to ≤ 1.0	Borderline - recommend repeat testing
> 1.0	Positive

Semi-Quantitative Protocol

Plot the mean absorbance value of each Calibrator against log₁₀ Calibrator concentration (see following table) on suitable graph paper. Concentrations of Controls and samples can then be read from the calibration curve; a typical plot is shown below for reference purposes, it must not be used for interpreting results. Weighted 4-parameter logistic (4PL), weighted 5-parameter logistic (5PL), smoothed spline, log/logit and lin/linfit curve fits are also satisfactory.

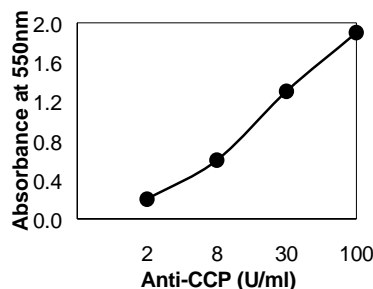
Samples with absorbances above Calibrator 5 (100 U/mL) are outside the range of the assay, and should be reported as >100 U/mL, diluted and re-assayed, correcting for this further dilution factor.

NB: As in any assay measuring antibodies, this assay determines the activity of the antibody present in the sample, rather than the concentration. Activity can be affected by a number of parameters, such as antibody avidity.

Calibrator Concentrations

Calibrator Number	Concentration U/mL
1	0
2	2
3	8
4	30
5	100

Typical Calibration Curve



QUALITY CONTROL

Ensure that adequate maintenance and calibration of the plate-reader is performed according to the manufacturer's instructions, and that the correct wavelength is employed.

Users should ensure they are fully acquainted with the instructions for the assay, particularly the Warnings and Precautions section, and the Handling and Procedural Notes. Users should demonstrate that they can obtain performance specifications for precision and reportable range of test results comparable to those established by the manufacturer before reporting patient test results. It is recommended that the pre-diluted Positive and Negative Controls are run in duplicate in all assays to monitor the quality of the test procedure. Run the ready-to-use Reference Control in duplicate in all qualitative assays.

Assuming the precision specifications described by the manufacturer are met, failure of any Control to meet the Control ratio specifications below renders the assay invalid and patient results should not be reported. The operator may repeat the assay, having reviewed their procedure, or contact the distributor/manufacturer. If repeating the assay, prepare a fresh dilution of each Control and sample. Laboratories may wish to include in-house controls in each assay run. Store such control material at or below -20 °C and avoid repeat freeze/thaw cycles. Preservatives such as sodium azide at 0.1% (w/v) will not affect sample results.

Levels of analytes identified in particular diseases are those established by the manufacturer for specific populations, and may not necessarily mirror the literature. Incidence levels, their relationship to specific diseases, reference ranges, and appropriate cut-off points should all be calculated for the specific populations serviced by users.

Control Ratio Specifications

Protocol	Specifications
Qualitative (ratios)	$\frac{\text{Positive Control Absorbance}}{\text{Reference Control Absorbance}}$ see Positive Control label
	$\frac{\text{Negative Control Absorbance}}{\text{Reference Control Absorbance}} < 0.95$
Semi-Quantitative	See Positive Control label for acceptable expected range (U/mL)
	Negative Control concentration < 2 U/mL

EXPECTED VALUES

200 serum samples from asymptomatic apparently healthy donors with an age range of 18-72 years, comprising approximately equal numbers of males [n = 105] and females [n = 95], were tested with the DIASTAT™ Anti-CCP ELISA test.

No differences attributable to gender or age were observed (calculated comparing age ranges of ≤ 40 years [n = 115] and > 40 years [n = 85]).

The overall mean anti-CCP concentration for this population was 0.63 ± 0.419 U/mL (range 0.05-3.8 U/mL).

On the basis of this reference population data and that of a clinical population, the suggested assay cut-off is:-

<i>Reference Range</i> ≤ 5 U/mL = Negative > 5 U/mL = Positive
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This reference range is suggested as a guideline only and each laboratory should establish a reference range appropriate to their patient populations and clinical practice. Please note that rheumatoid arthritis is twice as prevalent in females as in males.

PERFORMANCE DATA

Clinical Sensitivity in Clinically Confirmed RA

Clinical sensitivity data for the DIASTAT™ Anti-CCP ELISA was calculated as the percentage of clinically confirmed RA sera positive in the anti-CCP assay. Clinically confirmed RA was diagnosed according to the American College of Rheumatology (ACR) criteria. The collated data from four sites is shown below. The corresponding clinical sensitivity of RF IgM using a commercially available test at two of the sites is also given below.

Site	Confirmed RA (n)	Anti-CCP Positive (n)	Clinical Sensitivity	Confirmed RA (n)	RF IgM Positive (n)	Clinical Sensitivity
UK	283	220	78%	283	208	74%
UK	100	75	75%	100	95	95%
Europe	100	78	78%	-	-	-
USA	92	81	88%	-	-	-
Total	575	454	79%	383	303	79%

- Not tested

Clinical Specificity in Non-RA Disease States and Asymptomatics

Clinical specificity for the DIASTAT™ Anti-CCP ELISA was obtained by calculating the percentage of non-RA disease state sera negative in the anti-CCP assay. The collated data from five sites for non-RA disease and from four sites for asymptomatics is shown below.

Non-RA Disease States	n	Anti-CCP Negative (n)	Clinical Specificity
Systemic Lupus Erythematosus	227	209	92%
Scleroderma	92	86	93%
Polymyositis	21	21	100%
Sjögren's Syndrome	86	85	99%
Osteoarthritis	66	59	89%
Psoriatic Arthritis	49	48	98%
Reactive RA	40	38	95%
Polymyalgia Rheumatica	38	36	95%
Fibromyalgia	21	21	100%
Early Sinovitis	6	5	83%
Reiters' Syndrome	18	18	100%
Sarcoidosis	4	4	100%
Seronegative Inflammatory Arthropathy	13	10	77%
Ulcerative Colitis	40	39	98%
Crohn's Disease	43	43	100%
Autoimmune Thyroiditis	50	50	100%
Lyme Disease	45	44	98%
CREST	22	22	100%
Juvenile RA	9	8	89%
Infectious Mononucleosis	118	116	98%
Parvovirus	11	11	100%
Vasculitis	23	22	96%
Gout	11	11	100%
Dermatomyositis	10	10	100%
Ankylosing Spondylitis	30	30	100%
Totals	1,093	1,043	95.4%

Asymptomatics	n	Anti-CCP Negative (n)	Clinical Specificity
Totals	334	334	100%

Clinical Specificity of Anti-CCP and RF IgM

RF IgM and anti-CCP were measured in non-RA groups at three sites. The data from all sites has been collated below. All equivocal RF IgM results are excluded.

Disease	n	Anti-CCP		RF IgM	
		Negative n	Clinical Specificity	Negative n	Clinical Specificity
Systemic Lupus Erythematosus	147	128	87%	79	54%
Sjögren's Syndrome	50	49	98%	11	22%
Scleroderma	53	48	91%	14	26%
Polymyositis	15	15	100%	11	73%
Osteoarthritis	31	26	84%	20	65%
Autoimmune Thyroiditis	35	35	100%	25	71%
Lyme Disease	36	36	100%	26	72%
Infectious Mononucleosis	39	38	97%	11	28%
Totals	406	375	92%	197	49%

Asymptomatics	n	Anti-CCP		RF IgM	
		Negative n	Clinical Specificity	Negative n	Clinical Specificity
Totals	196	196	100%	158	81%

Agreement With Rheumatoid Factor IgM

The performance of the DIASTAT™ Anti-CCP ELISA was compared with that of a commercially available ELISA test for the measurement of Rheumatoid Factor IgM in an asymptomatic population [n = 196] and in a population of clinically confirmed RAs [n = 504].

Asymptomatic Population

		RF IgM ELISA	
		+	-
DIASTAT™	+	0	0
	-	37	159

Overall Agreement = 81%
n = 196

Clinically Confirmed RA

		RF IgM ELISA	
		+	-
DIASTAT™	+	365	33
	-	48	58

Overall Agreement = 84%
n = 504

Dilution Characteristics

Five dilutions of three patient samples were assayed using two kit batches. The following table shows the mean values obtained and the dilution-corrected recovery.

Sample	Dilution	Mean Value U/mL	Dilution Corrected % Recovery
1	1/100	66.6	100
	1/200	33.9	104
	1/400	17.9	113
	1/800	9.4	118
	1/1600	4.0	101
2	1/100	62.9	100
	1/200	31.1	101
	1/400	14.4	94
	1/800	7.4	97
	1/1600	3.2	84
3	1/100	77.8	100
	1/200	35.0	88
	1/400	16.0	82
	1/800	9.0	94
	1/1600	4.2	88

Imprecision

- Intra-assay imprecision** determined by testing four controls [n = 4], in twenty-five assays, using five operators and three kit batches.

Control	Mean Value U/mL	Root Mean Square %CV
1	5.4	7.6
2	15.5	10.5
3	22.4	8.7
4	34.1	9.7

The range of %CV for each control was 1.8 – 16.3% (QC1), 1.2 – 20.3 (QC2), 1.4 – 15.5% (QC3) and 3.2 – 14.9% (QC4). This spread is a reflection of the number of operators [n = 5] and kit batches [n = 3] used for this study.

- Inter-assay imprecision** determined by testing four controls [n = 4], in twenty-five assays, using five operators and three kit batches.

Control	Mean Value U/mL	SD	%CV
1	5.4	0.74	13.6
2	15.5	1.71	11.0
3	22.4	2.76	12.4
4	34.1	2.56	7.6

Lower Limit of Detection

The lower limit of detection, calculated as the mean of the zero standard plus two standard deviations, run in triplicate in 12 assays from three kit batches was 0.05 U/mL.

Interferences

Haemoglobin up to 400 mg/dl, bilirubin up to 0.2 mg/mL, intralipid up to 15 mg/mL and rheumatoid factor up to 200 IU/mL do not interfere with anti-CCP antibody results.

LIMITATIONS OF USE







1. Although the presence of antibodies to CCP is associated with Rheumatoid Arthritis, a positive result is not in itself diagnostic, the data must be considered in light of other clinical and laboratory findings.
2. Some individuals may have high levels of anti-CCP antibodies with little or no evidence of clinical disease. By contrast, some patients with active disease may have undetectable levels of these antibodies. The clinical significance of this information is currently unclear.
3. As the result of an anti-CCP assay is not diagnostic proof of the presence or absence of clinical disease, therapy should not be started on the basis of an anti-CCP positive result alone.
4. Initiation or changes in treatment should not be based on changes in anti-CCP autoantibody concentration but rather on clinical observation(s).
5. The clinical effectiveness of monitoring CCP autoantibody levels as an indication of progression/remission of Rheumatoid Arthritis has not been defined.
6. The value of anti-CCP in juvenile arthritis has not been determined.
7. Due to the specific characteristics of antigen/antibody interactions, it is not the concentration of antibody which is determined, but the activity. Since patient sera contain heterogeneous antibody populations, some samples may exhibit non-linearity, especially at very high sample dilutions.

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SUMMARY OF PROTOCOL

1. Dilute samples and Positive and Negative Controls 1:100. Do not dilute Standards or Reference Control.
2. Add 100µL of Reference Control/Standards in duplicate, pre-diluted Positive and Negative Controls and samples into referenced wells of the microtitre strip.
3. Incubate 60±10 minutes at 18-25°C.
4. Wash strips 3 times.
5. Add 100µL of Conjugate to each well.
6. Incubate 30±5 minutes at 18-25°C.
7. Wash strips 3 times.
8. Add 100µL of Substrate to each well.
9. Incubate 30±5 minutes at 18-25°C.
10. Add 100µL of Stop Solution to each well.
11. Read absorbance at 550nm.

IVD	<i>In vitro</i> diagnostic medical device
REF	Catalogue number
LOT	Lot
	96 tests
	Caution
	Consult instructions for use
	Use by
	Store at 2-8 °C
	Manufactured by
CONTROL +	Positive Control
CONTROL -	Negative Control
CONJ	Conjugate
SUBS	Substrate
SOLN STOP	Stop Solution
BUF WASH 16x	Wash Buffer
MTP 8 x 12	Microtitre Strips ri
DIL 5x	Diluent
CAL 1 - CAL 5	Calibrator 1-5
CONTROL REF	Reference Control