



**A x i s - S h i e l d**

**Anti-CCP**

**IVD**



**REF FCCP400**

**For professional use only**



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## ENGLISH:

## INTENDED USE

The Axis-Shield 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, serum separator tube (SST) 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 common, systemic autoimmune disease affecting 0.5-1.0% of the adult population. RA is characterised by chronic inflammation of the synovium which can lead to progressive joint destruction and in many cases lead to disability and reduction of quality of life.<sup>1</sup> It is generally accepted that early intervention is vital in preventing irreversible joint damage and it is therefore important to diagnose RA as early in the disease course as possible.<sup>2,3</sup> 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 (ACR) criteria for the classification of RA.<sup>4</sup> Although the RF test has good sensitivity it is not specific for RA, as it is often present in healthy individuals and patients with other rheumatic or inflammatory diseases, autoimmune diseases and chronic infections.<sup>5</sup>




For several years, it has been recognised that antibodies to anti-perinuclear factor (APF) and keratin (AKA) are highly specific for RA. It was subsequently reported that both these antibodies reacted with native filaggrin and now are referred to as anti-filaggrin antibodies (AFA).<sup>6,7,8</sup> Recent evidence has shown that all these antibodies are directed to citrulline containing epitopes.<sup>9</sup> Citrulline is a non-standard amino acid, as it is not incorporated into proteins during protein synthesis. It can however be generated via post-translational modification of arginine residues by the enzyme peptidylarginine deiminase (PAD).<sup>10</sup> In 1998, Schellekens and colleagues reported that autoantibodies reactive with linear synthetic peptides containing citrulline were highly specific for RA in an ELISA based assay.<sup>11</sup> Subsequent studies demonstrated that cyclic variants of these linear peptides, termed cyclic citrullinated peptides (CCP) were as specific for RA but with a higher sensitivity than the linear peptides.<sup>12</sup> In an effort to further improve the sensitivity of the CCP test, a dedicated library of citrulline-containing peptides were screened with RA sera and a new set of peptides (CCP2) were discovered which gave superior performance compared to the CCP1 test.<sup>13</sup> Over the last few years many published reports have confirmed the diagnostic performance of the CCP2 test.<sup>14</sup> Anti-CCP antibodies have been found to be present very early in the disease, often with the absence of clinical symptoms and many reports indicate that elevated levels of anti-CCP antibodies can predict the development of erosive disease.<sup>15,16,17,18,19,20</sup> These findings suggest an important role for cyclic citrullinated peptides in the diagnosis of RA at an early stage of the disease course.


The Axis-Shield 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 (CCP2 peptides). 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 polyclonal 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

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

<b>MTP</b> 8 x 12	8 x 12 well microtitre strips	Coated with synthetic citrullinated peptide, in a resealable foil pack with desiccant.
<b>DIL</b> 5x	1 x 25 mL	Phosphate buffer, protein stabiliser, 0.5% (w/v) sodium azide. <b>Dilute before use. N.B. HARMFUL.</b> 
<b>CAL</b> 1 - <b>CAL</b> 6	6 x 1.0 mL	Human plasma, buffer, < 0.1% (w/v) sodium azide. 0, 2, 8, 30, 100, 200 U/mL. <b>Ready-to-use.</b>
<b>CONTROL</b> REF	1 x 1.5 mL	Human plasma, buffer, < 0.1% (w/v) sodium azide. <b>Ready-to-use.</b>
<b>CONTROL</b> +	1 x 0.3 mL	Human plasma, < 0.1% (w/v) sodium azide. <b>Dilute 1:100 with diluted Sample Diluent before use, as for samples.</b>
<b>CONTROL</b> -	1 x 0.3 mL	

## STORAGE OF REAGENTS

### 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 (serum separator tube, EDTA, lithium heparin or 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.

## WARNINGS AND PRECAUTIONS

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),<sup>21</sup> 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.
6. 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.


7. 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.
8. 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*

1. 96 well plate/strip reader with 550 nm filter (540-565 nm is acceptable).
2. Precision pipettes to dispense 10 µL, 100 µL, 1 mL. Automatic pipette to dispense 100 µL. Automatic pipette to dispense 200 µL for manual washing, automatic plate washer optional.
3. Glass/plastic measuring cylinders: 1×100 mL, 1×400 mL.
4. 1 mL volume containers.
5. Distilled/deionised water.
6. Paper towels.
7. 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 (1-6), 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  $\log_{10}$  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. 4-parameter logistic (4PL), cubic spline, log/logit and point-to-point curve fits are also satisfactory.

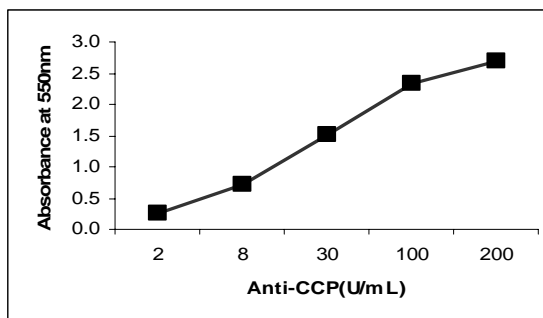
Samples with absorbances above Calibrator 6 (200 U/mL) are outside the range of the assay, and should be reported as > 200 U/mL, diluted and re-assayed, correcting for this further dilution factor.

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
6	200

#### 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  $-20^{\circ}\text{C}$  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)	$\frac{\text{Positive Control Absorbance}}{\text{Reference Control Absorbance}} \geq 1.1$
	$\frac{\text{Negative Control Absorbance}}{\text{Reference Control Absorbance}} < 0.95$
Semi-Quantitative	See Positive Control label for acceptance expected range (U/mL)
	Negative Control concentration < 2 U/mL

## EXPECTED VALUES

50 of the asymptomatic apparently healthy donor samples had gender and age details. The age range was 19-72 years, comprising equal numbers of males (n=25) and females (n=25). No difference could be attributed to gender or age when comparing the age range of  $\leq 40$  years (n=17) and  $> 40$  years (n=33). The overall mean anti-CCP concentration for this population was  $0.48 \pm 0.51$  U/mL (range 0.0 – 2.59 U/mL).

<i>Reference Range</i> $\leq 5$ 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 Axis-Shield 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.

Site	Confirmed RA (n)	FCCP200 Positive (n)	FCCP200 Clinical Sensitivity	Confirmed RA (n)	FCCP400 Positive (n)	FCCP400 Clinical Sensitivity
Axis-Shield	412	258	62%	412	267	64%

**Clinical Specificity in Non-RA Disease States and Asymptomatics**

Clinical specificity for the Axis-Shield Anti-CCP ELISA was obtained by calculating the percentage of non-RA disease state sera negative in the anti-CCP assay.

<b>Non RA Disease State</b>	<b>n</b>	<b>FCCP200 Negative</b>	<b>FCCP200 Clinical Specificity</b>	<b>FCCP400 Negative</b>	<b>FCCP400 Clinical Specificity</b>
Ankylosing spondylitis	10	10	100%	10	100%
Early synovitis	10	7	70%	7	70%
Osteoarthritis	18	18	100%	18	100%
Polymyositis	15	15	100%	14	93%
Polymyalgia rheumatica	6	4	67%	4	67%
Psoriatic arthritis	25	24	96%	24	96%
Raynaud	3	3	100%	3	100%
Reactive arthritis	2	2	100%	2	100%
Sarcoidosis	2	2	100%	2	100%
Scleroderma	23	21	91%	21	91%
Sjogren's Syndrome	23	21	91%	21	91%
SLE	104	93	89%	93	89%
Vasculitis	10	10	100%	10	100%
Viral	5	5	100%	5	100%
JIA	1	1	100%	1	100%
<b>Total</b>	<b>257</b>	<b>236</b>	<b>93.6%</b>	<b>235</b>	<b>93.2%</b>

<b>Non RA</b>	<b>n</b>	<b>FCCP200 Negative</b>	<b>FCCP200 Clinical Specificity</b>	<b>FCCP400 Negative</b>	<b>FCCP400 Clinical Specificity</b>
Asymptomatic	192	192	100%	190	99%

### Clinical Specificity of Anti-CCP

#### All FCCP200 Results Versus FCCP400 Results

FCCP400	FCCP200		Grand Total
	Negative	Positive	
Negative	570	0	570
Positive	14	277	291
Grand Total	584	277	861

Disease Group	n	% Positive Agreement (95% CI)	% Negative Agreement (95% CI)	% Total Agreement (95% CI)
All samples	861	100.0 (98.7 to 100.0)	97.6 (96.0 to 98.7)	98.4 (97.3 to 99.1)

#### *Dilution Linearity*

The anti-CCP assay is designed to have a mean recovery of  $100 \pm 20\%$  when analysing specimens in negative plasma. Three high anti-CCP specimens were diluted with negative plasma according to the CLSI document EP6-A. In a representative study, the concentration of anti-CCP was determined using the Anti-CCP assay, and the mean percentage recovery was calculated. The overall mean recovery was 108.1% (mean observed recovery range 98.3%-113.3%). Data from this study are summarised in the following table.

Sample	% Recovery per Dilution Factor (Low+High)						Mean % Recovery
	6 + 1	5 + 2	4 + 3	3 + 4	2 + 5	1 + 6	
1	111.0	120.0	106.0	116.0	114.0	113.0	113.3
2	114.0	111.0	115.0	117.0	112.0	106.0	112.5
3	94.0	105.0	98.0	101.0	95.0	97.0	98.3
<b>Overall Mean % Recovery</b>							<b>108.1</b>

### Precision

The anti-CCP assay is designed to have within and between precision of < 20% CV.

The human plasma based panel members were assayed in replicates of two at two separate times of the day, n=88 for each panel member, testing was performed using two kit lots.

Data from this study are summarised in the following tables

<b>Panel Member 1</b>						
Combination	n	Mean Conc Value (U/mL)	Within Run		Between Run	
			SD	CV(%)	SD	CV(%)
1	88	5.2	0.82	15.8	0.37	7.2
2	88	3.1	0.52	16.8	0.21	6.7

<b>Panel Member 2</b>						
Combination	n	Mean Conc Value (U/mL)	Within Run		Between Run	
			SD	CV(%)	SD	CV(%)
1	88	9.7	1.01	10.4	0.65	6.7
2	88	7.6	0.88	11.6	0.37	4.9

<b>Panel Member 3</b>						
Combination	n	Mean Conc Value (U/mL)	Within Run		Between Run	
			SD	CV(%)	SD	CV(%)
1	88	18.8	2.07	11	1.26	6.7
2	88	16.4	1.57	9.6	0	0

<b>Kit Positive</b>						
Combination	n	Mean Conc Value (U/mL)	Within Run		Between Run	
			SD	CV(%)	SD	CV(%)
1	88	27.2	2.57	9.4	0	0
2	88	26.0	2.5	9.6	0.63	2.4

<b>Panel Member 4</b>						
Combination	n	Mean Conc Value (U/mL)	Within Run		Between Run	
			SD	CV(%)	SD	CV(%)
1	88	55.4	8.07	14.7	1.71	3.1
2	88	47.5	5.7	12	1.44	3

<b>Panel Member 5</b>						
Combination	n	Mean Conc Value (U/mL)	Within Run		Between Run	
			SD	CV(%)	SD	CV(%)
1	88	129.2	25.47	19.7	0	0
2	88	110.7	18.57	16.8	2.06	1.9

### Analytical Sensitivity

The anti-CCP assay is designed to have a mean analytical sensitivity/limit of blank of  $\leq 1.0$  U/mL.

Analytical sensitivity (according to the CLSI document EP17-A) represents the highest value that is expected for a sample that contains no anti-CCP antibody.

### **Interference**

The anti-CCP assay is designed to have a mean potential interference from bilirubin, haemoglobin, intralipid and rheumatoid factor of < 15% at the levels indicated in the following table.

<b>Interfering Substance</b>	<b>Interfering Substance Concentration</b>
Bilirubin	0.2 mg/mL
Haemoglobin	4 mg/mL
Intralipid	15 mg/mL
Rheumatoid Factor	200 IU/mL

### **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.

## SUMMARY OF PROTOCOL

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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.

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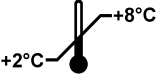

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<b>IVD</b>	<i>In vitro</i> diagnostic medical device
<b>REF</b>	Catalogue number
<b>LOT</b>	Lot
	96 tests
	Caution
	Consult instructions for use
	Use by
	Store at 2-8°C
	Manufactured by
<b>CONTROL +</b>	Positive Control
<b>CONTROL -</b>	Negative Control
<b>CONJ</b>	Conjugate
<b>SUBS</b>	Substrate
<b>SOLN STOP</b>	Stop Solution
<b>BUF WASH 16x</b>	Wash Buffer
<b>MTP 8 x 12</b>	Microtitre Strips
<b>DIL 5x</b>	Diluent
<b>CAL 1 - CAL 6</b>	Calibrator 1-6
<b>CONTROL REF</b>	Reference Control