A x i s - S h i e l d

Anti-CCP

IVD

Σ 96

REF **FCCP600**

For professional use only

CE

Axis-Shield Diagnostics Limited
The Technology Park, Dundee DD2 1XA, United Kingdom.
Tel: +44 (0) 1382 422000, *Fax:* +44 (0) 1382 422088.
E-mail: shield@axis-shield.com
Web: www.axis-shield.com

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 (including Serum Separator Tubes) or plasma (EDTA, lithium heparin, or sodium citrate). Detection of anti-CCP antibodies is used as an aid in the diagnosis of Rheumatoid Arthritis (RA), and should be used in conjunction with other clinical information. Autoantibody levels represent one parameter in a multi-criterion diagnostic process, encompassing both clinical and laboratory-based assessments. For in vitro diagnostic use.

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.[1] 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.[2,3] 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).[4] 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 or chronic infections.[5]

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).[6,7,8] Recent evidence has shown that all these antibodies are directed to citrulline containing epitopes.[9] Citrulline is a non-standard amino acid, as it is not incorporated into proteins during protein synthesis. It can however be generated via post-transitional modification of arginine residues by the enzyme peptidylarginine deiminase (PAD).[10] 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.[11] 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.[12] In an effort to further improve the sensitivity of the CCP test, a dedicated library of citrulline-containing peptides was screened with RA sera and a new set of peptides (CCP2) was discovered which gave superior performance compared to the CCP1 test.[13] Over the last few years many published reports have confirmed the diagnostic performance of the CCP2 test.[14] Anti-CCP antibodies, which are also often termed as anti-citrullinated protein/peptide antibodies (ACPA's), 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.[15,16,17,18,19,20] These findings suggest an important role for cyclic citrullinated peptides in the diagnosis of RA at an early stage of the disease course.

In 2010 the *ACR / EULAR Rheumatoid Arthritis Classification Criteria* were published and replaced the "old" ACR criteria of 1987 which were widely considered not to be suitable for the early diagnosis of RA. The revised classification criteria, jointly published by the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) recommend a point scoring system of between 0 and 10. The new classification criteria are to be applied to every individual presenting with definitive synovitis (undifferentiated inflammatory Arthritis). The four additional criteria were number of joints involved, serologic abnormality, acute-phase response and duration of symptoms in the involved joints. For the first time the serologic criteria included measurement of ACPAs, such as anti-CCP, as well as some definition of a low positive and high positive serology result.[21]

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 citrullinated 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 and 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 × 15.0 mL	Horseradish peroxidase-labelled goat polyclonal antibody to human IgG, 0.1% (w/v) p-Hydroxyphenylacetic acid, 0.15% (w/v) Proclin and 1% protein (bovine) stabiliser (w/v) in a HEPES buffer. **Ready-to-use. N.B. WARNING.** 
SUBS	1 × 15.0 mL	3,3',5,5'-Tetramethylbenzidine, buffer solution. **Ready-to-use.** Do not expose to light during storage. **N.B. WARNING.** 
SOLN STOP	1 × 15.0 mL	Sulphuric acid 0.25mol/L aqueous solution **Ready-to-use. N.B. DANGER.** 
BUF WASH 10 X	3 × 25.0 mL	Phosphate buffered saline, 1.3% (v/v)Tween 20 **Dilute before use.**
MTP 8 x 12	8 x 12 well microtitre (breakapart) strips	Coated with synthetic cyclic citrullinated peptide, in a resealable foil pack with desiccant.
SAMPLE DIL 5 X	1 × 25.0 mL	Phosphate buffer, protein (bovine) stabiliser, 0.5% (w/v) sodium azide. **Dilute before use.** **N.B. DANGER.** 
CAL 1	1 × 1.0 mL	Phosphate buffer, protein (bovine) stabiliser, < 0.1% (w/v) sodium azide. 0 U/mL. **Ready-to-use.**
CAL 2 - CAL 6	5 x 1.0 mL	Human plasma, Phosphate buffer, protein (bovine) stabiliser, < 0.1% (w/v) sodium azide. 2, 8, 30, 100, 300 U/mL. **Ready-to-use.**
CONTROL REF	1 × 1.5 mL	Human plasma, buffer, < 0.1% (w/v) sodium azide. **Ready-to-use.**
CONTROL + CONTROL -	1 × 0.3 mL 1 x 0.3 mL	Human plasma, < 0.1% (w/v) sodium azide. **Dilute 1:100 with diluted Sample Diluent before use, as for samples.**

STORAGE OF REAGENTS

Opened (In-Use) Kit Stability

A kit was opened, and reused on three occasions over a three month period with no adverse effect on kit performance. Following use, components must be returned to storage at 2-8°C.

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. Ensure microbal contamination of the diluted Wash Buffer and diluted Sample Diluent is avoided and return to 2-8°C after testing.
6. Replace surplus (unused) microtitre strips in the foil pack with the desiccant. Ensure seal is integral and return to 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 colourless to very pale blue in colour. Turbidity or precipitation in any component indicates deterioration and the component should be discarded.

If crystals are visible in the wash or sample diluent on removal from cold storage, these will dissolve upon inversion and equilibration to room temerature.

Sample Collection and Storage

The assay is recommended for human serum (including serum separator tube (SST)) or plasma (EDTA, lithium heparin, or sodium citrate) samples. Other tube types have not been tested for use in the assay. 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.

For preparation for analysis follow the tube manufacturer's instructions for the collection tubes. Samples may be stored undiluted at 2-8°C for four weeks; for longer storage store at -20°C or lower. Samples diluted at 1:100 in diluted Sample Diluent must be used within the 24 hours 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 or HCV RNA 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),[22] 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 and Sample Diluent 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. Material safety data sheets for all hazardous components contained in this kit are available on request from Axis-Shield Diagnostics.

Caution: Federal law restricts this device to sale by or on the order of a physician

 Warning **CONJUGATE**	**WARNING** H317 – **PREVENTION** P272 - P280 - P363 -	 May cause an allergic skin reaction. Contaminated work clothing should not be allowed out the workplace. Wear protective gloves/protective clothing/eye protection/face protection. Wash contaminated clothing before use.
 Warning **SUBSTRATE**	**WARNING** H302 – H312 – H315 – H319 – H332 – H335 – **PREVENTION** P260 – P280 – **RESPONSE** P301+310 – P304+340 – P305+351+338 –	 Harmful if swallowed. Harmful in contact with skin. Causes skin irritation. Causes serious eye irritation. Harmful if inhaled. May cause respiratory irritation. Do not breathe dust/fume/gas/mist /vapours spray. Wear protective gloves/protectiveclothing/eye protection/face protection. IF SWALLOWED : Immediately call a POISON CENTRE or doctor / physician. IF INHALED : Remove victim to fresh air and keep at rest in a position comfortable for breathing. IF IN EYES : Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing.
  Danger **SAMPLE DILUENT**	**WARNING** H302 – H318 – H412 – EUH032 – **PREVENTION** P264 – P280 – **RESPONSE** P301+310 – P305+351+338 – P330 –	 Harmful if swallowed. Causes serious eye damage. Harmful to aquatic life with long lasting effects. Contact with acids liberates very toxic gas. Wash hands thoroughly after handling. Wear protective gloves/protective clothing/eye protection/face protection. IF SWALLOWED : Immediately call a POISON CENTRE or doctor / physician. IF IN EYES : Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing. Rinse Mouth
 Danger **Stop Solution**	**WARNING** H314 – **PREVENTION** P260 – P273 – P280 – **RESPONSE** P301+330+331 – P303+361+353 – P304+340 – P305+351+338 –	 Causes severe skin burns and eye damage. Do not breathe dust/fume/gas/mist /vapours spray. Avoid Release to the environment. Wear protective gloves/protective clothing/eye protection/face protection. IF SWALLOWED: rinse mouth. Do NOT induce vomiting. IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. IF INHALED : Remove person to fresh air and keep comfortable for breathing. IF IN EYES : Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing.

P R E P A R A T I O N

Materials/Equipment Required but not Provided

1. 96 well plate/strip reader with 450 nm filter.
2. Precision pipettes to dispense 10 µL, 100 µL, 1 mL. Automatic pipette to dispense 100 µL. Automatic pipette to dispense 300 µL for manual washing; automatic plate washer optional.
3. Glass/plastic measuring cylinders: 1×100 mL, 1×500 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	225 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 2-8ºC 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.

A S S A Y P R O T O C O L

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

Semi-Quantitative protocol: assay Calibrators (1-6), Positive and Negative Controls, and samples.

1. Reference wells for identification.
2. Pipette in duplicate 100µL of Reference Control/Calibrators and pre-diluted (1:100) Positive and Negative Controls into appropriate wells. Pipette either in singleton or duplicate 100 µL of pre-diluted (1:100) patient samples into appropriate wells. Samples are recommended to be assayed in duplicate, but is optional according to local laboratory rules. Remember to change pipette tips between additions. This step should not exceed **10 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 **four times** with a minimum of 300 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 addition. Tap wells gently to mix and ensure there are no visible bubbles.
12. Read strips at 450 nm.
13. Read the assay within 60 minutes of completion of the test.

CALCULATION AND INTERPRETATION OF RESULTS

Consider each assay separately when calculating and interpreting results.

Qualitative Protocol

Calculate the mean absorbance value (optical density) ratio for the Positive and Negative Controls, and for each (mean) sample to the mean Reference Control Absorbance Value:

$$\text{Absorbance Ratio} = \frac{\text{Mean Control Absorbance Value}}{\text{Mean Reference Control Absorbance Value}}$$

$$\text{Absorbance Ratio} = \frac{\text{(Mean) Sample Absorbance Value}}{\text{Mean Reference Control Absorbance Value}}$$

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:

Absorbance Ratio	**Result Interpretation**
< 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. Mean concentrations of Positive and Negative Controls and (mean) samples can then be read from the calibration curve; a typical calibration curve plot is shown below for reference purposes, it must not be used for interpreting results. 4-parameter logistic (4PL) and Cubic Spline curve-fits are satisfactory. Other curve-fit models are not recommended.

Samples with absorbances above Calibrator 6 (300 U/mL) are outside the range of the assay, and should be reported as > 300 U/mL, diluted and re-assayed, correcting for this further dilution factor.

For the interpretation of Semi-Quantitative results and on the basis of the Axis-Shield reference* population data the following is suggested:

(Mean) Sample Result	**Result Interpretation**
≤ 5 U/mL	**Negative**
> 5 U/mL	**Positive**

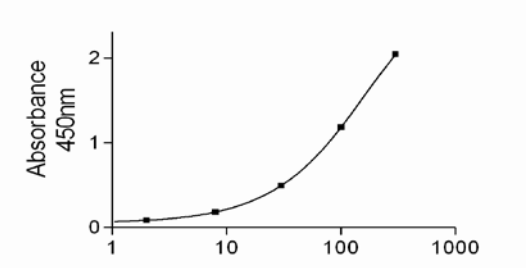
*This is suggested as a guideline only. It is recommended that users establish a reference range, which may be unique to the population.

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	300

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)	$\frac{\text{Positive Control Absorbance}}{\text{Reference Control Absorbance}} \geq 1.1$
	$\frac{\text{Negative Control Absorbance}}{\text{Reference Control Absorbance}} < 0.95$
Semi-Quantitative	See Positive Control vial label for acceptance 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 an Axis-Shield Anti-CCP assay (FCCP200).

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:

Reference Range
≤ 5 U/mL = Negative
> 5 U/mL = Positive

This reference range is suggested as a guideline only and each laboratory should establish a reference range, which may be unique to the population it serves depending upon geographical, patient, dietary, environmental factors or clinical practice. Please note that Rheumatoid Arthritis is twice as prevalent in females as in males.

PERFORMANCE DATA

Dilution Linearity

The Axis-Shield Anti-CCP assay is designed to be linear across the measurement range from LOD to 300 U/mL.

Based on a study performed by guidance from the CLSI document EP6-A,[23] the Axis-Shield Anti-CCP assay demonstrated linearity from 1.04 U/mL to 300 U/mL.*

* Representative data; results in individual laboratories may vary from these data

Samples > 300 U/mL exhibit mean recovery of ≤ 100% ± 15%* of the expected result when diluted into the assay range and using the correct dilution factor.

* Representative data; results in individual laboratories may vary from these data

Clinical Sensitivity and Specificity

The clinical sensitivity of the Axis-Shield Anti-CCP assay (FCCP600) was determined for 229 confirmed RA individuals, and clinical specificity was determined for 285 non-RA specimens (135 from patients with other rheumatic and non-rheumatic disorders and 150 from asymptomatic apparently healthy individuals). Using a cut-off of 5.0 U/mL, the sensitivity was calculated to be 78% with a specificity of 99%. The results are summarized in the following tables.*

Specimen Category	Total n	Positive n	% Sensitivity
RA	229	179	78

Specimen Category	Total n	Positive n	% Specificity
Non-RA Specimens in Total	285	4	98.6
Non-RA Healthy Asymptomatic	150	1	99.3
Non-RA Disease Specimens[+]	135	3	97.8

[+]Clinical specificity for 135 specimens from patients with other rheumatic and non-rheumatic disorders is catagorised in the following table.*

Non-RA Disease Specimens	Total n	Positive n	Clinical specificity
Total	135	3	97.8%
Inflammatory Polyarthritis	41	1	97.6%
EBV IgG Positive	18	1	94.4%
Hashimoto's Thyroiditis	17	0	100%
Sjögren's Syndrome	16	1	93.8%
Systemic Lupus Erythematosus	16	0	100%
Vasculitis	5	0	100%
Scleroderma	5	0	100%
Osteoarthritis	4	0	100%
Crohn's Disease	3	0	100%
Raynaud's Phenomenon	3	0	100%
Ulcerative Colitis	2	0	100%
Psoriatic Arthritis	2	0	100%
Reactive Arthritis	1	0	100%
Ankylosing Spondylitis and Polymyositis	2	0	100%

*Representative data; results in individual laboratories may vary from these data.

Method Comparison

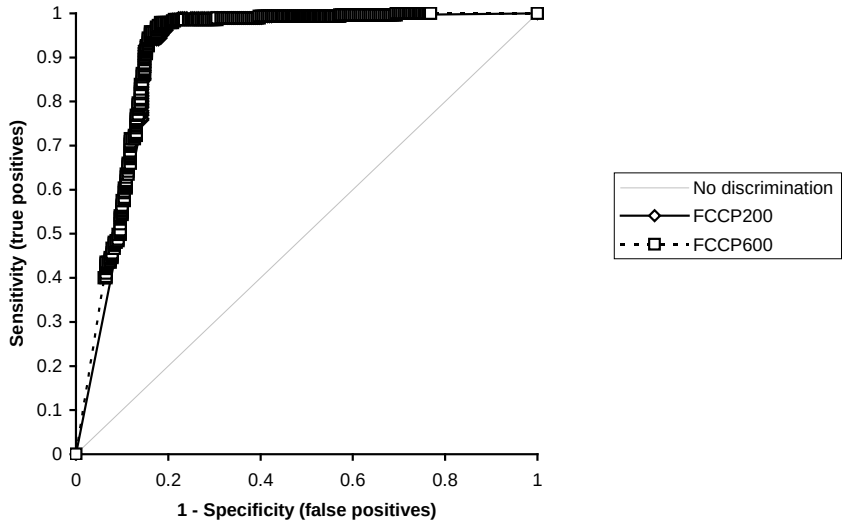
The Axis-Shield Anti-CCP assay (FCCP600) is designed to have a concordance of $\geq$ 99% for RA and non-RA specimens when compared to a comparator Axis-Shield Anti-CCP assay (FCCP200). The RA and non-RA specimens described in the Clinical Sensitivity and Specificity section were used to compare the Axis-Shield Anti-CCP (FCCP600) assay to the Axis-Shield Anti-CCP assay (FCCP200). The cut-off employed for the Axis-Shield Anti-CCP assay (FCCP200) was 5.0 U/mL, as stated in the manufacturer's package insert. Using a cut-off of 5.0 U/mL for the Axis-Shield Anti-CCP assay (FCCP600), the concordance was calculated to be 99%. The results are summarized in the following tables.*

All Samples (514)		FCCP200	
		Positive	Negative
FCCP600	Positive	179	4
	Negative	1	330

Comparison Method	FCCP600 vs FCCP200
Number of specimens	65
Slope of regression line	0.910
Y-Intercept	1.226
Correlation coefficient	0.94

*Representative data; results in individual laboratories may vary from these data.

A Receiver Operator Characteristic (ROC) analysis was carried out using the above data obtained for the two assays. The area under the curve (AUC) for the Axis-Shield Anti-CCP assay (FCCP600) was 0.910 (95% confidence interval: 0.881-0.940) and 0.903 (95% confidence interval: 0.871-0.934) for the comparator Axis-Shield Anti-CCP assay (FCCP200), thus indicating that both assays are comparable with respect to their clinical differentiation. The ROC analysis curve is shown below.*



*Representative data; results in individual laboratories may vary from these data.

Precision

A study was performed with guidance from the CLSI (formally NCCLS) Document EP5-A2.[24] Two anti-CCP controls, six QC panel members and one human serum sample were assayed using two lots of reagents, in replicates of two, at two separate times per day for 20 days (n=80). Data from this study are summarised in the following table as representative data (*rounded to 1 decimal place*):

Sample	Kit Lot	n	Mean (U/mL)	Within Run		Between Run		Between Day		Total	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV
Positive Control	001	80	20.30	1.05	5.2	1.24	6.1	0.00	0.0	1.63	8.0
	003		20.62	0.43	2.1	1.20	5.8	0.00	0.0	1.27	6.2
QC 1	001	80	3.72	0.33	8.8	0.17	4.5	0.13	3.6	0.39	10.5
	003		3.92	0.23	5.8	0.35	8.9	0.04	1.1	0.42	10.7
QC 2	001	80	8.17	0.34	4.2	0.72	8.8	0.00	0.0	0.80	9.8
	003		8.47	0.30	3.6	0.70	8.3	0.25	2.9	0.80	9.5
QC 3	001	80	15.30	0.37	2.4	0.93	6.0	0.30	1.9	1.04	6.8
	003		15.98	0.36	2.2	0.92	5.8	0.00	0.0	0.99	6.2
QC 4	001	80	53.55	2.30	4.3	3.19	6.0	1.71	3.2	4.29	8.0
	003		55.49	2.36	4.2	3.35	6.0	0.00	0.0	4.09	7.4
QC 5	001	80	94.26	3.17	3.4	7.31	7.8	2.01	2.1	8.22	8.7
	003		97.15	2.61	2.7	5.56	5.7	4.79	4.9	7.79	8.0
QC 6	001	80	134.77	4.58	3.4	5.84	4.3	5.74	4.3	9.38	7.0
	003		142.41	5.69	4.0	9.02	6.3	0.00	0.0	10.67	7.5
Ref Control	001	80	5.18	0.34	6.6	0.24	4.6	0.21	4.0	0.46	9.0
	003		5.09	0.26	5.1	0.21	4.1	0.21	4.2	0.39	7.7
Sample 1	001	80	4.83	0.16	3.3	0.38	7.9	0.24	5.0	0.48	9.9
	003		4.77	0.20	4.1	0.37	7.8	0.25	5.2	0.49	10.2

* Representative data; results in individual laboratories may vary from these data

Limit of Detection

The limit of detection (LOD) of the Axis-Shield Anti-CCP assay according to the CLSI (formally NCCLS) Document EP17-A[25] was found to be 1.04 U/mL*.

LOD determinations were performed using one negative anti-CCP sample (60 replicates) and six low-level anti-CCP samples (15 replicates each).

*Representative data; results in individual laboratories may vary from these data.

High Dose Hook

High dose hook is a phenomenon whereby very high level specimens may read within the dynamic range of the assay. For the Axis-Shield Anti-CCP assay, no high dose hook effect was observed when a sample containing approximately 3000 U/mL of anti-CCP antibody was assayed.*

* Representative data; results in individual laboratories may vary from these data.

Interference

The Axis-Shield Anti-CCP assay is designed to have a maximum deviation in anti-CCP concentration from the following potentially interfering compounds within:

- $\pm$ 15% for anti-CCP concentrations $\geq$ 10.0 U/mL
- $\pm$ 10% for anti-CCP concentrations $\geq$ 4.0 U/mL to < 10.0 U/mL
- <0.75 U/mL for anti-CCP concentrations < 4.0 U/mL

A study was performed based on guidance from the Clinical and Laboratory Standards Institute (CLSI) document EP7-A2[26] for the Axis-Shield Anti-CCP assay. Six samples with anti-CCP levels across the assay range were supplemented with the potentially interfering compounds listed in the table below. The maximum deviation of anti-CCP concentration observed in samples during these studies ranged from:

- -9.4% to 3.3% for anti-CCP concentrations $\geq$ 10.0 U/mL
- -7.3% to 4.8% for anti-CCP concentrations $\geq$ 4.0 U/mL to < 10.0 U/mL
- -0.6 U/mL to 0.05 U/mL for anti-CCP concentrations < 4.0 U/mL*

Potetial Interfering Substance	No interference found up to the following concentration
Haemoglobin	4 mg/mL
Bilirubin	0.2 mg/mL
Triglyceride (Intralipid Solution)	15 mg/mL
Rheumatoid Factor	200 IU/mL
Total Protein	120 mg/mL

*Representative data; results in individual laboratories may vary from these data.

L I M I T A T I O N S O F U S E

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.

R E F E R E N C E S

1. Feldmann M, Brennan FM, Maini RN. Rheumatoid Arthritis. *Cell* 1996;85:307-10.
2. Landewé 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. Arnett FC, Edworthy SM, Bloch DA, *et al*. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis*. Arthritis Rheum* 1988;31(3):315-24.
5. van Venrooij WJ, Hazes JM, Visser H. Anticitrullinated protein/peptide antibody and its role in the diagnosis and prognosis of early rheumatoid arthritis. *Neth J Med* 2002;60(10):383-8.
6. Nienhuis RL, Mandema E, Smids C. A new serum factor in patients with rheumatoid arthritis. The antiperinuclear factor. *Ann Rheum Dis* 1964;23:302-05.
7. Young BJ, Mallya RK, Leslie RD, *et al*. Anti-keratin antibodies in rheumatoid arthritis. *Br Med J* 1979;2:97-9.
8. Hoet RM, Boerbooms AM, Arends M, *et al.* Antiperinuclear factor, a marker autoantibody for rheumatoid arthritis: colocalisation of the perinuclear factor and profilaggrin. *Ann Rheum Dis* 1991;50:611-8.
9. Sebbag M, Simon M, Vincent C, *et al.* The antiperinuclear factor and the so-called antikeratin antibodies are the same rheumatoid arthritis-specific autoantibodies. *J Clin Invest* 1995;95:2672-9
10. Vossenaar ER, Zendman AJ, van Venrooij WJ, *et al*. PAD, a growing family of citrullinating enzymes: genes, features and involvement in disease. *BioEssays* 2003;25:1106-18
11. Schellekens GA, de Jong BA, van den Hoogen FH, *et al*. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. *J Clin Invest* 1998;101(1):273-81
12. Schellekens GA, Visser H, de Jong BA, *et al*. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. *Arthritis Rheum* 2000;43(1):155-63.
13. Vossenaar ER, van Venrooij WJ. Anti-CCP antibodies, a highly specific marker for (early) rheumatoid arthritis. *Clin Applied Immunol Rev* 2004;4:239-62.
14. Pruijn GJ, Vossenaar ER, Drijfhout JW, *et al*. Anti-CCP antibody detection facilitates early diagnosis and prognosis of rheumatoid arthritis. *Current Rheumatology Reviews* 2005;1(1):1-7.
15. Rantapaa-Dahlqvist S, de Jong BA, Berglin E, *et al*. Antibodies against cyclic citullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthirtis Rheum* 2003: 48(10):2741-49
16. Nielen MM, van Schaardenbur D, Reesink HW, *et al*. Specific autoantibodies precede the symptoms of rheumatoid arthritis. *Arthritis Rheum* 2004: 50 (2):380-386
17. van Gaalen, Linn-Rasker SP, van Venrooij WJ, *et al*. Autoantibodies to cyclic citrullinated peptides predict progression to rheumatoid arthritis in patients with undifferentiated arthritis. *Arthritis Rheum* 2004: 50(3):709-15
18 Meyer O, Labarre C, Dougados M, *et al*. Anticitrullinated protein / peptide antibody assays in early Rheumatoid Arthritis for predicting five year radiographic damage. *Ann Rheum Dis* 2003: 62:120-26
19. Forslind K, Ahlmén M, Eberhardt K, *et al*. Prediction of radiological outcome in early rheumatoid arthritis in clinical practice: role of antibodies to citrullinated peptides (anti-CCP). *Ann Rheum Dis* 2004: 63:1090-95
20. Kastbom A, Strandberg G, Lindroos A, *et al.* Anti-CCP antibody test predicts the disease course during 3 years in early rheumatoid arthritis (the Swedish TIRA project). *Ann Rheum Dis* 2004: 63:1085-89
21. Aletaha D, Neogi T, Silman AJ, *et al*. 2010 Rheumatoid Arthritis Classification Ciriteria. An American College of Rheumatology/European League against Rheumatism Collaborative Initiative. *Arthritis Rheum* 2010: 62 (9):2569-81
22. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline – Third Edition*. CLSI Document M29-A3. Wayne, PA: Clinical and Laboratory Standards Institute, 2005.
23. The National Committee for Clinical Laboratory Standards. *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline.* NCCLS document EP6-A. Wayne, PA: The National Committee for Clinical Laboratory Standards, 2003.
24. The National Committee for Clinical Laboratory Standards. *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition*. NCCLS document EP5-A2. Wayne, PA: The National Committee for Clinical Laboratory Standards, 2004.
25. The National Committee for Clinical Laboratory Standards. *Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline.* NCCLS document EP17-A Wayne, PA: The National Committee for Clinical Laboratory Standards, 2004.
26. Clinical and Laboratory Standards Institute. *Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition.* CLSI document EP7-A2. Wayne, PA: Clinical and Laboratory Standards Institute, 2005.

Axis-Shield Diagnostics Limited

The Technology Park, Dundee DD2 1XA, UK.

Tel: +44 (0) 1382 422000, *Fax:* +44 (0) 1382 422088.

E-mail: shield@axis-shield.com

Web: www.axis-shield.com

IVD	*In vitro* diagnostic medical device
REF	Catalogue number
LOT	Lot
Σ 96	96 tests
	Caution
	Consult instructions for use
	Protect from Light
	Use by
+8°C +2°C	Store at 2-8°C
Rx Only	For Prescription Use Only
	Manufactured by
CONTROL +	Positive Control
CONTROL -	Negative Control
CONJ	Conjugate
SUBS	Substrate
SOLN STOP	Stop Solution
BUF WASH 10 X	Wash Buffer
MTP 8 x 12	Microtitre (Breakapart) Strips
SAMPLE DIL 5 X	Sample Diluent
CAL 1	Calibrator 1
CAL 2 - CAL 6	Calibrator 2-6
CONTROL REF	Reference Control

Ver: 2016/08