

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

k110296

B. Purpose for Submission:

New device

C. Measurand:

Anti-cyclic citrullinated peptide (CCP) antibodies

D. Type of Test:

Semi-quantitative/qualitative enzyme-linked immunosorbent assay (ELISA)

E. Applicant:

Axis-Shield Diagnostics Limited

F. Proprietary and Established Names:

Axis-Shield Anti-CCP

G. Regulatory Information:

1. Regulation section:

21 CFR §866.5775 Rheumatoid factor immunological test system.

2. Classification:

Class II

3. Product code:

NHX, Antibodies, Anti-Cyclic Citrullinated Peptide (CCP)

4. Panel:

Immunology (82)

H. Intended Use:

1. Intended use(s):

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.

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

96-well plate/strip reader capable of measuring optical density (OD) at 450 nm

I. Device Description:

Each device contains the following components:

- A microtitre plate with 8 x 12-well break-apart strips coated with purified synthetic CCP containing modified arginine residues (CCP2 peptides);

- Ready-to-use calibrators (human plasma with or without IgG antibodies against CCP);
- Positive and negative assay controls (human plasma with or without IgG antibodies against CCP);
- Ready-to-use reference control;
- Goat anti-human IgG horseradish peroxidase conjugate;
- TMB substrate;
- Sample diluent (5x);
- Wash buffer (10x);
- Ready-to-use stop solution (0.25 mol/L sulphuric acid solution).

J. Substantial Equivalence Information:

1. Predicate device name(s) and 510(k) number(s):
Diastat™ Anti-CCP assay (k023285)
2. Comparison with predicate:

Similarities		
Item	Device	Predicate
	Axis-Shield Anti-CCP (FCCP600)	Diastat™ Anti-CCP (FCCP200)
Intended Use/ Indications for Use	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. For in vitro diagnostic use	Same
Indications for Use	To aid in the diagnosis of rheumatoid arthritis	Same
Technology	ELISA, 96-well plate; 8 x 12 well microtitre strips	Same
Capture antigen	Synthetic cyclic citrullinated peptide (CCP), second generation.	Same
Cut-off	5.0 U/mL	Same
Quantitation	Results determined from a standard calibration curve (0, 2, 8, 30, 100, 300 U/mL) generated on each microtitre plate	Same
Specimen Type	Human serum (SST) or plasma (EDTA, lithium heparin or sodium citrate)	Same
Expected values in asymptomatic population	0.05 – 3.8 U/mL	Same
Control	Negative and positive kit controls	Same
Calibration	Qualitative protocol: The amount of Conjugate bound by the sample is compared with that bound by the	Same

Similarities		
Item	Device	Predicate
	Reference Control. Semi-quantitative protocol: The concentration of anti-CCP autoantibody can be estimated by interpolation from a dose-response curve based on calibrators	
Storage conditions	Store kit components at 2-8°C. Do not freeze kits.	Same
Interference	Hemoglobin (up to 400 mg/dL), bilirubin (up to 0.2 mg/mL), intralipid (up to 15 mg/mL), rheumatoid factor (up to 200 IU/mL) do not interfere with anti-CCP antibody results	Same

Differences		
Item	Device	Predicate
	Axis-Shield Anti-CCP (FCCP600)	Diastat™ Anti-CCP (FCCP200)
Conjugate Antibody	Horseradish peroxidase-labeled goat polyclonal antibody to human IgG	Alkaline phosphatase-labeled murine monoclonal antibody to human IgG
Substrate	3,3',5,5'-Tetramethylbenzidine (TMB).	Mg ²⁺ , phenolphthalein monophosphate (PMP)
Calibration	0, 2, 8, 30, 100, 300 U/mL	0, 2, 8, 30, 100 U/mL
Assay End-Point	Color, read at 450 nm	Color, read at 540-565 nm
Calibrator Range	0-300 U/mL	0-100 U/mL
Assay Range	1.04-300 U/mL	0.05-100 U/mL
Analytical Sensitivity	Limit of detection = 1.04 U/mL.	Lower limit of detection (Mean + 2 SD of zero calibrator) = 0.05 U/mL.
Imprecision	Within-run CV: 2.1% to 8.8% Between-run CV: 4.1 to 8.9% from 3.7 to 205.2 U/mL	Intra-assay CV: 7.6% to 10.5% Inter-assay CV: 7.6% to 13.6% from 5.4 to 34.1 U/mL
Interference	Total Protein up to 120 mg/mL does not interfere with anti-CCP antibody results	Not reported or assessed

K. Standard/Guidance Document Referenced (if applicable):

CLSI EP05-A2 Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition

CLSI EP06-A Evaluation of the Linearity of Quantitative Measurement

CLSI EP07-A2 Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition

CLSI EP09-A2 Method Comparison and Bias Estimation Using Patient Samples;

Approved Guideline

CLSI EP17-A Protocols for Determination of Limits of Detection and Limits of Quantitation

L. Test Principle:

The Axis-Shield Anti-CCP assay is an ELISA based on the detection of IgG autoantibodies in human serum or plasma towards a synthetic CCP containing modified arginine residues (CCP2 peptides). The test provides an additional tool in the diagnosis of patients with RA. The wells of the microtitre strips are coated with purified CCP2 peptides. Patient sample is added and 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-labeled polyclonal antibody to human IgG, binds to any surface-bound autoantibodies. After further washing, specific autoantibodies are detected by incubation with the Substrate. Addition of Stop Solution terminates the reaction, resulting in a colored 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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:* Two kit combinations were used throughout the study including reagents, positive and negative controls, quality control (QC) controls and reference control. Testing was carried out by at least two operators. The sample and controls were assayed in replicates of two, twice daily, for 20 days (n=80 for each control/sample) and the sampling order was randomized on the individual testing days throughout the study.

Quantitative assay: nine human plasma or serum based samples were used for the study (20 days x 2 runs per day x2 replicates=80 determinations per sample; 40 per kit lot combination). The sample concentrations are detailed in the table below:

Sample	Target Concentration (U/mL)
Assay positive control	25.0
Assay reference control	5.0
Serum sample 1	5.0
QC 1 (plasma)	4.1
QC 2 (plasma)	7.8
QC 3 (plasma)	16.0
QC 4 (plasma)	56.0
QC 5 (serum)	97.0
QC 6 (serum)	146.0

Five additional samples (A1-A5) were tested to verify imprecision of the study. These samples were prepared from processed human plasma to achieve target CCP concentrations of 5.2, 3.9, 185.0, 191.4, 205.2 U/mL. Assays were

run in duplicates, twice daily for 5 days, using 2 operators and 2 kit combinations, resulting in a total of n=40 observations for each sample.

Precision data for each of the studies is summarized in the tables below:

20-Day Imprecision Data N=80		Between- Reagent		Between- Day		Between- Run		Within- Run		Total	
Sample I.D.	Mean (U/mL)	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Positive control	20.4	0.1	0.7	0.0	0.0	1.2	6.0	0.8	3.9	1.7	7.2
QC 1	3.8	0.1	3.5	0.1	2.6	0.3	7.2	0.3	7.4	0.4	11.2
QC 2	8.3	0.2	2.1	0.0	0.0	0.7	8.5	0.3	3.9	0.8	9.6
QC 3	15.6	0.5	2.9	0.0	0.0	0.9	5.9	0.4	2.3	1.1	7.0
QC 4	54.5	1.2	2.3	0.9	1.7	3.3	6.0	2.3	4.2	4.3	7.9
QC 5	95.7	1.5	1.6	3.7	3.8	6.5	6.8	2.9	3.0	8.2	8.5
QC 6	138.6	5.2	3.7	4.0	2.9	7.6	5.5	5.2	3.7	11.3	8.1
Ref Control	5.1	0.0	0.0	0.2	4.1	0.2	4.3	0.3	5.9	0.4	8.4
Sample 1	4.8	0.0	0.0	0.3	5.1	0.4	7.8	0.2	3.7	0.5	10.1

5-Day Imprecision Data N=40		Between- Reagent		Between- Day		Between- Run		Within- Run		Total	
Sample I.D.	Mean (U/mL)	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Sample 1A	5.2	0.0	0.0	0.0	0.0	0.3	5.8	0.2	4.0	0.4	7.0
Sample 2A	3.9	0.0	0.3	0.0	0.0	0.3	7.5	0.1	3.5	0.3	8.2
Sample 3A	185.0	0.0	0.0	4.0	2.1	10.4	5.6	6.2	3.4	12.7	6.9
Sample 4A	191.4	3.5	1.8	0.7	0.4	8.8	4.6	8.8	4.6	12.9	6.8
Sample 5A	205.2	3.3	1.6	0.0	0.0	11.4	5.5	6.8	3.3	13.6	6.6

Qualitative precision: Three samples were evaluated for the repeatability/reproducibility of the assay. The data is presented as positive, negative or borderline outcome as a percent of the 40 samples run in that study. The results are summarized in the table below:

Sample	Lot	N	Mean (U/mL)	Overall mean (U/mL)	% Negative	% Borderline	% Positive
Serum sample 1	001	40	4.8	4.8	36.3	41.3	22.5
	003	40	4.8		42.5	32.5	25.0
QC 1 (plasma)	001	40	3.7	3.8	97.5	1.3	1.3
	003	40	3.9		97.5	2.5	0.0
QC 2 (plasma)	001	40	8.2	8.4	0.0	0.0	100.0
	003	40	8.5		0.0	0.0	100.0

b. *Linearity/assay reportable range:*

Linear range was determined using a total of 13 samples pools with high anti-CCP levels (4 plasma sample pools, 2 serum sample pools, 4 spiked serum sample pools and 2 spiked plasma sample pools) diluted using an anti-CCP negative sample, according to the dilution scheme recommended in CLSI document EP6-A. Samples were tested in triplicates. Polynomial regression was used to assess linearity. The observed concentrations (Y) were plotted against the expected concentrations (X) at each dilution, for each of the samples. The % recoveries were 76.6 to 114.1%. The results are summarized in the table below:

Sample I.D	Dilution Range (GPL-U/mL)	Slope (95% CI)	Y-intercept (95% CI)	R ²	%CV Range
1	0.0 – 247.9	0.9826 (0.9412, 1.0239)	+1.3762 (-4.8899, 7.6422)	0.991	0.76 – 4.10
2	0.4 – 97.9	0.9573 (0.9114, 1.0032)	-0.1462 (-2.8103, 2.5178)	0.996	0.57 – 27.11
3	0.7 – 15.1	1.0069 (0.9670, 1.0467)	-0.0546 (-0.4179, 0.3088)	0.9973	0.78 – 20.37
4	0.6 – 48.1	1.0279 (0.9744, 1.0814)	-0.7815 (-2.3107, 0.7477)	0.9953	0.37 – 10.63
5	0.2 – 123.2	1.0234 (0.9705, 1.0763)	-1.6047 (-5.4605, 2.2512)	0.9953	0.42 – 22.33
8	0.0 – 181.1	0.997 (0.9598, 1.0341)	-2.304 (-6.2855, 1.6775)	0.9976	0.52 – 5.40
9	0.0 – 283.6	1.0208 (0.9566, 1.0850)	-5.6586 (-16.4355, 5.1183)	0.9931	0.73 – 6.31
10	0.0 – 377.2	0.9599 (0.8765, 1.0433)	-9.9781 (-28.5916, 8.6355)	0.9869	1.35 – 6.67
11	0.0 – 254.5	1.0026 (0.9533, 1.0519)	-4.2706 (-11.6998, 3.1580)	0.9958	0.52 – 6.79
12	0.0 – 268.5	1.0196 (0.9771, 1.0622)	-9.0985 (-15.8556, 2.3414)	0.9969	1.58 – 8.14
13	0.0 – 306.8	1.0107 (0.9616, 1.0599)	-9.7967 (-18.722, 0.8715)	0.9959	1.37 – 9.10

Axis-Shield anti-CCP assay claimed reportable range is 1.04 U/mL (LoD) - 300.0 U/mL.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Traceability: There is no recognized standard reference material for anti-CCP.

Calibrators and Controls (positive and negative) are prepared in-house and arbitrary units are assigned during the development process. The Calibrators and Controls for the Axis-Shield anti-CCP submission device are the same final formulation as those previously cleared for use with Diastat™ anti-CCP device (k023285) with the exception of the additional 300U/mL calibrator. Calibrator values are summarized in the table below:

Calibrator	Anti-CCP U/mL
1	0
2	2
3	8
4	30
5	100
6	300

Control values are summarized in the table below:

Control	Target concentration	Concentration Range
Negative	< 2.0 U/mL	< 2.0 U/mL
Positive	25.0 U/mL	13.0 – 37.0 U/mL
Reference	5.0 U/mL	2.7 – 7.3 U/mL

Stability: The claimed shelf life is 52 weeks at 2-8°C. The claimed open vial stability is 3 months from first opening. Real-time stability studies are ongoing; current data supports 13 weeks but testing will continue until at least 108 weeks.

d. *Detection limit:*

Limit of Blank (LoB): An anti-CCP negative serum sample was diluted 1/100 in sample diluent and run for a total of n=60. $LoB = \text{Result at position } [0.95 \times N_B + 0.5]$, where N_B is the number of blank measurements (n=60). The LoB was the average of the ranked results at positions 57 and 58. LOB was determined to be 0.348U/mL.

Limit of Detection (LoD): four serum samples (mean concentrations of 1.35, 0.70, 0.68 and 0.52 U/mL) were assayed by two operators in replicates of eight, using two lots of kit components for a total of 15 replicates per sample and n=60. $LoD = LoB + (1.643 \times \text{pooled SD})$. The claimed LoD is 1.04 U/mL.

e. *Analytical specificity:*

Endogenous Interference: Endogenous interferents tested included hemoglobin, bilirubin, triglyceride (intralipid solution), rheumatoid factor and total protein (bovine serum albumin, BSA). Six samples with differing anti-CCP concentrations (range from 2.7 U/mL to 261 U/mL) were spiked with at least two different concentrations of the interfering substances. The results are summarized in the table below:

Potential Interfering compound	No interference found up to the following concentrations
Hemoglobin	4 mg/mL
Bilirubin	0.2 mg/mL
Triglyceride (Intralipid Solution)	15 mg/mL
Rheumatoid factor	200 IU/mL
Total Protein (BSA)	120 mg/mL

Hook Effect/Over the Range Results: Two plasma samples with elevated anti-CCP concentrations (2809 U/mL and 2736 U/mL) were used to establish whether a possible hook effect is observed in the Axis-Shield Anti-CCP (FCCP600) assay. Samples were diluted to create a range from 3000 U/mL to < 100 U/mL. Calibrators, kit controls and the samples dilutions were tested in duplicate and the data generated was plotted for visual assessment of assay high dose hook. The percentage recoveries of the dilution-corrected samples on the test device are within $\pm 20\%$ of the actual concentrations determined on the predicate device. No high dose hook effect was observed.

- f. Assay cut-off:
The Axis-Shield Anti-CCP (FCCP600) uses the same cut-off value as the predicate device: k023285 (FCCP200)

Semi-Quantitative Protocol:

Samples with results ≤ 5 U/mL are defined as negative.
Samples >5 U/mL are defined as positive.

Qualitative Protocol:

Absorbance ratio	Result interpretation
< 0.95	Negative
≥ 0.95 to ≤ 1.0	Borderline – recommend repeat testing
> 1.0	Positive

2. Comparison studies:

- a. *Method comparison with predicate device:*

Specimens were collected from 514 subjects including 229 RA patients, 150 asymptomatic, apparently healthy and 135 non-RA patients with other diseases. Diagnosis of RA was made according to the 1987 American College of Rheumatology (ACR) revised criteria. Specimens spanned the Axis-Shield Anti-CCP (FCCP600) measurement range.

Using a cutoff of 5.0 U/ml, concordance analysis was performed on all sample (n=514). The results are summarized below:

		Diastat™ Anti-CCP (FCCP200)		
		Positive	Negative	Total
Axis-Shield Anti-CCP (FCC600)	Positive	179	4	183
	Negative	1	330	331
	Total	180	334	514

% Positive agreement = 99.4% (179/180)

% Negative agreement = 98.8% (330/334)

% Total agreement = 99.0% (509/514)

In addition, 65 of the 514 samples that were within the measurement range of both the new and predicate device (1.04 U/mL to 100 U/mL) and 8 additional

samples around the assay cut-off were assayed in duplicate with both Diastat™ Anti-CCP FCCP200 and Axis-Shield Anti-CCP FCCP600 assays. Results of the regression analysis of the 73 samples are summarized in the table below:

n	Slope	Slope 95% CI	Intercept	Intercept 95% CI	r
73	0.920	0.83 to 0.99	1.06	0.57 to 1.73	0.94

b. Matrix comparison:

Nineteen matched serum and plasma samples were collected in the following anticoagulant tubes: serum clot tube, serum separator tube (SST), Potassium EDTA plasma tube, Lithium Heparin plasma tube, Sodium Citrate plasma tube. Specimens spanned the Axis-Shield Anti-CCP (FCCP600) measurement range. In order to obtain samples with varying CCP concentrations covering the measurable range of the assay (1.04 U/mL – 300 U/mL), 15 of the 19 serum/plasma samples were spiked with a high anti-CCP positive sample and 4 samples were tested unmodified. In addition to these samples, 16 spiked-in serum/plasma matched specimens with anti-CCP concentrations at approximately 2.5 U/mL and 5.5 U/mL for the different tube type and anticoagulant were evaluated. Samples with results <LoD were excluded from the study analysis.

For all samples, Passing Bablok regression plots were generated by plotting the mean concentration observed from the control tube type (serum) versus the mean concentration for each test collection tube. The corresponding regression slopes and intercepts as well as correlation coefficients are summarized in the table below:

Matrix Comparison	N	Slope (95% CI)	Intercept (95% CI)	Correlation (95% CI)
Serum vs SST	32	0.957 (0.94, 1.01)	0.176 (-0.10, 0.28)	0.996 (0.991, 0.998)
Serum vs EDTA	33	0.928 (0.92, 1.10)	1.23 (-0.15, 0.19)	0.996 (0.991, 0.998)
Serum vs Lithium Heparin	32	0.946 (0.92, 0.96)	0.20 (0.13, 0.32)	0.999 (0.998, 1.000)
Serum vs Sodium Citrate	31	0.955 (0.94, 1.00)	0.052 (-0.11, 0.14)	0.998 (0.996, 0.999)

3. Clinical studies:

a. Clinical Sensitivity:

A total of 229 frozen retrospective sera with clinical characterization were assayed. Diagnosis of RA was made according to the American College of Rheumatology's (ACR) criteria. The results are summarized below:

Samples	Confirmed RA (n)	FCC 600 Positive	FCC600 Clinical Sensitivity	95% CI
Early RA	43	27	63%	50.55 to 78.44
Established RA	186	152	82%	86.34 to 94.91
All RA	229	179	78%	88.19 to 95.63

b. *Clinical specificity:*

Samples from non-RA diseased (n=135) and healthy asymptomatic (n=150) donors were tested. The results are summarized below:

Healthy asymptomatic	149/150 = 99.3%	95% CI = 85.4 to 94.4 %
Non RA disease states	132/135 = 97.8%	95% CI = 83.8 to 93.7 %
Non RA total	281/285 = 98.6%	95% CI = 91.8 to 96.9%

The non-RA specimens are categorized 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%

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off:

Same as assay cut-off.

5. Expected values/Reference range:

Expected values in the normal population should be negative. Each laboratory should establish a reference range appropriate to their patient populations and clinical practice.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.