510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM

A. 510(k) Number:

K153551

B. Purpose for Submission:

New Device

C. Measurand:

Anti-cyclic citrullinated peptide (CCP) IgG autoantibodies

D. Type of Test:

Semi-quantitative chemiluminescent immunoassay (CIA)

E. Applicant:

Axis-Shield Diagnostics Ltd.

F. Proprietary and Established Names:

ADVIA Centaur® Anti-CCP IgG Assay
ADVIA Centaur® Anti-CCP IgG Quality Controls
ADVIA Centaur® Anti-CCP IgG Master Curve Materials

G. Regulatory Information:

1. Regulation section:

   21 CFR §866.5775 – Rheumatoid Factor Immunological Test System
   21 CFR §862.1150 – Calibrator
   21 CFR §862.1660 – Quality Control Material (Assayed and Unassayed)

2. Classification:

   Class II – Assay and Calibrator
   Class I – Control

3. Product code:

   NHX – Antibodies, Anti-Cyclic Citrullinated Peptide (CCP)
   JJX – Single (Specified) Analyte Controls (Assayed and Unassayed)

4. Panel:

   Immunology (82) (Assay)
   Clinical Chemistry (75) (Calibrators and Controls)

H. Intended Use:

1. Intended use(s):

   The ADVIA Centaur® Anti-CCP IgG (aCCP) assay is for in vitro diagnostic use in the semi-quantitative determination of the IgG class of autoantibodies specific to cyclic citrullinated peptide (CCP) in human serum and plasma (K2-EDTA, lithium heparin) using the ADVIA Centaur XP system. 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-criteria diagnostic process, encompassing both clinical and laboratory-based assessments.

   The ADVIA Centaur® Anti-CCP IgG (aCCP) quality control material is for in vitro diagnostic use to monitor the precision and accuracy of the ADVIA Centaur aCCP assay using the ADVIA Centaur systems.

   The ADVIA Centaur® Anti-CCP IgG (aCCP) Master Curve Material (MCM) is for in vitro diagnostic use in the verification of calibration and reportable range of the ADVIA Centaur aCCP assay.

2. Indication(s) for use:

   Same as Intended Use above

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   ADVIA Centaur XP system

I. Device Description:

The ADVIA Centaur® Anti-CCP IgG assay contains the following materials for 100 tests:

a. One ReadyPack primary reagent pack contains

- Lite Reagent: Monoclonal anti-human IgG (0.1 μg/mL) labeled with acridinium ester in buffer with surfactant and preservatives; 10 mL/reagent pack
- Solid Phase Reagent: Paramagnetic microparticles coated with streptavidin

coupled with biotinylated CCP (0.2 mg/mL) in buffer with surfactant and Proclin 300 (0.1%); 20.0 mL/reagent pack
- aCCP Ancillary Reagent: Buffer with surfactants and Prolin 300 (0.1%); 25 mL/reagent pack

b. One vial aCCP ADVIA Centaur low calibrator (0 U/mL)
c. One vial aCCP ADVIA Centaur high calibrator (150 U/mL)
d. AVDIA Centaur systems aCCP Calibrator Assigned Value card

The ADVIA Centaur aCCP Quality Control is sold separately and contains:

a. One vial of negative control: Processed human plasma with anti-CCP concentration of 3.5 U/mL; 7.0 mL/vial
b. One vial of positive control: Processed human plasma with anti-CCP concentration of 25 U/mL; 7.0 mL/vial
c. Lot-specific value sheet and barcode labels

The ADVIA Centaur aCCP Master Curve Material (MCM) is sold separately and contains five vials of MCMs:

a. MCM1: Buffer with BSA and sodium azide (< 0.1%) with anti-CCP concentration of 0 U/mL, 2 mL/vial
b. MCM2–5: Human plasma containing anti-CCP antibody at concentration of 45.0, 90.0, 135.0 and 180 U/mL, respectively, each level containing BSA, buffer and sodium azide (< 0.1%), 2 mL/vial

J. Substantial Equivalence Information:

1. Predicate device name(s) and 510(k) number(s):

   ARCHITECT Anti-CCP Assay, K083868

2. Comparison with predicate:

Similarities		
Item	Device ADVIA Centaur® aCCP	Predicate ARCHITECT Anti-CCP
Intended Use	Semi-quantitative determination of anti-CCP antibodies in human serum and plasma. An aid in the diagnosis of rheumatoid arthritis (RA).	Same
Sample type	Serum and Plasma (Li-Heparin, K2-EDTA)	Same
Analyte	Anti-CCP IgG antibodies	Same

Similarities		
Item	Device ADVIA Centaur® aCCP	Predicate ARCHITECT Anti-CCP
Measurement	Semi-quantitative	Same
Assay Technology	Chemiluminescent Microparticle immunoassay (CMIA)	Same
Capture antibody	CCP, second generation	Same
Conjugate	Acridinium-labeled mouse anti-human IgG	Same
Detection Method	Chemiluminescent immunoassay	Same
Assay cut-off	5.0 U/mL	Same
Storage condition	2–8°C	Same

Differences		
Item	Device: ADVIA Centaur® aCCP	Predicate: ARCHITECT Anti-CCP
Instrument	ADVIA Centaur XP system	ARCHITECT *i* system
Assay Dilution	Manual (1:10)	Auto-dilution (1:6)
Calibration curve	10-point master calibration curve stored on the lot-specific aCCP master curve card	6-point calibration curve stored on the instrument.
Assay Measuring Range (AMR)	0.4–200 U/mL	0.5–200 U/mL

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

- EP5-A2, Evaluation of Precision of Quantitative Measurement Procedures, Approved Guideline, Third Edition.
- EP6-A, Evaluation of Linearity of Quantitative Measurement, Approved Guideline, Second Edition.
- EP7-A2, Interference Testing in Clinical Chemistry, Approved Guideline, Second Edition
- EP9-A3, Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline, Third Edition.
- EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline, Second Edition
- EP24-A2, Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves; Approved Guideline, Second Edition
- EP25-A, Evaluation of Stability of In vitro Diagnostic Reagents, Approved Guideline

L. Test Principle:

The ADVIA Centaur aCCP assay is a fully automated, two-step immunoassay using chemiluminescent technology. The patient sample is diluted with Ancillary Reagent and mixed with the Solid Phase which consists of magnetic latex microparticles coated with

streptavidin-biotinylated CCP complex. Anti-CCP antibodies in the sample binds to the CCP coated microparticles. After washing, the particles are mixed with the Lite Reagents which contains acridinium ester labeled anti-human IgG. After incubation, the particles are washed and then added with Acid Reagent and Base Reagent to initiate the chemiluminescent reaction. The result is measured as relative light units (RLUs). The amount of anti-CCP IgG present in the patient sample is calculated for U/mL based on obtained RLU against the lot-specific master curve.

M. Performance Characteristics (if/when applicable):

1. Analytical performance: The results met the sponsor's pre-determined acceptance criteria for each study.

   a. Precision/Reproducibility:

   Precision: The precision of the ADVIA Centaur aCCP assay was evaluated by testing eight serum samples containing various concentrations of anti-CCP antibody. Each sample was run in duplicate, twice a day, for 20 days with one reagent lot (total of 80 replicates per sample). The results are summarized in the table below.

Sample	Mean (U/mL)	Within-Run		Between-Run		Between-Day		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	2.37	0.09	3.9	0.00	0.0	0.04	1.8	0.10	4.3
2	3.75	0.12	3.1	0.06	1.5	0.04	1.0	0.13	3.6
3	6.73	0.22	3.3	0.00	0.0	0.10	1.5	0.25	3.6
4	42.01	1.09	2.6	0.27	0.6	0.59	1.4	1.27	3.0
5	55.34	1.73	3.1	0.00	0.0	1.02	1.8	2.01	3.6
6	111.53	3.3	3.0	1.50	1.3	0.35	0.3	3.64	3.3

   Reproducibility: To evaluate lot-to-lot reproducibility, six samples with anti-CCP antibody concentration at various levels across the measuring range (2.12, 3.48, 6.29, 40.73, 54.15, and 111.48 U/mL) were tested. Each sample was tested in replicates of five per run, two runs on two instruments using three difference reagent lots. Mean and %CV for each sample were calculated and %CV values for between-lot reproducibility were from 5.4% to 6.9% for all samples.

   Site-to site reproducibility was evaluated by testing a total of five samples at three sites with one reagent and calibrator lot and one quality control (QC) lot. The samples were run in replicates of five per day for five days at each site, to generate a total of 75 data points for each sample. Data were analyzed for within-run, between-run, between site and total precision. The results are summarized in the table below.

Sample	N	Mean (U/mL)	Within-Run		Between-Run		Between Sites		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	75	2.28	0.07	2.9	0.08	3.5	0.06	2.8	0.12	5.4
2	75	3.79	0.12	3.1	0.16	4.2	0.00	0.0	0.20	5.3
3	75	6.85	0.16	2.3	0.27	3.9	0.00	0.0	0.31	4.6
4	75	55.71	1.45	2.6	1.04	1.9	0.00	0.0	1.79	3.2
5	75	112.67	3.55	3.1	3.18	2.8	0.00	0.0	4.76	4.2

b. *Linearity/assay reportable range:*

Linearity: Linearity was evaluated according to the CLSI protocol EP6-A. Three serum samples containing high levels of anti-CCP IgG were mixed with a pool of negative serum. Two sample dilution series were tested in replicates of five using two lots of reagent. One sample dilution series with concentration ranging from 0.18 to 76.18 U/mL was tested using one lot of reagent. The results are summarized as follows:

Sample	Reagent Lot	Dilution Range (U/mL)	Slope (95% CI)	Y-intercept (95% CI)	R^2
1	Lot 1	0.10–190.63	0.99 (0.97–1.01)	-3.55 (-6.15– -0.94)	1.00
	Lot 2	0.10–200.69	0.96 (0.97–1.07)	-3.93 (0.77–12.27)	0.98
2	Lot 1	1.00–311.04	0.99 (0.97–1.00)	1.81 (-1.11–4.72)	1.00
	Lot 2	1.01–289.97	0.98 (0.95–1.00)	-4.18 (-8.54–0.17)	0.99
3	Lot 3	0.18–76.18	1.03 (1.02–1.04)	-0.004 (-0.03–0.02)	1.00

The ADVIA Centaur aCCP assay is linear from 0.40–200.00 U/mL.

Hook effect: To evaluate a potential hook effect, three high positive serum samples with anti-CCP antibody concentrations above the assay measuring range (6,007.38, 4,426.99, and 2,138.78 U/mL) were examined. No hook effect was observed up to 3,170.88 U/mL.

Dilution study: The study was done to evaluate the use of the ADVIA Centaur Multi-diluent 1 as an appropriate sample diluent for the assay as well as to characterize any recovery bias due to the matrix used upon dilution. Two anti-CCP serum samples with anti-CCP concentrations of approximately 140 and 180 U/mL were used. A manual dilution of 1/10 for each sample was prepared using ADVIA Centaur Multi-diluent 1. Samples were tested neat and manually diluted in the ADVIA Centaur

aCCP assay in replicates of four. The study supports a 1/10 dilution of sample for use in the ADVIA Centaur aCCP assay

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

Traceability: There is no recognized standard or reference material for anti-CCP antibodies. The ADVIA Centaur aCCP assay is standardized using internal reference standards.

Value assignment: The ADVIA Centaur in-house aCCP Master Standards and ADVIA Centaur aCCP Adjusters are expressed in arbitrary Axis-Shield units (U/mL) and prepared gravimetrically from an in house reference standard. The ADVIA Centaur aCCP Master Standards are manufactured using a pool of high titer anti-CCP positive plasmas with a known assigned anti-CCP IgG value in U/mL. The pool is tittered to fixed target concentrations in order to achieve the ten ADVIA Centaur aCCP Master Standard levels. The Master standards contain ten levels (0, 0.5. 7.5, 10, 15, 25, 50, 100, 150, 200 U/mL) of anti-CCP IgG target concentrations spanning the assay range of 0.0 to 200 U/ml. The standards are used to generate a lot-specific Master Curve by performing the testing on multiple instrument systems over several days to ensuring standard curve determines the relationship between the defined analyte levels and the RLUs. These concentrations and RLUs become the lot-specific Master Curve which is provided as barcodes on the Master Curve Card.

The ADVIA Centaur aCCP calibrators, quality controls, and MCMs are manufactured to target anti-CCP IgG levels by using a pool of high titer anti-CCP positive plasmas. The value assignment is performed by testing against the ADVIA Centaur Master Standards and the target CCP concentration is assigned to each of calibrators, quality controls and MCMs.

Stability:

The un-opened (shelf life), open-vial, and on-board stability studies were each performed using multiple lots of regent, calibrators and quality controls. The results support the following stability claims:

Kit Reagent Pack	Un-opened	12 months at 2–8°C
	Open-vial	60 days at 2–8°C
	On-board	60 days
Calibrators	Un-opened	12 months at 2–8°C
	Open-vial	90 days at 2–8°C
	On-board	8 hours
Calibration interval		35 days
Quality Controls	Un-opened	12 months at 2–8°C
	Open-vial	90 days at 2–8°C
	On-board	8 hours

The stability of MCMs was determined to have the same stability claim as calibrator materials:

MCMs	Un-opened	12 month at 2–8°C
	Open-vial	90 days at 2–8°C
	On-board	8 hours at 2–8°C

Sample stability: The sample stability was established in K083868. The specimens are stable up to 22 hours at room temperature, 14 days at 2–8°C and up to two freeze/thaw cycles.

d. Detection limit:

Limit of Blank (LoB) was determined by assaying four serum samples in five replicates per sample over three days with two lots of reagents, calibrators and kit controls. Sixty data points per lot were generated. LoB for each lot was calculated separately at the 95th percentile using the non-parametric method. The LoB for two lots was determined to be 0.22 U/mL and 0.24 U/mL. The claimed LoB value is 0.24 U/mL.

The Limit of Detection (LoD) was determined by assaying four samples with low level anti-CCP antibody concentration. Each sample was tested in replicates of five over three days on two reagent lots. LoD value was calculated as the LoB + 1.645 x SD of the replicates for the low level samples. The LoD of the aCCP assay for the two lots were determined to be 0.30 and 0.32 U/mL. The claimed LoD is 0.40 U/mL.

The Limit of Quantification (LoQ) was determined by assaying additional four samples with low concentration level of anti-CCP antibodies using two lots of reagent. The determined LoQ were equal to the LoD and not higher than 0.40 U/mL. The lower limit of measuring range is determined as 0.4 U/mL.

e. Analytical specificity:

Endogenous Interference: Three serum samples (negative, low positive, and high positive) were spiked separately with endogenous interferents. The control samples were prepared for each interfering substance by spiking with the same volume of diluents. Each sample was tested in five replicates and the percent difference was calculated by comparing to a corresponding control sample. The interferents tested were: Bilirubin (conjugated and unconjugated; 20 and 40 mg/dL), Hemoglobin (500 and 1000 mg/dL), Triglycerides (2450 mg/dL), Lipemia (500 and 1500 mg/dL), Rheumatoid Factor (200 IU/mL), IgG (3 and 6 g/dL), Total Protein (12 g/dL), and Biotin (30 and 500 ng/dL). No interference was detected in the samples up to the concentrations listed in the table below.

Potential Interfering Substances	Maximum Concentration	Range of % Difference
Bilirubin (conjugated)	40 mg/dL	-3.0– -1.2
Bilirubin (unconjugated)	40 mg/dL	-3.0–4.7
Hemoglobin	1000 mg/dL	-2.5–2.7
Triglycerides	2450 mg/dL	8.8–9.1
Lipemia (Interlipid)	1500 mg/dL	3.2–10.1
IgG	6 g/dL	-10.0–7.0
Biotin	500 ng/dL	-2.9–2.8
Total Protein	12 g/dL	-8.5–1.3
Rheumatoid Factor	200 IU/mL	-2.5–2.7

Analytical cross-reactivity: Cross-reactivity with other autoantibodies commonly found in autoimmune disease was tested in the presence and absence of anti-CCP IgG using the ADVIA Centaur aCCP assay. Autoantibodies evaluated in the study included Sm, RNP, SSB, Scl-70, Jo-1, Ribosomal P, M2, TPO, dsDNA, SSA and Chromatin. Five serum samples with anti-CCP concentration at 3.32, 5.35, 10.33, 39.80 and 103.58 U/mL were tested by spiking the serum samples containing each of the autoimmune analytes. Each sample was tested in five replicates and the percent difference was calculated by comparing to corresponding control (unspiked) sample. No interference was observed for the tested autoimmune analyses.

f. Assay cut-off:

The assay cut-off was determined as follows:

	Positive	Negative
ADVIA Centaur aCCP	≥ 5 U/mL	< 5 U/mL

2. Comparison studies:

a. Method comparison with predicate device:

Samples for method comparison analysis included 767 samples from the clinical validation study. All these samples were tested on both the ADVIA Centaur aCCP and the predicate ARCHITECT anti-CCP assay. From the total sample size of 767, results for 253 samples were within the measuring ranges of the assays. The analysis of the results comparing the ADVIA Centaur aCCP to the predicate is summarized below:

		ARCHITECT anti-CCP		
		Positive	**Negative**	**Total**
ADVIA Centaur aCCP	**Positive**	121	4	125
	Negative	4	124	128
	Total	125	128	253

Positive agreement: 96.80% (95% CI: 92.06–98.75%)
Negative agreement: 96.88% (95% CI: 92.24–98.78%)
Overall agreement: 96.84% (95% CI: 93.89–98.39%)

b. Matrix comparison:

A study was performed to compare anti-CCP IgG values in matched samples collected in five different types of collection tubes: plastic serum tube, plastic serum separator tube (SST), plastic K2-EDTA plasma tube, and plastic Lithium Heparin tube. These matched serum/plasma samples with concentration ranging from 0.41 to 180.82 U/mL were tested using one lot of reagent. The Passing-Bablok regression analysis was performed by comparing the results of samples with different matrix to the results of corresponding serum samples. The results were shown as follows.

	n	**Slope (95% CI)**	**Intercept (95% CI)**	**r**
Serum vs. Serum (SST)	50	1.01 (0.98–1.04)	0.05 (-0.15–0.47)	1.00
Serum vs. K2-EDTA plasma	51	0.98 (0.97–1.00)	0.03 (-0.05–0.49)	1.00
Serum vs. Li-Heparin plasma	49	1.00 (0.96–1.02)	0.11 (-0.33–1.05)	0.98

3. Clinical studies:

a. Clinical Sensitivity and Clinical Specificity:

The 767 serum samples used in the clinical validation for the ADVIA Centaur aCCP assay include 307 samples from patients diagnosed with RA and 460 samples from patients with other potentially cross-reacting diseases. RA subjects were classified according to the 2010 ACR (American College of Rheumatology) criteria. Clinical sensitivity and specificity to aid in diagnosis of RA are summarized in the following table:

		Clinical Diagnosis of RA		
		Positive	Negative	Total
ADVIA Centaur aCCP	Positive	209	13	222
	Negative	98	447	545
	Total	307	460	767

Clinical Sensitivity: 68.08% (95% CI: 62.54–73.26%)
Clinical Specificity: 97.17% (95% CI: 95.22–98.49%)

The table below shows the results for non-RA samples tested with ADVIA Centaur aCCP:

Non-RA Control Diseases	N	No (%) Positive
Ankylosing Spondylitis	26	0 (0.0%)
Osteoarthritis	74	5 (6.8%)
Polymyalgia Rheumatica	13	0 (0.0%)
Polymytosis	3	0 (0.0%)
Psoriasis Arthritis	27	2 (7.4%)
Reactive RA	24	0 (0.0%)
Systemic Lupus Erythematosus	92	4 (4.3%)
Scleroderma	8	0 (0.0%)
Sjögren's syndrome	22	1 (4.5%)
Hashimoto's	10	0 (0.0%)
Vasculitis	6	0 (0.0%)
Fibromyalgia	4	0 (0.0%)
Gout	2	0 (0.0%)
Vasculitis	6	0 (0.0%)
Myeloma/Oncology	23	0 (0.0%)
Crohn's Disease	13	0 (0.0%)
Ulcerative colitis (UC)	12	0 (0.0%)
Lyme Disease	7	0 (0.0%)
West Nile Virus	10	0 (0.0%)
Chikungunya	15	0 (0.0%)
Dengue fever	13	0 (0.0%)
Eostein-Barr Virus (EBV) Positive	15	0 (0.0%)
Hepatitis C	41	1 (2.4%)
TOTAL	**460**	**13 (2.8%)**

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

Not applicable

4. Clinical cut-off**:**

See assay cut-off.

5. Expected values/Reference range:

The expected value in the normal population is "negative". Anti-CCP antibody levels were analyzed in a cohort of 127 apparently healthy donors (47 females and 80 males, ages 21 to 77 years, with an average and median age of 41 years) using the ADVIA Centaur aCCP. The results are shown as follows:

	Total (n=127)	Male (n=80)	Female (n=47)
Median	0.06 U/mL	0.05 U/mL	0.07 U/mL
95% percentile	0.37 U/mL	0.35 U/mL	0.37 U/mL
99% percentile	1.27 U/mL	1.66 U/mL	0.49 U/mL

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.