

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

k063347

B. Purpose for Submission:

New application on an approved system

C. Measurand:

Anti-cyclic citrullinated peptide (CCP)

D. Type of Test:

Microparticle Enzyme Immunoassay (MEIA) Semi-quantitative

E. Applicant:

AXIS-SHIELD DIAGNOSTICS, LTD.

F. Proprietary and Established Names:

AxSYM® Anti-CCP Reagent kit

AxSYM® Anti-CCP Standard Calibrator kit

AxSYM® Anti-CCP Control kit

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
Antibodies, Anti-Cyclic Citrullinated Peptide (CCP) (NHX)	Class II	21 CFR § 866.5775, Rheumatoid factor immunological test system.	82 Immunology
Calibrator, Multi-Analyte Mixture (JIX)	Class II	21 CFR § 862.1150	75 Chemistry
Quality Control Material (Assayed and Unassayed) (JJY)	Class I	21 CFR § 862.1660	75 Chemistry

H. Intended Use:

1. Intended use(s):

AxSYM® Anti-CCP is a Microparticle Enzyme Immunoassay (MEIA) for the semi-quantitative determination of the IgG class of autoantibodies specific to cyclic citrullinated peptide (CCP) in human serum and plasma on the AxSYM 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 multicriterion diagnostic process, encompassing both clinical and laboratory-based assessments.

The AxSYM® Anti-CCP Standard Calibrators are for the standard calibration of the AxSYM System when used for the semi-quantitative determination the IgG

class of autoantibodies specific to cyclic citrullinated peptide (CCP) in human serum and plasma.

The AxSYM® Anti-CCP Controls are for the use in quality control to monitor the accuracy and precision of the AxSYM® Anti-CCP assay when used for the semi-quantitative determination of the IgG class of autoantibodies specific to cyclic citrullinated peptide (CCP) in human serum and plasma on the AxSYM System.

2. Indication(s) for use:
Same as above
3. Special conditions for use statement(s):
This device is for prescription use only.
4. Special instrument requirements:
For use with AxSYM System Software version 5.0 cleared under k974651

I. Device Description:

Each AxSYM® Anti-CCP Reagent kit contains 1 bottle of each component of Conjugate, Microparticles, Sample Diluent and Matrix Cell Blocker.

- Conjugate: 1 Bottle (12.2 mL) Mouse Anti-Human IgG:Alkaline Phosphatase Conjugate in TRIS buffer containing detergent with protein (Bovine) stabilizers. Minimum concentration 0.1 µg/mL. Preservative: Sodium Azide. (Reagent Bottle 1)
- Microparticles: 1 Bottle (4.8 mL) CCP-Coated Microparticles in phosphate buffer with protein (Bovine) stabilizers. Minimum concentration: 0.1% solids (w/v). Preservative: Sodium Azide. (Reagent Bottle 2)
- Sample Diluent: 1 Bottle (24.7 mL) Sample Diluent containing detergent with protein (Bovine) stabilizers. Preservative: Sodium Azide. (Reagent Bottle 3)
- Matrix Cell Blocker: 1 Bottle (50.2 mL) Matrix Cell Blocker containing detergent. Preservative: Sodium Azide. (Reagent Bottle 4)

Each Anti-CCP Standard Calibrator kit contains six bottles of AxSYM® Anti-CCP Standard Calibrators (4.3 mL each). Calibrator A is a phosphate buffer with protein (Bovine) stabilizers. Calibrators B-F contains anti-CCP positive human plasma in phosphate buffer with protein (Bovine) stabilizers to yield the concentrations (U/mL) shown in the following table:

Standard Calibrator	Anti-CCP Concentration (U/mL)
Cal A	0
Cal B	5
Cal C	25
Cal D	50
Cal E	100
Cal F	200

Each AxSYM® Anti-CCP Control kit contains two bottles (7.0 mL each) of controls with one positive and the other negative human plasma in phosphate buffer to yield the following concentrations (U/mL):

Bottle	Anti-CCP Concentration (U/mL)	Range (U/mL)
Positive Control	24.0	14.0 – 34.0
Negative Control	0.0	≤ 3.0

Calibrators and controls are sold separately.

J. Substantial Equivalence Information:

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

Similarities

Parameter	Submission Device Axis-Shield AxSYM Anti-CCP	Predicate Device Axis-Shield DIASTAT Anti-CCP
Intended Use	Aid in the diagnosis of Rheumatoid Arthritis (RA)	Same
Specimen Type	Human serum or plasma (sodium citrate, lithium heparin)	Same
Capture Antigen	Cyclic citrullinated peptide (CCP), second generation	Same
Storage Conditions	The AxSYM Anti-CCP Reagent Pack, Standard Calibrator Pack and Control Pack must be stored at 2°-8°C	Same
Suggested Cut-off	5.0 U/mL	Same

Differences

Parameter	Submission Device Axis-Shield AxSYM Anti-CCP	Predicate Device Axis-Shield DIASTAT Anti-CCP
Technology Format	Automated, sandwich format, Microparticle Enzyme Immunoassay (MEIA)	Manual, microtitre plate format, Enzyme-linked Immunosorbent Assay (ELISA)
Conjugate Antibody	Mouse anti-human IgG: alkaline phosphatase	Alkaline phosphatase-labelled murine monoclonal antibody to human IgG
Substrate	4-Methylumbelliferyl phosphate	Phenolphthalein monophosphate
Assay End-Point	Fluorescence	Color, read at 540-565nm
Quantitation	Results are determined from a standard calibration curve (0, 5, 25, 50, 100, 200 U/mL) generated and stored on the instrument	Results are determined from a standard calibration curve (0, 2, 8, 30, 100 U/mL) generated on each microtiter plate
Calibrator Range	0-200 U/mL Auto-dilute (1/10) up to 2000 U/mL	0-100 U/mL
Calibration	Semi-quantitative assay	Semi-quantitative/qualitative assay
Calibrators and Controls	Sold separately	Kit components
Expected Values in Asymptomatic Population	< 1.0 to 2.9 U/mL in a representative study	0.05 – 3.8 U/mL from a reference population

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

STANDARDS

Title and Reference Number

Other Standards

GUIDANCE

Document Title	Office	Division	Web Page

L. Test Principle:

The AxSYM® Anti-CCP is based on Microparticle Enzyme Immunoassay (MEIA) technology. The AxSYM® Anti-CCP reagents and sample are pipetted in the following sequence:

SAMPLING CENTER

- Sample and all AxSYM Anti-CCP reagents required for one test are pipetted by the Sampling Probe into various wells of a Reaction Vessel (RV).
- AxSYM Line Diluent and sample are pipetted into the Incubation Well of the RV.
- The AxSYM Anti-CCP Sample Diluent and diluted sample are pipetted into the Sample Well of the RV.
- The AxSYM Anti-CCP Matrix Cell Blocker is pipetted into the Buffer Well of the RV.
- The AxSYM Anti-CCP Mouse Anti-Human IgG:Alkaline Phosphatase Conjugate is pipetted into Reagent Well 3 of the RV.
- AxSYM Line Diluent and CCP-Coated Microparticles are pipetted into Reagent Well 2 of the RV.
- A reaction mixture is formed by combining diluted sample and diluted microparticles coated with CCP in Reagent Well 1 of the RV.
- When anti-CCP antibody is present in the sample, it binds to the CCP-Coated Microparticles, forming antigen-antibody complexes on the microparticles.
- The RV is immediately transferred into the Processing Center. Further pipetting is done in the Processing Center by the Processing Probe.

PROCESSING CENTER

- An aliquot of Matrix Cell Blocker is transferred to the Matrix Cell.
- An aliquot of the reaction mixture, containing microparticles and bound antigen-antibody complex, is transferred to the Matrix Cell. The microparticles bind irreversibly to the glass fiber matrix.
- The Matrix Cell is washed to remove materials not bound to the microparticles.
- The AxSYM Anti-CCP Mouse Anti-Human IgG:Alkaline Phosphatase Conjugate is dispensed onto the Matrix Cell and it binds with the antigen-antibody complexes.
- The Matrix Cell is washed to remove conjugate not bound to the microparticles.
- The substrate, 4-Methylumbelliferyl Phosphate, is added to the Matrix Cell. The alkaline phosphatase-labeled conjugate catalyzes the removal of a phosphate group from the substrate, yielding the fluorescent product, 4-Methylumbelliferone. This fluorescent product is measured by the MEIA optical assembly.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Four samples covering the measuring range of the device were assayed in replicates of two at two separate times of the day for twenty days (n=80). Testing was performed on two AxSYM Systems, using one reagent lot. The precision acceptance criteria for total %CV and within-run %CV was <15%. Data from the studies are summarized below:

N	Mean (mg/dL)	Within run %CV	Total %CV
80	5.2	6.9	9.3
80	4.3	10.7	11.1
80	13.8	7.1	8.4
80	15.0	5.7	7.7
80	24.4	7.0	8.6
80	25.6	6.6	8.3
80	139.8	6.8	10.0
80	149.7	7.1	8.5

b. *Linearity/assay reportable range:*

Linearity testing was demonstrated by testing five high patient sample pools, with anti-CCP concentrations between 100 and 200 U/mL. These samples were serially diluted with AxSYM Anti-CCP Negative control at the following dilutions: 1:2, 1:4, 1:6, 1:8, and 1:10. Each dilution was assayed in triplicate and a median is calculated. Data was analyzed using linear regression analysis (x-axis: expected concentration and y-axis: actual concentration). The data showed the following regression equation:

Sample Pool 1	$y = 1.0842x + 2.3294, r^2 = 0.991$
Sample Pool 2	$y = 1.0025x + 4.6563, r^2 = 0.9873$
Sample Pool 3	$y = 0.9507x + 6.6989, r^2 = 0.9869$
Sample Pool 4	$y = 1.0288x + 3.1923, r^2 = 0.9813$
Sample Pool 5	$y = 1.000x + 1.9658, r^2 = 0.9903$

The AxSYM Anti-CCP assay showed linearity from 0 to 200 U/mL.

In addition, an automated dilution protocol was designed to assist in quantitation of test results using the AxSYM system. The AxSYM system performs a 1:10 dilution of the specimen prior to analysis. The system automatically calculates the concentration of the diluted specimen and reports the result. Five high sample pools were used in this study. Each sample was diluted manually and auto-dilute using Anti-CCP Negative Control. The assay was designed to have a mean auto-dilute recovery of $100 \pm 20\%$. The % recoveries for the five sample pools were 107.3, 90.0, 86.3, 91.3, 100.2, and 95.0. The results support the use of both automated and manual 1:10 dilution for the AxSYM Anti-CCP assay.

High dose hook effect

The possibility of high dose hook effect occurring when using the device was evaluated with two serum samples with concentrations above the assay range (1200U/mL and 2400 U/mL). The samples were analyzed on two AxSYM instruments in triplicate with two reagent lots. No hook effect was observed at concentrations 12 times that of Calibrator F (200 U/mL).

- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
An international reference material for anti-CCP antibodies is not available. The calibrators and controls are assigned relative arbitrary Axis-Shield units (U/mL).

The AxSYM Anti-CCP Reference Calibrators were manufactured using a pool of high titer anti-CCP positive plasmas that has been assigned a value in U/mL. The AxSYM Anti-CCP Standard Calibrators were prepared from an anti-CCP stock (positive plasma) diluted in Calibrator diluent. This is tested by rate matching to the Reference Calibrator and should be within the target range of $\pm 2.5\%$. If acceptable, this becomes Calibrator F and is then further diluted to produce calibrators B-E.

Storage conditions

The AxSYM Anti-CCP Reagent Pack must be stored at 2-8°C. When stored and handled as directed, reagents are stable until the expiration date.

- d. *Detection limit:*

The AxSYM assay is designed to have a mean analytical sensitivity/limit of blank (LoB) of ≤ 1.0 U/mL. The LoB was calculated as the concentration at the 95th percentile above the Standard Calibrator A (0.0 U/mL) and was found to be 0.067 U/mL. The design specification (≤ 1.0 U/mL) was met.

- e. *Analytical specificity:*

Interference: Interference testing was performed using guidance supplied by CLSI EP7-A, "Interference testing in Clinical Chemistry". No significant interference was observed in:

- Hemoglobin up to 500 mg/dL
- Bilirubin up to 20 mg/dL
- Triglycerides up to 1500 mg/dL
- Total Protein up to 12 g/dL
- Rheumatoid Factor up to 200 IU/mL
- RBC up to 0.4%

Crossreactivity

To assess the potential cross-reactivity of the CCP antigen used in the AxSYM Anti-CCP assay with other autoantibodies, the assay was evaluated with 16 samples, all with high levels of various autoantibodies and negative for CCP. The following autoantibodies (2 samples each) were tested: SSA, SSB, RNP, dsDNA, Jo-1, and Ribosomal-P. The study showed no significant cross-reactivity of the CCP antigen with any of these autoantibodies.

- f. *Assay cut-off:*

See Expected Value.

2. Comparison studies:

a. *Method comparison with predicate device:*

Nine hundred thirty one (931) specimens were tested at Axis-Shield using the AxSYM® Anti-CCP assay and its predicate device, DIASTAT™ Anti-CCP assay. Specimens were received from five sites covering a range of relevant disease states. Sample distribution and percent concordance between the two devices for each disease group are shown below:

Disease Group	Total N	%American	%European	%Concordance
Asymptomatic Healthy	100	100	0	100
EBV	25	100	0	100
Infectious disease	25	100	0	100
SLE	76	14	86	99
Sjögren's Syndrome	8	50	50	100
Scleroderma	9	0	100	100
Polymyositis/Dermatomyositis	6	33	67	100
Osteoarthritis	22	0	100	100
Psoriatic Arthritis	31	0	100	100
Inflammatory Polyarthritis	80	0	100	100
Ankylosing Spondylitis	20	25	75	100
Rheumatoid arthritis	529	18	82	95
Total	931	37	63	96

The following table summarized the % concordance between the two assays:

RA (n = 529)

AxSYM	DIASTAT	
	+	-
+	384	0
-	23	122

Total Agreement (95% CI) = 95.7% (93.5-97.2%)

Positive Agreement (95% CI) = 94.3% (91.6-96.4%)

Negative Agreement (95% CI) = 100% (97.6-100%)

Non-RA (n = 402)

AxSYM	DIASTAT	
	+	-
+	21	1
-	1	379

Total Agreement (95% CI) = 99.5% (98.2-99.9%)

Positive Agreement (95% CI) = 95.5% (77.2-99.9%)

Negative Agreement (95% CI) = 99.7% (98.5-100%)

All Samples (n = 931)

Total Agreement (95% CI) = 97.3% (96.1-98.3%)

Positive Agreement (95% CI) = 94.4% (91.8-96.4%)

Negative Agreement (95% CI) = 99.8% (98.9-100%)

b. Matrix comparison:

Matrix comparison studies using matched serum (clot), serum separator, EDTA, lithium and sodium citrate plasma samples covering the dynamic range were performed on the AxSYM instrument using AxSYM® Anti-CCP assay. The results of the linear regression analyses are seen on the table below.

	N	Slope (95% CI)	Intercept (95% CI)	Correlation Coefficient
Serum (clot vs. Serum separator)	10	1.0618 (0.9943-1.1294)	-1.2470 (-4.8136-2.3197)	1.00
Serum vs. Lithium Heparin	10	0.9531 (0.9246-9816)	0.3433 (-11598-1.8465)	1.00
Serum vs. Sodium Citrate	10	0.9880 (0.9617-1.0142)	0.3122 (-1.0726-1.6970)	1.00
Serum vs. EDTA	10	0.9452 (0.9211-0.9693)	0.4536 (-0.8189-1.7261)	1.00

3. Clinical studies:

a. Clinical Sensitivity and Specificity

Using the same samples from the method comparison study, % sensitivity and specificity were calculated. The following results were obtained:

Disease Group	N	Anti-CCP positive	%Sensitivity	% Specificity
All RA samples	529	382	72.2	
All Non-RA samples	402	22		94.5

The lower sensitivity of 72.2% was accepted for the following reasons:

1. Anti-CCP assays from different manufacturers showed variable ranges from 69% to 87.8%.
2. Literature showed sensitivity ranging from 65% to 85% in established RA depending on cohort tested.

References:

Autoantibodies to citrullinated antigens in (early) rheumatoid arthritis. W.J. van Venrooij, A.J.W. Zendman, GJN Pruijn. *Autoantibody Reviews*: 6 (2006); 37-41.

Anti-cyclic citrullinated peptide antibodies: diagnostic, predictive and monitoring value in RA. PH Schur. *Int. J. Advances in Rheumatology*: 3 (2005); 77-83.

Diagnostic and predictive value of anti-CCP antibodies in rheumatoid arthritis: a systematic literature review. J Avouac, L Gossec, M Dougados. *Ann. Rheum. Dis.* (2006). Online: doi:10.1136/ard.2006.051391.

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

None

4. **Clinical cut-off:**

Not applicable

5. **Expected values/Reference range:**

In a representative study, 100 serum specimens from asymptomatic healthy donors with an age range of 19-73 years, comprised of males (n=51) and females (n=49), were tested in the AxSYM Anti-CCP assay. No differences attributable to gender or age were observed. Specimen values range from <1.0 to 2.9 U/mL. On the basis of this reference population data, the suggested cut-off is 5.0 U/mL. Values ≥ 5.0 U/mL is considered positive and ≤ 5.0 U/mL is considered negative.

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.