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

A. 510(k) Number:

k121576

B. Purpose for Submission:

New assay

C. Measurand:

Anti-cyclic citrullinated peptide (CCP) IgG antibodies

D. Type of Test:

Semi-quantitative chemiluminescent immunometric assay

E. Applicant:

Siemens Healthcare Diagnostics

F. Proprietary and Established Names:

IMMULITE 2000 Anti-CCP IgG Assay

IMMULITE 2000 Anti-CCP IgG Calibration Verification Material

G. Regulatory Information:

1. Regulation section:

   21 CFR §866.5775, Rheumatoid factor immunological test system

   21 CFR §862.1150, Calibrator

2. Classification:

   Class 2

3. Product code:

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

   JIT, Calibrator, Secondary

4. Panel:

   Immunology (82)

   Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   The IMMULITE 2000 Anti-CCP IgG Assay is an in vitro diagnostic immunoassay for the semi-quantitative determination of the IgG class of autoantibodies specific to cyclic citrullinated peptide (CCP) in human serum or plasma on the IMMULITE 2000 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 IMMULITE 2000 Anti-CCP IgG Calibration Verification Material (CVM) is for in vitro diagnostic use, as a control for calibration verification of the IMMULITE 2000 Anti-CCP IgG Assay on the IMMULITE 2000 system.

2. Indication(s) for use:

   See Intended Use above

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   Only for use on the IMMULITE 2000 Analyzer (k970227)

I. Device Description:

The IMMULITE 2000 Anti-CCP IgG assay consists of the following components:

- Anti-CCP IgG bead pack coated with cyclic citrullinated peptide (CCP) antigen
- Anti-CCP IgG reagent wedge containing alkaline phosphatase from bovine calf intestine conjugated to a monoclonal murine anti-human IgG antibody
- Anti-CCP IgG adjustors, low and high, containing lyophilized human serum with IgG reactive to CCP
- Anti-CCP IgG controls, negative and positive, containing human serum
- Autoantibody sample diluent containing protein/buffer matrix

The following reagents are required for the test and supplied separately; chemiluminescent substrate, probe wash, probe cleaning kit and disposable reaction tubes.

The IMMULITE 2000 Calibration Verification Materials are sold separately. This kit consists of four vials, containing low, intermediate and high levels of lyophilized human serum with IgG reactive to cyclic citrullinated peptide (CCP), and an anti-CCP-free sample.

J. Substantial Equivalence Information:

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

   DIASTAT™ Anti-Cyclic Citrullinated Peptide (anti-CCP) ELISA, k023285

   Elecsys anti-CCP CalCheck, k091601

2. Comparison with predicate:

ASSAY SIMILARITIES		
Item	**Device**	**Predicate**
Intended Use	For the semi-quantitative determination of the IgG class of autoantibodies specific to cyclic citrullinated peptide (CCP) in human serum or plasma on the IMMULITE®2000 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.	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. The test is intended to aid in the diagnosis of Rheumatoid Arthritis (RA) and is not definitive in isolation. Autoantibody levels represent one parameter in a multi-criterion diagnostic process, encompassing both clinical and laboratory-based assessments.
Antigen	Synthetic cyclic citrullinated peptide, second generation	Same
Detecting Antibody	Murine monoclonal antibody to human IgG	Same
Controls	Negative and positive kit controls	Same
Sample Matrix	Serum or plasma (EDTA, lithium heparin)	Same

ASSAY DIFFERENCES		
Item	**Device**	**Predicate**
Assay format	Solid-phase, chemiluminescent immunometric assay	Enzyme-linked immunosorbent assay (ELISA)
Instrument Platform	IMMULITE 2000, automated	Spectrophotometer, manual
Sample Matrices	Serum or plasma (EDTA, lithium heparin)	Serum or plasma (EDTA, lithium heparin, sodium citrate)
LoD	1.50 U/mL	0.05 U/mL
Reportable Range	1.50 - 200 U/mL	0.05 – 100 U/mL
Assay Cut-off	≥4.00 U/mL = Reactive	>5 U/mL = Positive

Calibrator Verification Materials SIMILARITIES		
Item	**Device**	**Predicate**
Intended Use	The IMMULITE 2000 Anti-CCP IgG Calibration Verification Material (CVM) is for in vitro diagnostic use, as a control for calibration verification of the IMMULITE 2000 Anti-CCP IgG assay on the IMMULITE 2000 system.	For use in the verification of the calibration established by the Elecsys Anti-CCP reagent on the indicated Elecsys and cobas e immunoassay analyzers.
Format	Lyophilized human serum with anti-CCP antibodies	Same

Calibrator Verification Materials DIFFERENCES		
Item	**Device**	**Predicate**
Instrumentation	Siemens IMMULITE 2000	Roche Elecsys and cobas e
Levels	Four	Three

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

CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition.

CLSI EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach

CSLI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline

L. Test Principle:

The IMMULITE® 2000 Anti-CCP IgG assay is a solid phase, chemiluminescent immunometric assay. The solid phase, a polystyrene bead, is coated with a cyclic citrullinated peptide (CCP) antigen. The liquid phase consists of protein-based buffer and alkaline phosphatase (bovine calf intestine) conjugated to a monoclonal murine anti-human IgG antibody in buffer. In the first cycle, the patient sample and the buffer are incubated together with the coated bead for 30 minutes. During this time, human IgG in the sample binds to CCP on the bead. Unbound sample is then removed by centrifugal washes. In the second cycle, the enzyme conjugated monoclonal murine antihuman IgG is added to the original reaction tube for additional 30 minutes incubation. The enzyme conjugated monoclonal murine anti-human IgG antibody binds to immobilized anti-CCP IgG on the bead. The unbound enzyme conjugate is removed by centrifugal washes. Finally, chemiluminescent substrate is added to the reaction tube containing the bead and the signal is generated in proportion to the bound enzyme. The signal is converted to a value from an internal standard curve and is reported in U/mL.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision was evaluated according to CLSI EP05-A2. Eleven samples were tested: the positive control supplied in the kit, five serum samples, and five lithium heparin plasma samples. Precision panels were prepared by pooling the samples and spiking to the desired concentration with a stock Anti-CCP reactive sample with a concentration of 1382.29 U/mL.

Each sample was tested in duplicate in two runs per day over 20 days. Two instruments were used, and two lots of the device were tested for a total of 320 replicates per sample. Precision was calculated using Nested Analysis of Variance:

Sample	Mean (U/mL)	Within Run		Between Run		Between Day	
		SD	%CV	SD	%CV	SD	%CV
Hi Control	47.2	1.84	3.9	0.90	1.9	0.00	0.0
Serum 2	2.1	0.20	9.4	0.15	6.8	0.15	6.9
Serum 3	4.3	0.28	6.5	0.17	3.9	0.13	3.1
Serum 4	8.5	0.34	4.0	0.24	2.9	0.18	2.1
Serum 5	37.6	1.54	4.1	1.12	3.0	0.96	2.6
Serum 6	144.0	5.45	3.8	4.17	2.9	1.25	0.9
Plasma 2	2.4	0.20	8.2	0.17	7.1	0.17	6.8
Plasma 3	4.5	0.29	6.4	0.20	4.5	0.17	3.6
Plasma 4	8.4	0.35	4.1	0.31	3.7	0.05	0.6
Plasma 5	38.5	1.57	4.0	1.04	2.7	0.54	1.4
Plasma 6	141.5	5.52	3.9	3.87	2.7	2.23	1.6

Sample	Mean (U/mL)	Between Lot		Total	
		SD	%CV	SD	%CV
Hi Control	47.2	0.18	0.4	2.05	4.3
Serum 2	2.1	0.20	9.3	0.29	13.6
Serum 3	4.3	0.08	1.8	0.35	8.2
Serum 4	8.5	0.00	0.0	0.46	5.4
Serum 5	37.6	0.66	1.8	2.13	5.7
Serum 6	144.0	2.29	1.6	6.98	4.8
Plasma 2	2.4	0.16	6.7	0.31	12.8
Plasma 3	4.5	0.18	3.8	0.39	8.6
Plasma 4	8.4	0.00	0.0	0.47	5.6
Plasma 5	38.5	0.11	0.3	1.96	5.0
Plasma 6	141.5	1.06	0.7	7.11	5.0

b. Linearity/assay reportable range:

The linearity study was performed according to CLSI EP6-A using a pool of three high anti-CCP positive samples (in equal amounts) mixed with a pool of three non-reactive samples. The dilution series was by combining the samples in different

ratios to produce dilutions covering the claimed assay range. Each sample was read in triplicate. Sample concentration tested ranged from 0.74-211.64 U/mL. Samples outside the assay range (1.5-200 U/mL) were not included in the calculation of linearity. Regression analysis yielded $y = 1.03x – 0.40$, $R^2 = 0.999$ in 11 samples ranging from 2.4 U/mL to 182.9 U/mL.

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

Traceability:

There is no standard reference material for anti-CCP. The IMMULITE 2000 anti-CCP IgG assay, Calibrator Verification Material (CVM) Adjustors, and control materials are traceable to an internal standard material and manufactured using qualified materials and measurement procedures.

The instrument-stored assay calibration curve is based on a series of calibrators covering the claimed assay range and is specific for each lot of the assay.

Assay and component stability:

Real time stability testing to support the following claims for unopened devices and opened components:

Acceptance criteria for all components of the assay was the dose value of the component should be within 20% for sample values >6 U/mL and 30% for sample values ≤6 U/mL of the Day 0 values except if below the LoD of 1.46 U/mL where recovery cannot be properly determined. At each time point, all Control results must be within Quality Control established range, to validate the run. Percent recovery was calculated by comparing dose values of Adjustors from the latest time point to Day 0 dose value.

Closed vial testing (kit stored at 2 – 8 °C) was conducted at 0, 1, 2, 7, 30, 45, 60, 90, 120, 150, 180, 210, 224, 238, 360, and 390 days. Four replicates for the Low and High Adjustors and two replicates for each of the Kit Controls (LPIC1-2) and in-house reference panel (CCP1-5) were run on each day of the study. Dose values of the controls and samples were calculated from the Day 0 adjustment and from the adjustment performed on each subsequent day of testing.

Open vial/kit testing of components stored at 2 – 8°C was conducted at Days 0, 2, 7, 14, 21, 28, 30, and 35. Open vial testing of aliquotted components stored at -20°C was conducted on Days 0, 30, 60, 90, 180, 270, 300, 360, and 390. Four replicates of each Adjustor and three replicates of each Control were run on each day of the study.

Component	**Storage**	**Stability Claim**
Kit, unopened	2 – 8°C	12 months
Bead Pack, open	2 – 8°C	90 days
Reagent wedge, open	2 – 8°C	90 days
Sample diluent, open	2 – 8°C	30 days
Sample diluent, open frozen aliquotted	-20°C	6 months

Component	Storage	Stability Claim
Adjustors open (LPIL, LPIH)	2 – 8°C	30 days
Adjustors (LPIL, LPIH) frozen aliquotted	-20°C	6 months
Controls open (LPIC1, LPIC2)	2 – 8 °C	30 days
Controls open (LPIC1, LPIC2) frozen aliquotted	-20°C	90 days
CVM, unopened	2 – 8°C	12 mo
CVM, opened	2 – 8°C	30 days

Sample stability:

The sample stability study supports the claim of stability for two days at 20 - 25°C and 7 days at 2 – 8ºC for serum and plasma. Serum was shown to be stable for 6 months at -20ºC while plasma was stable for 4 months at -20ºC.

Adjustment interval (calibration curve) stability:

Real time testing supported the recommended adjustment interval of two weeks.

d. Detection limit:

Limit of Blank (LoB): An Anti-CCP nonreactive donor sample was used as a blank and was analyzed on three IMMULITE® 2000 instruments over 5 days. The sample was tested twice daily using two lots of reagent with two replicate per run. The LoB result was determined by applying a nonparametric principle based on ranked ordered value using the following equation where p= (100-α) = 95 and NB = number of blank measurements = 732:

$$LoB = [N_B(p/100)+0.5]$$

The LoB was the average of the ranked results at positions 695 and 696. The LoB was determined to be 0.26 U/mL.

Limit of Detection (LoD): Five Anti-CCP serum samples (mean concentrations ranging from 0.26 to 2.5 U/mL) were assayed in replicates of 6 using 2 reagent kit lots run for 5 days with 2 runs per day. Two instruments and 2 operators generated 960 observations. The LoD was determined by applying the following equation:

$$LoD = LoB + \text{median} - 5^{th} \text{ percentile}$$

The LoD was determined to be 1.46 U/mL.

The functional sensitivity of the assay is 2.34 U/mL, where functional sensitivity is defined as the concentration at which the percent (%) CV is ≤ 20%.

e. Analytical specificity:

Endogenous interferents:

Six serum pooled with concentrations across assay range were prepared then spiked separately with endogenous interferents. A control sample was prepared for each

interfering substance by spiking the appropriate diluent at the same volume as the interfering substance; each sample was tested in triplicate. The interferents tested were: Human Serum Albumin (12g/dL); Triglycerides (1.5 g/dl); Hemoglobin (500 mg/dL); Bilirubin: Conjugated and Unconjugated (0.2 mg/mL each); and Rheumatoid Factor (200 IU/mL).

While no interference (≤ 10% different than control sample) was found when the means of control samples were compared to the means of the spiked samples, occasional individual samples spiked with bilirubin or RF showed > 10% interference. The sponsor has included limitations to this effect in the package insert: "Bilirubin concentrations ≥ 0.23 mg/mL may have an unpredictable effect on patient sample results" and "Rheumatoid Factor (RF) may have unpredictable effects on patient samples with low concentrations of anti-CCP antibodies."

Samples spiked with a five-fold excess of EDTA or heparin anticoagulants were compared to normal serum to investigate the effect of a short blood draw on anti-CCP assay results. Eight samples were tested; all but one showed recovery within ± 10% of the serum control value. The sample that did not recover within ± 10% of the serum control value was a sample below the assay cut-off; clinical results were not changed by the discrepancy.

f. Assay cut-off:

The cutoff of the IMMULITE® 2000 Anti-CCP IgG assay was determined with positive and negative patient samples by a ROC analysis, with a balanced consideration of sensitivity and specificity. A total of 388 samples were used, consisting of 212 apparently healthy samples, 106 RA positive samples, and 70 anti-CCP positive samples (as determined by a predicate method). A result ≥ 4 U/mL indicates that anti-CCP IgG antibodies were detected in the sample. A result of < 4 U/mL indicates that anti-CCP IgG antibodies were not detected in the sample.

2. Comparison studies:

a. Method comparison with predicate device:

Serum specimens were obtained from 1966 subjects including RA patients and patients with diseases other than RA were tested on the Siemens anti-CCP assay and the predicate. Each sample was tested at one of four sites: Siemens or one of the clinical trial sites (see below). Samples outside the measuring range of either assay were excluded from the analysis, leaving 255 samples (245 R, 5 SLE, 2 psoriatic arthritis, and 3 non-RA diseases) for comparison:

		Predicate		
		Positive	Negative	Total
IMMULITE 2000 Anti-CCP IgG	Reactive	199	14	213
	Non-reactive	30*	12	42
	Total	229	26**	255

*29 were RA patients and 21 of these patients were on anti-RA medication. The highest discrepant sample was 24 U/mL by the predicate.

**23 samples were RA patients; 14 were purchased from a commercial vendor. The highest

discrepant sample was 29.6 U/mL by IMMULITE anti-CCP

Positive agreement	199/213 = 86.9%	95% CI: 81.0% – 91.5%
Negative agreement	12/26 = 46.2%	95% CI: 28.8% – 64.5 %

The low percent negative agreement is likely due to the limited number of negative samples in this study. To further support substantial equivalence, samples from the clinical study were tested also with the predicate for comparison of clinical sensitivity and specificity.

b. Matrix comparison:

Thirty-nine sets of matched serum and plasma samples were collected in the following anti-coagulant tubes: serum clot tube, lithium heparin plasma tube, serum separator tube (SST), and EDTA plasma tube. Twenty-one of the 39 sample sets were spiked to achieve Anti-CCP levels across the assay measuring range. Serum sample concentrations ranged from <1.5 to 184.4 U/mL, including several samples around the cut-off. Data was analyzed using Deming regression plots:

Serum vs.	Slope	Intercept	Correlation Coefficient
SST Serum	1.02	-0.52	1.00
EDTA Plasma	1.01	-0.31	1.00
Lithium heparin	1.02	-0.46	1.00

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

A total of 1512 patient serum samples with RA (1048 samples) or potentially cross-reacting diseases (464 samples) were collected for the study. The samples were collected from two clinical sites and two vendors (US and Canada) and were tested at four different test sites (three external, one internal). The samples were enrolled on the basis of diagnosis alone; their predicate anti-CCP status and RF status were not known by the sponsor. Roughly 65% of the samples were from women, and approximately half of the samples were from patients that were between 50 and 70 years of age.

At the clinical trial sites, samples were collected from patients enrolled by board certified rheumatologists following the 1987 ACR guideline. Samples from vendors were provided as diagnosed with RA. Of the 791 RA samples collected prospectively from the clinical sites, 204 had been diagnosed less than two years before sample collection. Of note, most of the prospectively enrolled patients were taking anti-rheumatic medication(s).

		RA Status		
		Positive	Negative	Total
IMMULITE 2000	Reactive	667	14	681
	Non-reactive	381	450	831
Anti-CCP IgG	Total	1048	464	1512

Sensitivity: 667/1048 = 63.6% 95% CI: 60.0% - 66.6%

Specificity: 450/464 = 97.0% 95% CI: 95.0% - 98.3%

The findings in this clinical study are in line with sensitivity and specificity reported in the clinical peer-reviewed literature[1,2,3].

The non-RA negative samples (i.e. potentially cross-reacting) consisted of the following:

Non-RA Disease State	**n**	**Clinical Specificity**
Ankylosing Spondylitis	31	100%
Autoimmune Thyroiditis / Hashimoto's Disease	16	94%
Crohn's Disease	9	100%
Dermatomyositis	6	83%
Epstein-Barr Virus	5	100%
Gout	10	100%
Lyme Disease	1	100%
Microscopic polyangiitis	1	0%
Osteoarthritis	93	96%
Other*	78	100%
Polyarthralgia	5	80%
Polymyalgia Rheumatica	26	96%
Polymyositis	3	100%
Psoriatic Arthritis	49	96%
Reactive Arthritis	3	100%
Scleroderma	15	100%
Sjögren's Syndrome	18	100%

[1] Kunihiro N et al., Meta-analysis: Diagnostic Accuracy of Anti–Cyclic Citrullinated Peptide Antibody and Rheumatoid Factor for Rheumatoid Arthritis. Annals of Internal Medicine. 146:797-808. 2007.

[2] Penny F. Whiting PF et al., Systematic Review: Accuracy of Anti–Citrullinated Peptide Antibodies for Diagnosing Rheumatoid Arthritis. Annals of Internal Medicine. 152:456-464. 2010.

[3] Zintzaras E et al., The reporting quality of studies investigating the diagnostic accuracy of anti-CCP antibody in rheumatoid arthritis and its impact on diagnostic estimates. BMC Musculoskeletal Disorders 13:113. 2012.

Non-RA Disease State	n	Clinical Specificity
Still's disease	2	100%
Systemic Lupus Erythematosus	79	96%
Ulcerative Colitis	8	100%
Wegener's granulomatosis	6	100%
Grand Total	464	97%

* Other includes: osteoporosis (8), various vasculitides (13), undifferentiated connective tissue disease (4), fibromyalgia (4), pseudogout (4), miscellaneous or undifferentiated arthritides (14), and miscellaneous others (31).

The samples tested at the external clinical sites (n= 1255) were also tested with the predicate for comparison of clinical sensitivity and specificity:

	Predicate	IMMULITE anti-CCP
Sensitivity (95% CI)	63.8% (60.4 – 67.2%)	58.9% (55.4 – 62.4%)
Specificity (95% CI)	96.1% (93.9 – 97.7%)	97.0% (95.0 – 98.3%)

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

Not applicable.

4. Clinical cut-off**:**

Not applicable.

5. Expected values/Reference range:

A total of 200 serum samples from presumed healthy male and female donors were analyzed using the IMMULITE® 2000 Anti-CCP IgG assay. Three samples (1.5%, two female and one male) were positive. The median of the test samples was <1.5 U/mL while the nonparametric 99th percentile range was 4.06 U/mL.

The device labeling cautions that 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.