510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k052264

B. Purpose for Submission:
This is a new submission.

C. Measurand:
Anti-Cyclic Citrullinated Peptide (CCP) Antibodies

D. Type of Test:
ELISA (Semi-quantitative)

E. Applicant:
INOVA Diagnostics, Inc.

F. Proprietary and Established Names:
QUANTA Lite™ CCP3 IgG ELISA

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:
   (82) Immunology

H. Intended Use:

1. Intended use(s):
   The QUANTA Lite™ CCP3 IgG ELISA is a semiquantitative enzyme-linked immunosorbent assay for the detection of IgG anti-CCP3 (Cyclic Citrullinated Peptide 3) antibodies in patient sera or EDTA plasma. The presence of these antibodies, when considered in conjunction with other laboratory and clinical findings, is an aid in the diagnosis of rheumatoid arthritis.

2. Indication(s) for use:
   Same as above

3. Special conditions for use statement(s):
   The device is for prescription use only.

4. Special instrument requirements:
   Microwell plate reader capable of measuring OD at 450nm (and 620nm for dual wavelength readings)

I. Device Description:
The device consists of the following: a foil package containing 12 (1 x 8) polystyrene microwell ELISA plate coated with purified synthetic CCP3 antigen with holder, a negative control, a low and high positive controls, 5 levels of calibrators, HRP sample diluent, HRP wash concentrate, HRP IgG (goat), anti-human conjugate, TMB chromogen and HRP stop solution.

The end user has the option to run a standard curve instead of a single point calibrator. The low calibrator is given a value of 25 ELISA units (EU). For the single point method, the value in EU for the patient is obtained by dividing the OD of the patient by the OD of the calibrator. To use the standard curve, five calibrators are included. The value of the patient sample is extrapolated form the standard curve.

J. Substantial Equivalence Information:
1. Predicate device name(s):
   QUANTA Lite™ CCP IgG ELISA
2. Predicate 510(k) number(s):
   k020414
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	QUANTA Lite™ CCP3 IgG ELISA	**QUANTA Lite™ CCP IgG ELISA**
Intended Use	Detection of specific autoantibodies	Same
Assay type	ELISA	Same
Type of test	Semi-quantitative	Same
Detection method	Colorimetric	Same
Conjugate	Horse radish peroxidase	Same
Solid Phase Capture	Microwells	Same

Differences		
Item	Device	Predicate
Antigen	3rd generation anti-CCP	2nd generation anti-CCP
Sample type	Serum or EDTA plasma	Serum only
Microwells	Color-coded breakaway wells	Plain microwells

K. Standard/Guidance Document Referenced (if applicable):
None referenced.

L. Test Principle:
The antigen used in the QUANTA Lite™ CCP3 IgG ELISA test is a synthetic cyclic citrullinated peptide. This antigen is bound to the surface of a microwell plate. Pre-diluted controls and diluted patient sample are added to separate wells, allowing any CCP3 IgG antibodies present to bind to the immobilized antigen. Unbound sample is washed away and an enzyme labeled anti-human IgG conjugate is added to each well. A second incubation allows the enzyme labeled anti-human IgG to bind to any patient antibodies that have become attached to the microwells. After washing away any unbound enzyme labeled anti-human IgG, the remaining enzyme activity is measured by adding a chromogenic substrate and measuring the intensity of the color that develops. The assay can be evaluated spectrophotometrically by measuring and comparing the color intensity that develops in the patient wells with the color in the

control wells.

M. Performance Characteristics (if/when applicable):
  1. <u>Analytical performance:</u>
    a. *Precision/Reproducibility:*

The between-assay variation was measured by running duplicates of negative, low positive, and strong positive samples in 6 separate assays on 6 different days. The within-assay variation was measured by running 5 samples 9 times each on the same ELISA plate. Results are shown below.

Between-run Variation

	Neg		Low 1		Low 2		High	
	1 pt	5 pt	1 pt	5 pt	1 pt	5 pt	1 pt	5 pt
Mean	5 U	0 U	28 U	31 U	35 U	42 U	110 U	226 U
SD	0.2	0.0	1.6	2.4	1.1	1.8	4.4	9.2
CV%	4%	0	6%	8%	3%	4%	4%	4%

Within-Run Variation

	Neg		Low 1		Low 2		High	
	1 pt	5 pt	1 pt	5 pt	1 pt	5 pt	1 pt	5 pt
Mean	5 U	0 U	26 U	28 U	32 U	37 U	114 U	226 U
SD	0.1	0.0	0.6	0.9	1.6	2.6	1.7	6.4
CV%	3%	0	3%	3%	5%	7%	1%	3%

Lot to lot reproducibility
Twenty (20) positive samples and 24 negative samples were tested for comparison between two lots. All negatives remained negative for both lots using both the 1 point and 5 point method of calculation. All samples that are high positive remained the same on both lots with both methods.

    b. *Linearity/assay reportable range:*
Serial dilution of positive sera and EDTA plasma, and citrated plasma were assayed to determine assay linearity. The following results are obtained:

5-Point Calculation, citrated plasma (203 U) with 5 serial dilutions:
$y = 1.0189x - 15.44$ $r^2 = 0.974$
1-Point calculation, citrated plasma (203 U) with 5 serial dilutions:
$y = 1.061x - 21.798$ $r^2 = 0.958$

5-Point Calculation, EDTA plasma (268 U) with 5 serial dilutions:
$y = 1.024x - 7.8656$ $r^2 = 0.991$
1-Point Calculation, EDTA plasma (268U) with 5 serial dilutions:
$y = 1.031x - 19.772$ $r^2 = 0.953$

5-Point Calculation, serum specimen (305 U) with 5 serial dilutions:
$y = 0.627x - 14.095$ $r^2 = 0.992$
1-Point Calculation, serum specimen (350U) with 5 serial dilutions:
$y = 0.752x - 30.584$ $r^2 = 0.944$

The reportable range of the device is 15.62 to 250 units.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
There is no international reference material for anti-CCP antibodies. The calibrators and controls are assigned relative arbitrary units (U).
Negative, low positive and high positive controls and calibrators are included.

d. *Detection limit:*
A high titer serum was diluted to endpoint in order to determine the detection and dynamic range of the assay. The high limit of detection is 3 OD. When the sample is diluted until it does not change upon further dilution, the OD is 0.041. This corresponds to a value of 1 unit calculated by the 5 point method, and a value of 3 units by the 1 point method.

e. *Analytical specificity:*
Cross-reactivity
To assess cross-reactivity of CCP3 antigen with other autoantibodies, 16 samples having high levels of other autoantibodies were tested. Included in this group were 2 samples each that reacted with SS-A, SS-B, Sm, RNP, Scl-70, Jo-1, Ribo-P and DNA. These are autoantibodies found in people with rheumatic diseases such as SLE, Sjögren's syndrome, scleroderma, and Polymyositis, but not usually in RA patients. The study shows no significant cross-reactivity of the QUANTA Lite™ CCP3 IgG ELISA with these autoantibodies.
Interfering substances
Some sera have high levels of hemoglobin, bilirubin, cholesterol, or triglycerides. To assess whether any of these substances could cause a false positive on the Quanta Lite CCP3 ELISA, sera with known quantities of interferent (1000 mg/dL hemoglobin, 29.7 mg/dL bilirubin, 354 mg/dL cholesterol, and 2173 mg/dL triglycerides) were mixed with a normal serum and run on the ELISA. To determine if these substances could cause a false negative, these substances were also mixed with a high positive CCP serum. Results showed no significant interference. The product insert included a statement "High levels of hemoglobin, bilirubin, cholesterol, or triglycerides do not cause false negative or false positive results" under Limitations of the Procedure.

f. *Assay cut-off:*
INOVA uses a cutoff between positive and negative of 20 Units for continuity with the predicate devices.

2. Comparison studies:
a. *Method comparison with predicate device:*
Five hundred twenty one (521) clinically defined patient samples were tested with the new device and the predicate device. These samples included 156 patients with a clinical diagnosis of RA and sera from 166 random blood donors. Of the 146 where age and sex were available, 59 were female and the average age was 38.3 years. Disease controls included 113 patients with other rheumatic diseases: 78 with SLE, 11 with Sjogren's syndrome, and 24 with scleroderma. 86 samples have antibodies to infectious diseases such as

hepatitis C (62), herpes simplex virus (8) cytomegalovirus (8), toxoplasmosis (6) and rubella (2). The table below summarizes the comparison results.

All Samples N=521		CCP ELISA		
		+	-	Total
CCP3 ELISA	+	112	19*	131
	-	2**	388	390
	Total	114	407	521

*10 of these patients are diagnosed RA while 3 of the others are positive for IgM and/or IgA RF.
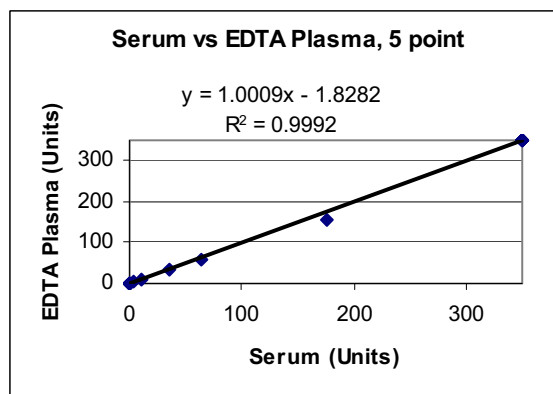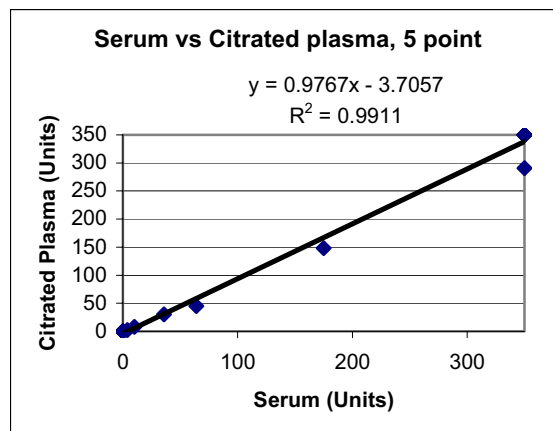**These 2 samples are diagnosed RA patients.
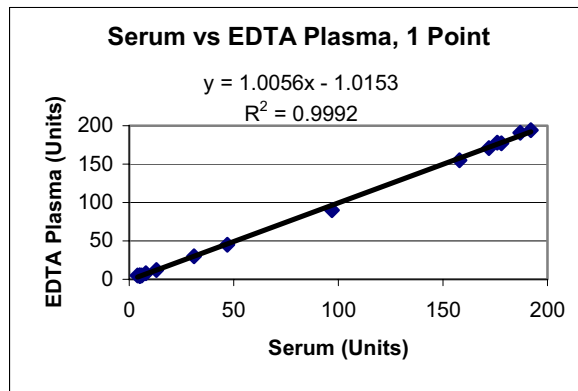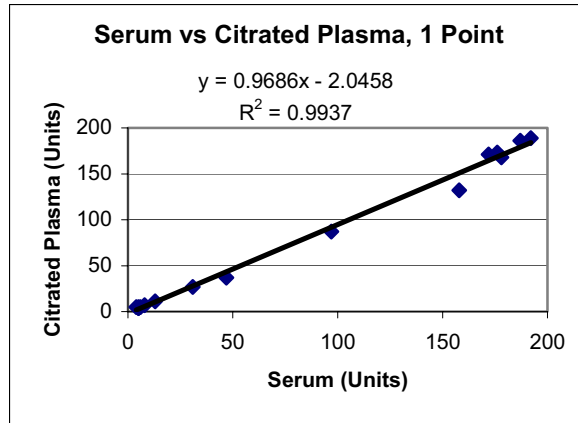
% Positive Agreement (95% CI) = 98% (94-100%)
% Negative Agreement (95% CI) = 95% (93-97%)
% Total Agreement =96.0%

b. *Matrix comparison:*
Three fresh serum and EDTA samples covering the entire measuring range were collected from each 16 individuals. Each sample was analyzed singly and calculated using both 5-point and 1-point method. The results are shown below.

Serum vs Citrated plasma, 5 point

$y = 0.9767x - 3.7057$
$R^2 = 0.9911$

Citrated Plasma (Units) vs Serum (Units)

Serum vs EDTA Plasma, 5 point

$y = 1.0009x - 1.8282$
$R^2 = 0.9992$

EDTA Plasma (Units) vs Serum (Units)

Serum vs Citrated Plasma, 1 Point

y = 0.9686x - 2.0458
$R^2 = 0.9937$

Citrated Plasma (Units) vs Serum (Units)

Serum vs EDTA Plasma, 1 Point

y = 1.0056x - 1.0153
$R^2 = 0.9992$

EDTA Plasma (Units) vs Serum (Units)

3. Clinical studies:
   a. *Clinical Sensitivity and specificity:*
      Samples used in the method comparison study were used to determine
      sensitivity and specificity of the assay. Results of the predicate device and the
      new device are summarized below.

Samples N=521	CCP3 ELISA		CCP ELISA		Sensitivity (95% CI)	Specificity (95% CI)
	+	-	+	-		
RA (N=156)	115	41	107	49	CCP3 = 74% (67-81%)	
					CCP = 69% (61-76%)	
Blood Donors (N=166)	4	162	1	165		
ORD (N=113)	12	101	6	107		
Infectious Disease (N=86)	0	86	0	86		
Total Controls (N=365)	16	349	7	358		CCP3 = 96% (93-98%)
						CCP = 98% (96-99%)

   b. *Other clinical supportive data (when a. is not applicable)*:
      Not applicable.
4. Clinical cut-off**:**
   See assay cut-off.
5. Expected values/Reference range:

The values shown below are suggested values only. Each laboratory should establish its own normal range.

	Units
Negative	<20
Weak Positive	20 – 39
Moderate Positive	40 – 59
Strong Positive	≥60

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.