510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
  k072944
B. Purpose for Submission:
  New device
C. Measurand:
  Anti-cyclic citrullinated peptide 3 (CCP3)
D. Type of Test:
  Semiquantitative enzyme-linked immunosorbent assay (ELISA)
E. Applicant:
  INOVA Diagnostics, Inc.
F. Proprietary and Established Names:
  QUANTA Lite™ CCP3.1 IgG/IgA ELISA
G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
NHX – Antibodies, anti-cyclic citrullinated peptide	Class II	21 CFR 866.5775 Rheumatoid factor immunological test system	Immunology 82

H. Intended Use:
  1. Intended use(s):
     The QUANTA Lite CCP3.1 IgG/IgA ELISA is a semiquantitative enzyme-linked immunosorbent assay for the detection of IgG and IgA anti-CCP3 (Cyclic Citrullinated Peptide 3) antibodies in patient sera or citrated 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 (RA), including RA diagnosed within 2 years of presentation of symptoms.
  2. Indication(s) for use:
     Same as Intended Use
  3. Special conditions for use statement(s):
     For prescription use only
  4. Special instrument requirements:
     Microplate reader capable of measuring OD at 450/620 nm
I. Device Description:
  The QUANTA Lite CCP3.1 IgG/IgA ELISA consists of a polystyrene microwell ELISA plate coated with purified, synthetic CCP3 antigen; ELISA negative, low positive and high positive (Calibrator A) controls; calibrators A–E (250, 125, 62.5, 31.25 and 15.62 Units); sample diluent; high specificity wash concentrate; goat anti-human IgG/IgA horseradish peroxidase conjugate; TMB chromogen; and stop solution.
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
	CCP3.1 IgG/IgA ELISA	**CCP IgG ELISA**
Indications for Use	To aid in the diagnosis of rheumatoid arthritis, including RA diagnosed within 2 years of presentation of symptoms	To aid in the diagnosis of rheumatoid arthritis
Method	ELISA	Same
Serum dilution and volume	1:101; 100 µl	Same
Calculation of results	Ratio method: compared to low control unit value; Standard curve: unknown concentrations read from plot of calibrators	Same
Result interpretation	<20 U/mL = negative, 20 - 39 = weak positive, 40 - 59 = mod. positive ≥ 60 = strong positive	Same
Units	Arbitrary ELISA units	Same
Solid phase	Coated polystyrene microwell plates	Same
Sample diluent	Tris-buffered saline, Tween 20, absorbents and protein stabilizers	Same
Controls	Negative, low positive, and high positive	Same

Differences		
Item	Device	Predicate
	CCP3.1 IgG/IgA ELISA	**CCP IgG ELISA**
Analyte measured	Anti-cyclic citrullinated peptide 3	Anti-cyclic citrullinated peptide 2
Capture antigen	Synthetic CCP3	Synthetic CCP2
Conjugate	HRP conjugated goat anti-human IgG/IgA	HRP conjugated goat anti-human IgG
Test matrix	Serum and citrated or EDTA plasma	Serum
Wash concentrate	10X High specificity	40X Tris-buffered saline and Tween 20

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

L. Test Principle:
The assay utilizes plastic microwells as a solid phase for attachment of the synthetic cyclic citrullinated peptide. Pre-diluted controls and diluted patient samples are added to separate wells, allowing any CCP IgG and/or IgA antibodies present to bind to the immobilized antigen. Unbound sample is washed away and an enzyme labeled anti-human IgG/IgA conjugate is added to each well. A second incubation allows the enzyme labeled anti-human IgG/IgA to bind to any patient antibodies that have become attached to the microwells. After washing away any unbound enzyme labeled anti-human IgG/IgA, the remaining enzyme activity is measured by adding a chromogenic substrate and measuring the intensity of the color that develops. The assay is evaluated by spectrophotometrically measuring and comparing the color intensity that develops in the patient wells with the color in the control wells (ratio). Alternatively, calibrator absorbance results are plotted and unknowns interpreted by reading the corresponding absorbance from the standard curve and calculating the units. Results determined with the assay are interpreted as negative, weak positive, moderately positive, or strong positive and are reported in arbitrary units.

M. Performance Characteristics (if/when applicable):
1. Analytical performance:
   a. *Precision/Reproducibility:*
      Intra-assay performance for the assay was measured by running 9 samples 9 or 10 times each on the same ELISA plate. Percent CVs ranged from 3-13%. Representative results are shown below.

	Negative		Low 1		Low 2		High 1	
	1 pt.*	5 pt.**	1 pt.	5 pt.	1 pt.	5 pt.	1 pt.	5 pt.
Mean units	4	2	22	24	26	30	68	81
SD	0.2	0.2	0.9	1.1	0.9	1.0	1.9	3.2
CV%	4%	13%	4%	5%	3%	3%	3%	4%

* Ratio method test results; ** Standard curve test results

      Inter-assay performance for the assay was determined by testing 16 samples: negative, low positive and strong positive samples in 6 separate assays spanning up to 9 different days. Overall percent CVs ranged from 1 to 11%. Representative results are shown below.

	Negative		Low 1		Low 2		High 1	
	1 pt.*	5 pt.**	1 pt.	5 pt.	1 pt.	5 pt.	1 pt.	5 pt.
Mean units	6	4	22	23	28	30	69	85
SD	0.4	0.5	0.7	0.6	0.8	1.2	1.7	1.2
CV%	8%	11%	3%	3%	3%	4%	2%	1%

* Ratio method test results; ** Standard curve test results

   b. *Linearity/assay reportable range:*

No claims were made regarding linearity for the assay. Reactivity is related to the quantity of antibody present in a non-linear fashion. While increases and decreases in patient antibody concentration will be reflected in a corresponding rise or fall in reactivity, the change is not proportional (i.e. a doubling of the antibody concentration will not double the reactivity). Specimens giving OD readings above the readable range of the plate reader may be reported as greater than the highest measurable OD. The assay has the option of interpretation of results from a standard curve but results are still semi-quantitative. Values calculated with the ratio method will be different than those calculated by the standard curve. High values will differ more than low values. The CCP3.1 IgG/IgA High Positive will not yield a value of 250 Units with the ratio method.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
There is no recognized standard reference material for CCP.
Based on results from accelerated stability testing, components were assigned expiration date of one year.

d. *Detection limit:*
The determination of detection limit/analytical sensitivity was not performed or relevant for this assay.

e. *Analytical specificity:*
Interfering substances:
To assess the possibility of false positive results from high levels of hemoglobin, bilirubin, cholesterol or triglycerides, sera with known quantities of the potential interferant were mixed with normal sera and run on the assay. To evaluate the potential for false negative results, the substances were mixed with a positive CCP3.1 IgG/IgA serum.

Substance added	Normal serum (Units)	Result Interpretation	Positive serum (Units)	% Difference
None	5	Negative	62	
Hemoglobin 1000 mg/dL	10	Negative	59	4.8
Bilirubin 29.7 mg/dL	7	Negative	56	9.7
Cholesterol 354 mg/dL	5	Negative	54	12.9
Triglycerides 1016 mg/dL	6	Negative	58	6.5

For samples closer to the assay cut-off (25-40 Units), % recoveries ranged from 84-110% for the single point and 87-112% for the 5 point curve interpretations.

Cross-reactivity
To assess potential cross-reactivity with other autoantibodies, the new assay was evaluated with 16 samples containing high levels of various other autoantibodies. Included in this group were 2 samples each that were positive

for SS-A, SS-B, Sm, RNP, Scl-70, Jo-1, ribosomal P, and dsDNA autoantibodies. All samples were negative for anti-CCP3.1 antibodies.

   f. *Assay cut-off:*

   To determine the reference interval (cut-off) a total of 275 samples from random blood donors were tested. At a cut-off of ≥20 Units, 2 samples were positive for a specificity of 99.3%.

2. Comparison studies:

   a. *Method comparison with predicate device:*

   Testing was performed on 942 specimens that included 275 from healthy individuals; 495 rheumatoid arthritis (RA) patient sera including 86 RA (presented with symptoms <2 years); 74 other rheumatic disease patient sera (24 scleroderma, 16 Sjögren's syndrome, and 34 SLE samples); and 98 infectious disease sera specimens (58 HCV, 14 HSV, 14 CMV6 toxoplasmosis and 6 rubella).

		INOVA QUANTA Lite IgG ELISA		
		Positive	Negative	Total
INOVA QUANTA Lite CCP3.1 IgG/IgA ELISA	Positive	320	38*	358
	Negative	8**	576	584
	Total	328	614	942

   * 33 of these samples are diagnosed RA
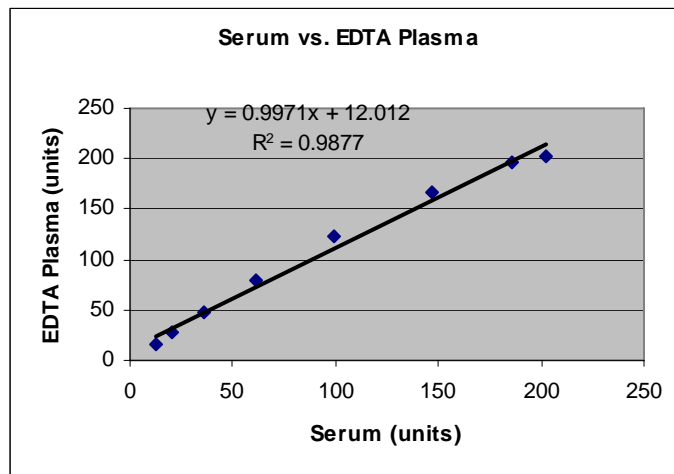   ** 5 of these samples are diagnosed RA

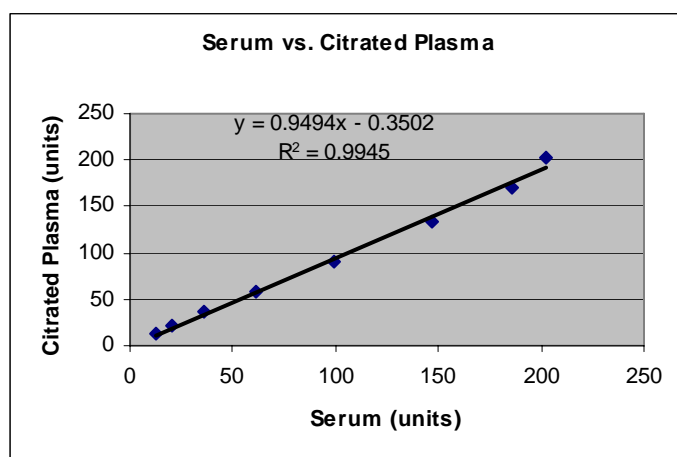|		
Positive percent agreement:	320/328 = 97.6%	95% CI: 95.3-98.9%
Negative percent agreement:	576/614 = 93.8%	95% CI: 91.6-95.6%
Overall agreement:	896/942 = 95.1%	

   b. *Matrix comparison:*

   Seven serum, EDTA plasma, and citrated plasma were serially diluted to demonstrate that the matrices showed similar results. The comparisons are shown below. Serum versus EDTA plasma yielded $y=0.9971x + 12.012$, $R^2 = 0.9877$.

   Serum versus citrated plasma yielded $y=0.9494x - 0.3502$, $R^2 = 0.9945$.

Serum vs. Citrated Plasma

$y = 0.9494x - 0.3502$
$R^2 = 0.9945$

(Chart: Citrated Plasma (units) vs. Serum (units))

3. Clinical studies:
   a. *Clinical Sensitivity and Specificity:*
      The clinical studies involved testing 942 specimens that included 275 from healthy individuals; 495 rheumatoid arthritis (RA) patient sera including 86 early RA (presented with symptoms <2 years); 74 other rheumatic disease patient sera; and 98 infectious disease sera specimens. The following results were obtained:

		Rheumatoid Arthritis		
		Positive	Negative	Total
INOVA QUANTA	Positive	348	10	358
Lite CCP3.1	Negative	147	437	584
IgG/IgA ELISA	Total	495	447	942

Clinical sensitivity:	348/495 = 70.3%	95% CI: 66.1-74.3%
Clinical specificity:	437/447 = 97.8%	95% CI: 95.9-98.9%
Overall agreement:	785/942 = 83.3%	

   The clinical sensitivity for the 86 early RA patients was 55/86 (64%).
   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 expected value in the normal population is negative. However, the presence of autoantibodies related to RA increases with age. Using the established cut-off of <20 Units as negative, in a study group of 272 normal blood donors, 99.3% were negative for the presence of anti-CCP3.1 autoantibodies. There are suggested cut-offs in the labeling with a recommendation that each laboratory establish its own normal range.

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.