

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY  
ASSAY AND INSTRUMENT COMBINATION TEMPLATE**

**A. 510(k) Number:**

k083080

**B. Purpose for Submission:**

New assay and instrument platform

**C. Measurand:**

Rheumatoid Factor (RF) IgA and RF IgM

Anti-cyclic citrullinated peptide (CCP) IgG autoantibodies

**D. Type of Test:**

Qualitative, microarray based multiplex assay

**E. Applicant:**

SQI Diagnostics

**F. Proprietary and Established Names:**

**IgX PLEX** Rheumatoid Arthritis Qualitative Assay and SQiDworks™ Diagnostics Platform

**G. Regulatory Information:**

1. Regulation section:

21 CFR§ 866.5775 - Rheumatoid Factor Immunological System

21 CFR § 862.2570 - Instrumentation for Clinical Multiplex Test

2. Classification:

Class II

Class II

3. Product code:

DHR - System, test, rheumatoid factor

NHX - Antibodies, Anti-cyclic citrullinated peptide

NSU - Instrumentation for Clinical Multiplex Test Systems

4. Panel:

Immunology (82)

**H. Intended Use:**

1. Intended use(s):

See Indications for Use below.

2. Indication(s) for use:

The IgX PLEX Rheumatoid Arthritis Qualitative Assay and SQiDworks™ Diagnostics Platform is an in vitro diagnostic test system for the qualitative detection of the IgA, and IgM classes of Rheumatoid Factors, and the IgG class of anti-cyclic citrullinated peptide antibodies (CCP-proprietary third generation equivalent formulation) in human serum specimens.

The IgX PLEX Rheumatoid Arthritis Qualitative Assay is intended for use in clinical laboratories as an aid in the diagnosis of Rheumatoid Arthritis in conjunction with other laboratory and clinical findings, and requires the SQiDworks Diagnostics Platform.

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:  
SQiDworks™ Diagnostics Platform

**I. Device Description:**

The IgX PLEX Rheumatoid Arthritis Qualitative Assay is supplied as two boxes requiring separate storage conditions: the Refrigerator Box must be refrigerated at 2-8°C, and the Freezer Box must be stored in a -15°C or lower freezer. A microarray plate and wash buffer concentrate are stored refrigerated while the Reporter mix, Calibrators, Positive Control #1, Positive Control #2, Negative Control, and Sample Diluent are stored refrigerated. The microarray plate consists of an array of protein and antibody replicate spots, covalently bound to the surface of coated glass within each well of a standard 96-well assay plate. All plates have the identical configuration of microarray elements, containing:

- 21 capture spots for Rheumatoid Factor Fcs and 21 capture spots for anti-CCP antibodies.
- Internal normalization curve and control spots for internal consistency confirmation.

The outer plate holder is labeled with a barcode indicating the assay type and lot number which is read by the platform. The reporter mix is comprised of diluted fluorescently labeled mouse monoclonal antibodies to human immunoglobulin G, A and M (marker antibody). Each type of marker antibody is labeled with a dye in a different spectral range allowing for the detection of both sub-classes of RF in one well; CCP is detected with a different dye in different wells. Each signal is analyzed to provide the end result for each analyte. The system reports each result independently.

The SQiDworks Diagnostics Platform is a multiplex immunoassay instrument that fully automates the process of a specific IgX PLEX Assay from serum transfer to reporting of all assay markers for each individual patient sample. The instrument automates the entire immunoassay protocol from end-to-end including sample pipetting, serum dilution, incubation, washing, and drying. Once the assay's biochemical reactions have completed, the instrument automatically performs a multi-color fluorescent scan of each well in the microarray, analyzes the data, and generates a report containing results for all assay markers. The SQiDworks Diagnostics Platform also includes numerous internal quality checks and user safety features with fail-safe and interlock mechanisms.

The instrument integrates an automated pipetting station, a fluorescent scanner, washing and drying stations, and other ancillary hardware components using dedicated instrument control. In addition, the software provides scheduling, self-verification, data acquisition, data management, analysis algorithms and reporting software.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):  
QUANTA Lite™ RF IgA  
QUANTA Lite RF IgM  
QUANTA Lite CCP3 IgG  
BioPLEX™ 2200 Multi-Analyte Detection System
2. Predicate 510(k) number(s):  
K983084  
K971614

K052264

K041658

3. Comparison with predicate:

| <b>IgX PLEX™ Rheumatoid Arthritis Qualitative Assay</b> |   | <b>Predicate(s)</b>             |
|---|---|---------------------------------|
| <b>Aspect/Feature/<br/>Characteristic</b>               | <b>IgX PLEX RF IgA</b>                      | <b>QUANTA Lite™ RF IgA</b>      |
| Intended Use  | As aid in diagnosis of rheumatoid arthritis | Same                            |
| Sample Matrix   | Serum                                       | Same                            |
| Methodology   | Microarray-based fluorescent detection      | ELISA                           |
| Calibration   | On each plate                               | Same                            |
| Concentration Determination                             | Qualitative                                 | Semi-Quantitative               |
| Expected Value  | Cut-off is 12.0 IU/mL                       | Cut-off is 6 U/mL               |
| Assay Substrate   | 96-well microarray plates                   | 96-well microtiter plates       |
| Multiplexed Assay                                       | Yes   | No                              |
| <b>Aspect/Feature/<br/>Characteristic</b>               | <b>IgX PLEX RF IgM</b>                      | <b>QUANTA Lite RF IgM</b>       |
| Intended Use  | As aid in diagnosis of rheumatoid arthritis | Same                            |
| Sample Matrix   | Serum                                       | Same                            |
| Methodology   | Microarray-based fluorescent detection      | ELISA                           |
| Calibration   | On each plate                               | Same                            |
| Concentration Determination                             | Qualitative                                 | Semi-Quantitative               |
| Expected Value  | Cut-off is 18.2 IU/mL                       | Cut-off is 6 U/mL               |
| Assay Substrate   | 96-well microarray plates                   | 96-well microtiter plates       |
| Multiplexed Assay                                       | Yes   | No                              |
| <b>Aspect/Feature/<br/>Characteristic</b>               | <b>IgX PLEX Anti-CCP<br/>IgG test</b>       | <b>QUANTA Lite CCP3<br/>IgG</b> |
| Intended Use  | As aid in diagnosis of rheumatoid arthritis | Same                            |
| Sample Matrix   | Serum                                       | Same                            |
| Methodology   | Microarray-based fluorescent detection      | ELISA                           |
| Calibration   | On each plate                               | Same                            |
| Concentration Determination                             | Qualitative                                 | Semi-Quantitative               |

|   |   |  |
|---|---|--|
| Expected Value                            | Cut-off is 11.7 U/mL                                | Cut-off is 20 U/mL                                       |
| Assay Substrate                           | 96-well microarray plates                           | 96-well microtiter plates                                |
| Multiplexed Assay                         | Yes   | No   |
| <b>Aspect/Feature/<br/>Characteristic</b> | <b>SQiDworks Microarray<br/>Diagnostic Platform</b> | <b>BioPlex™ 2200 Multi-<br/>Analyte Detection System</b> |
| Multi-analyte                             | Yes   | Same   |
| Multiplexing Method                       | Based on multiplex microarray<br>based technology   | Based on multiplex, bead-<br>based technology            |
| Detection Type                            | Fluorescence  | Same   |
| Target of Detection                       | Microarray spot                                     | Magnetic bead  |
| Detector                                  | Multichannel fluorescence<br>CCD camera scanner     | Laser monochromatic bead<br>flow cytometry-like reader   |
| Sample Handling and<br>Processing         | Automated   | Same   |
| Reagent Storage                           | No reagent storage                                  | On-board, refrigerated<br>reagent storage                |
| Internal Controls                         | Blank, serum verification                           | Same   |
| Internal Controls                         | Reaction condition<br>verifications                 | No   |
| Assay Substrate                           | 96-well microarray plates                           | Individual cuvettes                                      |
| Calibration                               | External calibrators                                | Same   |
| Laboratory Environment                    | Traditional laboratory<br>environment               | Same   |

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

None referenced.

**L. Test Principle:**

RF antibodies as well as any anti-CCP antibodies present in the serum sample or control materials bind to immobilized human IgG Fc fragments or the cyclic citrullinated peptide respectively to form antigen-antibody complexes. Unbound antibody and other substances in the sample are washed from the wells. Next, anti-human IgA, anti-human IgG, and anti-human IgM antibodies labeled with fluorescent dye are added as a mix. Excess reporter antibodies are washed away, and excess wash buffer is removed from the wells. Each well is scanned for each of the three dye wavelengths. The intensity of the generated signal is proportional to the amount of IgM, IgG, and IgA antibodies bound to the printed spots in the wells.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

a. *Precision/Reproducibility:*

The reproducibility of the four assays was determined by choosing clinical samples that were negative, slightly below the assay cutoff, slightly above the assay cutoff, or well above the cutoff for each assay. The positive samples were from rheumatoid arthritis (RA) patients diagnosed using the established American College of

Rheumatology (ACR) criteria. The same negative sample was used for all three assay reproducibility determinations.

Each sample was tested according to the standard assay procedure by three different operators, one platform, and four different assay kit lots. Each sample was tested in duplicate, twice a day, for twenty days (40 runs), for a maximum of 80 results per level (2 x 2 x 20 = 80).

**Qualitative reproducibility of IgX PLEX RF IgA Assay**

|                                     | <b>RF IgA (Cut-off = 12.0 IU/mL)</b> |                                  |                                  |                 |
|-------------------------------------|--------------------------------------|----------------------------------|----------------------------------|-----------------|
|                                     | <b>Negative</b>                      | <b>Negative Close to Cut-off</b> | <b>Positive Close to Cut-Off</b> | <b>Positive</b> |
| <b>Mean IU/mL</b>                   | <1.3                                 | 8.1                              | 16.3                             | 53.7            |
| <b>No of tests</b>                  | 80                                   | 80                               | 80                               | 80              |
| <b># positive</b>                   | 0                                    | 1                                | 76                               | 80              |
| <b># negative</b>                   | 80                                   | 79                               | 4                                | 0               |
| <b>Reproducibility (% Expected)</b> | 100%                                 | 98.8%                            | 95%                              | 100%            |

**Qualitative reproducibility of IgX PLEX RF IgM Assay**

|                                     | <b>RF IgM (Cut-off = 18.2 IU/mL)</b> |                                  |                                  |                 |
|-------------------------------------|--------------------------------------|----------------------------------|----------------------------------|-----------------|
|                                     | <b>Negative</b>                      | <b>Negative Close to Cut-off</b> | <b>Positive Close to Cut-Off</b> | <b>Positive</b> |
| <b>Mean IU/mL</b>                   | <5.7                                 | 12.6                             | 26.4                             | 32.9            |
| <b>No of tests</b>                  | 80                                   | 80                               | 80                               | 80              |
| <b># positive</b>                   | 0                                    | 3                                | 79                               | 80              |
| <b># negative</b>                   | 80                                   | 77                               | 1                                | 0               |
| <b>Reproducibility (% Expected)</b> | 100%                                 | 96.3%                            | 98.8%                            | 100%            |

**Qualitative reproducibility of IgX PLEX anti-CCP Assay**

|                                     | <b>anti-CCP (Cut-off = 11.7 IU/mL)</b> |                                  |                                  |                 |
|-------------------------------------|--|----------------------------------|----------------------------------|-----------------|
|                                     | <b>Negative</b>                        | <b>Negative Close to Cut-off</b> | <b>Positive Close to Cut-Off</b> | <b>Positive</b> |
| <b>Mean IU/mL</b>                   | 6.9                                    | 8.8                              | 16.0                             | 22.6            |
| <b>No of tests</b>                  | 80                                     | 80                               | 60                               | 80              |
| <b># positive</b>                   | 0                                      | 7                                | 50                               | 80              |
| <b># negative</b>                   | 80                                     | 73                               | 10                               | 0               |
| <b>Reproducibility (% Expected)</b> | 100%                                   | 91.3%                            | 83.3%                            | 100%            |

- b. *Linearity/assay reportable range:*  
Not applicable for qualitative assays.

- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*  
 Calibrators: The external calibrators (standards) are eight dilutions of a sample derived from human sera containing an appropriate representation of each of the analytes to be reported. The assay standards (secondary standards) for RF (IgA, and IgM) are traceable to the WHO/First British Standard 64/2 (primary standards) and internally calculated in IU/mL. The CCP IgG results are internally calculated in U/mL and are comparable to other assays on the market. The quantitative results are converted to qualitative results (positive and negative), based on assay-specific cutoff values. No dilutions or reconstitutions are needed.

Controls: The positive and negative control samples are derived from human sera clinically diagnosed as positive and normal respectively. The platform treats the controls as samples and does not perform any quality assessment based on the results. Although expected results for each applicable analyte of each control are provided, each lab is expected to follow their own quality procedures for assessing controls.

A real-time stability study is on-going. Current data supports a three-month shelf life. Serum samples were found stable at room temperature for 6 hours and for 1 week at 4°C.

- d. *Detection limit:*  
 Not applicable for qualitative assays.
- e. *Analytical specificity:*  
 Several RA samples with low or intermediate levels were evaluated with different levels of endogenous interferents above physiological ranges. The table below lists the levels (mg/mL) of interferents at which the bias relative to the unspiked sample is < ±15%:

|         | Bilirubin | Hemoglobin | Triglycerides | Human IgG |
|---------|-----------|------------|---------------|-----------|
|         | mg/mL     |            |               |           |
| RF IgA  | 3         | 25         | 100           | 10        |
| RF IgM  | 1.2       | 12.5       | 12.5          | 1.3       |
| CCP IgG | 3         | 100        | 100           | 10        |

- f. *Assay cut-off:*  
 The sponsor established the cutoffs of the assays using an ROC analysis of a data set consisting of serum specimens from 135 rheumatoid arthritis patients and 150 normal donors (n=285) collected from commercial sources representative of the United States, Canadian and European populations, along with the corresponding clinical and demographic information. These samples were not included in any other analysis (method comparison or clinical study).

### Demographics of Samples Used to Establish IgX PLEX Cut-Offs

| Samples       | Gender |        | Demographic Data |     |        | Age (years) |     |         |
|---------------|--------|--------|------------------|-----|--------|-------------|-----|---------|
|               | Male   | Female | Canada           | US  | Europe | Min         | Max | Average |
| <b>RA</b>     | 40     | 95     | 30               | 41  | 64     | 23          | 84  | 58.6    |
| <b>Normal</b> | 75     | 75     | 0                | 150 | 0      | 18          | 61  | 35.3    |

The ROC analysis provided a list of potential cut-off values with sensitivity and specificity calculated for each. The sponsor examined the proposed values which had balanced sensitivity and specificity and then chose the one with the best confidence interval and accuracy. The chosen cut-offs were: 12.0 IU/mL for RF IgA, 18.2 IU/mL for RF IgM, and 11.7 U/mL for CCP IgG.

2. Comparison studies:

a. *Method comparison with predicate device:*

Concordance between the IgX PLEX RA Assays and the predicate methods was calculated using 2x2 comparison tables with determination of positive percent agreement, negative percent agreement, and overall agreement, based on the cut-off level for each assay system. 350 clinical specimens were used; 250 patients diagnosed with RA according to ACR criteria and 100 normal donors. Two results were invalidated due to a within-well normalization failure.

#### Agreement and Confidence Intervals (CI) of IgX PLEX RF IgA Results

|                    |        | Quanta LITE IgA |     | SUMMARY             |               |
|--------------------|--------|-----------------|-----|---------------------|---------------|
|                    |        | POS             | NEG | %Agreement and (CI) |               |
| IgX PLEX<br>RF IgA | POS    | 178             | 44  | Positive            | 95% (87-103%) |
|                    | NEG    | 9               | 117 | Negative            | 73% (65-80%)  |
|                    | Totals | 187             | 161 | Overall             | 85% (77-92%)  |

#### Agreement and Confidence Intervals (CI) of IgX PLEX RF IgM Results

|                    |        | Quanta LITE RF IgM |     | SUMMARY             |              |
|--------------------|--------|--------------------|-----|---------------------|--------------|
|                    |        | POS                | NEG | %Agreement and (CI) |              |
| IgX PLEX<br>RF IgM | POS    | 214                | 17  | Positive            | 87% (79-96%) |
|                    | NEG    | 31                 | 86  | Negative            | 84% (75-92%) |
|                    | Totals | 245                | 103 | Overall             | 86% (78-95%) |

#### Agreement and Confidence Intervals (CI) of IgX PLEX anti-CCP Results

|                             |               | Quanta LITE CCP3 |     | SUMMARY             |               |
|-----------------------------|---------------|------------------|-----|---------------------|---------------|
|                             |               | POS              | NEG | %Agreement and (CI) |               |
| <b>IgX PLEX<br/>CCP IgG</b> | <b>POS</b>    | 190              | 10  | Positive            | 96% (91-101%) |
|                             | <b>NEG</b>    | 8                | 140 | Negative            | 93% (88-98%)  |
|                             | <b>Totals</b> | 198              | 150 | Overall             | 95% (90-100%) |

*b. Matrix comparison:*

Serum is the only claimed matrix for this assay.

3. Clinical studies:

The objective of this study was to establish the clinical sensitivity and specificity of the IgX PLEX RA assay, which detects RF IgA, RF IgM, and CCP IgG in serum samples. The clinical sensitivity and specificity were calculated by comparing the IgX PLEX RA assay result interpretations to the clinical diagnosis categories of RA patients, presumptive normal samples, and patients with other diseases both autoimmune and non-autoimmune. The available clinical diagnosis for the RA patients was defined by ACR criteria.

909 clinical specimens, collected from 360 patients with RA, 399 patients with other diseases—both autoimmune and otherwise—and 150 presumptively normal donors. Of the 909 samples assayed, 14 were invalidated by the system for various reasons, predominately because of within well normalization failure. Therefore 895 clinical samples were available for analysis.

*a. Clinical Sensitivity:*

358 RA samples were available for calculation of clinical sensitivity:

|                          | TP  | FN | Sensitivity  | +/- 95% CI    |
|--------------------------|-----|----|--------------|---------------|
| <b>IgX PLEX RF IgA</b>   | 306 | 52 | <b>85.5%</b> | 83.6% - 87.3% |
| <b>IgX PLEX RF IgM</b>   | 334 | 24 | <b>93.3%</b> | 92.0% - 94.6% |
| <b>IgX PLEX anti-CCP</b> | 278 | 80 | <b>77.7%</b> | 75.5% - 79.9% |

*b. Clinical specificity:*

Normal samples and samples from other diseases and conditions were tested (number in parentheses): Systemic Lupus Erythematosus (43), Sjogren's Syndrome (26). MCTD (43), Progressive Systemic Sclerosis (53), Arthropathies (26), Osteoarthritis (43), Joint Pain, CTS (37). Myalgia & Myositis (38), Vasculitis (17). Other Autoimmune Diseases (61).

|                              |                   | # samples | FP  | TN  | Specificity  | +/- 95% CI    |
|------------------------------|-------------------|-----------|-----|-----|--------------|---------------|
| <b>IgX PLEX<br/>RF IgA</b>   | other disease     | 387       | 93  | 294 | <b>76.0%</b> | 74.1% - 77.8% |
|                              | normals           | 150       | 11  | 139 | <b>92.7%</b> | 90.5% - 94.8% |
|                              | <i>all non-RA</i> | 537       | 104 | 433 | <b>80.6%</b> | 78.9% - 82.3% |
| <b>IgX PLEX<br/>RF IgM</b>   | other disease     | 388       | 110 | 278 | <b>71.6%</b> | 69.7% - 73.6% |
|                              | normals           | 150       | 9   | 141 | <b>94.0%</b> | 92.1% - 95.9% |
|                              | <i>all non-RA</i> | 538       | 119 | 419 | <b>77.9%</b> | 76.1% - 79.7% |
| <b>IgX PLEX<br/>anti-CCP</b> | other disease     | 388       | 49  | 339 | <b>87.4%</b> | 85.9% - 88.8% |
|                              | normals           | 150       | 6   | 144 | <b>96.0%</b> | 94.4% - 97.6% |
|                              | <i>all non-RA</i> | 538       | 55  | 483 | <b>89.9%</b> | 88.5% - 91.1% |

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

Not applicable

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

The expected value in the normal population is negative. However, apparently healthy asymptomatic individuals may have RF, usually of low titer. RF titers are also occasionally seen in other autoimmune diseases than RA. Anti-CCP antibodies appear to be fairly specific for RA although, as with most autoantibodies, some apparently healthy individuals are anti-CCP positive. The incidence of false positives to all three analytes increases with age and is similar in females and males.

**N. Instrument Name:**

SQiDworks Diagnostics Platform

**O. System Descriptions:**

1. Modes of Operation:

Semi-automated.

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes  or No

3. Specimen Identification:

Serum samples are labeled with barcodes. The system initialization process includes automatic instrument verifications including identification and confirmation of the barcodes on the patient sample tubes and additional operator confirmations.

4. Specimen Sampling and Handling:

Specimen sampling and handling is fully automated after the sample is loaded. The instrument performs quality control checks on the process.

5. Calibration:

Normalization of the captured signal intensity occurs in each well to bring all reaction results to an internally calibrated value (AEC). This process is used to normalize variances in assay kinetics and minor inter-lot variances between the microarray wells. Standardization then occurs at the plate level using eight defined assay standards (calibrators) provided with the assay. Patient sample results are then calculated from this standard curve. The value is compared to the cut-off and a determination of positive or negative is made.

6. Quality Control:

A set of internal quality control rules are invoked to evaluate the data that is produced. These rules were identified by safety and hazard analysis to mitigate risks such as missing human sera and general failures in the reaction. They include quality control rules for checking the fitness of the normalization and standardization curves, as well as thresholds of controls measuring reporter activity and human sera presence. When data is found that does not fit the required control levels or rules, further processing of the data is halted and the value “No Result” is reported. The internal quality control rules, or invalidation rules, are at each level of data processing. Single analyte results may be invalidated due to high CV of the replicate capture spots, results for a well may be invalidated due to improper normalization curve or control threshold, and results for an analyte may be invalidated due to an improper standardization curve.

**P. Other Supportive Instrument Performance Characteristics Data Not Covered In The “Performance Characteristics” Section above:**

None

**Q. Proposed Labeling:**

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**R. Conclusion:**

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.