

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM**

A. 510(k) Number:

k142781

B. Purpose for Submission:

New device

C. Measurand:

IgG autoantibodies specific for SS-A (Ro) protein
IgG autoantibodies specific for SS-B (La) protein
IgG autoantibodies specific for Sm protein
IgG autoantibodies specific for RNP protein

D. Type of Test:

Immunoassay, semi-quantitative

E. Applicant:

IMMCO Diagnostics Inc.

F. Proprietary and Established Names:

ImmuLisa Enhanced™ SS-A (Ro) Antibody ELISA
ImmuLisa Enhanced™ SS-B (La) Antibody ELISA
ImmuLisa Enhanced™ Sm Antibody ELISA
ImmuLisa Enhanced™ RNP Antibody ELISA

G. Regulatory Information:

1. Regulation section:
21 CFR§866.5100, Antinuclear antibody immunological test system
2. Classification:
Class II
3. Product code:
LLL, Extractable antinuclear antibody, antigen and control
LKP, anti-Sm antibody, antigen and control

4. Panel:

Immunology (82)

H. Intended Use:

1. Intended use(s):

ImmuLisa Enhanced™ SS-A (Ro) Antibody ELISA: Enzyme linked immunoassay (ELISA) for the qualitative and semi-quantitative detection of SS-A (Ro) (52 kD and 60 kD) IgG antibodies in human serum as an aid in diagnosis of Systemic Lupus Erythematosus (SLE) and Sjögren's Syndrome in conjunction with clinical findings and other laboratory tests.

ImmuLisa Enhanced™ SS-B (La) Antibody ELISA: Enzyme linked immunoassay (ELISA) for the qualitative and semi-quantitative detection of SS-B (La) IgG antibodies in human serum as an aid in diagnosis of Systemic Lupus Erythematosus (SLE) and Sjögren's Syndrome in conjunction with clinical findings and other laboratory tests.

ImmuLisa Enhanced™ Sm Antibody ELISA: Enzyme linked immunoassay (ELISA) for the qualitative and semi-quantitative detection of Sm IgG antibodies in human serum as an aid in diagnosis of Systemic Lupus Erythematosus (SLE) in conjunction with clinical findings and other laboratory tests.

ImmuLisa Enhanced™ RNP Antibody ELISA: Enzyme linked immunoassay (ELISA) for the qualitative and semi-quantitative detection of RNP IgG antibodies in human serum as an aid in diagnosis of Systemic Lupus Erythematosus (SLE) and Mixed Connective Tissue Disease (MCTD) in conjunction with clinical findings and other laboratory tests.

2. Indication(s) for use:

Same as intended use

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

An ELISA microplate reader capable of reading absorbance values at 450 nm. If dual wavelength microplate reader is available, the reference filter should be set at 600-650 nm. An automatic microplate washer capable of accurately dispensing 200 µL of fluid is also required.

I. Device Description:

Each kit consists of 12- 1 x 8 antigen coated microwell strips, negative control, positive control, five assay calibrators, anti-human IgG-horse radish peroxidase conjugate, TMB substrate, stop solution, wash buffer and diluent. The results are read by a spectrophotometer at 450 nm. Results are expressed in ELISA units per milliliter (EU/mL) and reported as positive or negative.

J. Substantial Equivalence Information:

1. Predicate device name(s):
QUANTA Lite® SS-A ELISA
QUANTA Lite® SS-B ELISA
QUANTA Lite® Sm ELISA
QUANTA Lite® RNP ELISA

2. Predicate 510(k) number(s):
k922830
k922832
k922831
k922832

3. Comparison with predicate:

ImmuLisa™ SS-A (Ro) Antibody ELISA

| Similarities | | |
|--------------|--|---|
| Item | ImmuLisa™ SS-A | Predicate Device |
| Intended Use | Enzyme linked immunoassay (ELISA) for the qualitative and semi-quantitative detection of SS-A (Ro) (52 kD and 60 kD) IgG antibodies in human serum as an aid in diagnosis of Systemic Lupus Erythematosus (SLE) and Sjögren’s Syndrome in conjunction with clinical findings and other laboratory tests. | QUANTA Lite™ SS-A is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of SS-A (60 kDa and 52 kDa) antibodies in human serum. The presence of SS-A antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of Systemic Lupus Erythematosus (SLE) and related connective tissue diseases, such as Sjögren’s Syndrome. |
| Assay Type | ELISA | Same |
| Type of Test | Semi-quantitative and qualitative | Semi-quantitative |

| Similarities | | |
|---------------------|--|-------------------------|
| Item | ImmuLisa™ SS-A | Predicate Device |
| Conjugate | HRP conjugated anti-human IgG | Same |
| Substrate | TMB | Same |
| Traceability | International Reference Preparation is not available. Results are traceable to in-house standards. | Same |
| Sample Type | Serum | Same |
| Screening Dilution | 1:101 | Same |
| Cut-off | 20 EU/mL | 20 units |
| Conjugate | HRP conjugated anti-human IgG | Same |
| Instrumentation | Spectrophotometer 450 nm | Same |
| Signal | Optical density | Same |

| Differences | | |
|--------------------|---|-------------------------|
| Item | ImmuLisa™ SS-A | Predicate Device |
| Calibrators | Set of 5; values in EU/mL: 160, 80, 40, 20, 1 | Single point calibrator |
| Linear Range | 2.0 EU/mL – 160 EU/mL | Not specified |
| Limit of Detection | 2.0 EU/mL | Not specified |
| Capture Antigen | Recombinant SS-A | Purified SS-A |

ImmuLisa™ SS-B Antibody ELISA

| Similarities | | |
|---------------------|--|---|
| Item | ImmuLisa™ SS-B | Predicate Device |
| Intended Use | Enzyme linked immunoassay (ELISA) for the qualitative and semi-quantitative detection of SS-B (La) IgG antibodies in human serum as an aid in diagnosis of Systemic Lupus Erythematosus (SLE) and Sjögren’s Syndrome in conjunction with clinical findings and other laboratory tests. | QUANTA Lite™ SS-B is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of SS-B antibodies in human serum. The presence of SS-B antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of Systemic Lupus Erythematosus (SLE) and related connective tissue diseases, such as Sjögren’s Syndrome. |
| Assay Type | ELISA | Same |
| Quantitation | Semi-quantitative and qualitative | Semi-quantitative |
| Conjugate | HRP conjugated anti-human IgG | Same |
| Substrate | TMB | Same |
| Traceability | International Reference Preparation is not available. Results are traceable to in-house standards. | Same |
| Sample Type | Serum | Same |
| Screening Dilution | 1:101 | Same |
| Cut-off | 20 EU/mL | 20 units |
| Conjugate | HRP conjugated anti-human IgG | Same |
| Instrumentation | Spectrophotometer 450 nm | Same |
| Signal | Optical density | Same |

| Differences | | |
|--------------------|--|-------------------------|
| Item | ImmuLisa™ SS-B | Predicate Device |
| Calibrators | Set of 5; values in EU/mL: 160, 80 40, 20, 1 | Single point calibrator |
| Linear Range | 1.6 EU/mL – 160 EU/mL | Not specified |
| Limit of Detection | 1.6 EU/mL | Not specified |
| Capture Antigen | Recombinant SS-B | Purified SS-B |

ImmuLisa™ Sm Antibody ELISA

| Similarities | | |
|---------------------|--|---|
| Item | ImmuLisa™ Sm | Predicate Device |
| Intended Use | Enzyme linked immunoassay (ELISA) for the qualitative and semi-quantitative detection of Sm IgG antibodies in human serum as an aid in diagnosis of Systemic Lupus Erythematosus (SLE) in conjunction with clinical findings and other laboratory tests. | QUANTA Lite™ Sm is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of Sm antibodies in human serum. The presence of Sm antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of Systemic Lupus Erythematosus (SLE) and related connective tissue diseases. |
| Assay Type | ELISA | Same |
| Type of Test | Semi-quantitative and qualitative | Semi-quantitative |
| Conjugate | HRP conjugated anti-human IgG | Same |
| Substrate | TMB | Same |
| Capture Antigen | Purified Sm | Same |
| Traceability | International Reference Preparation is not available. Results are traceable to in-house standards. | Same |
| Sample Type | Serum | Same |

| Similarities | | |
|---------------------|-------------------------------|-------------------------|
| Item | ImmuLisa™ Sm | Predicate Device |
| Screening Dilution | 1:101 | Same |
| Cut-off | 20 EU/mL | 20 units |
| Conjugate | HRP conjugated anti-human IgG | Same |
| Instrumentation | Spectrophotometer 450 nm | Same |
| Signal | Optical density | Same |

| Differences | | |
|--------------------|---|-------------------------|
| Item | ImmuLisa™ Sm | Predicate Device |
| Calibrators | Set of 5; values in EU/mL: 160, 80, 40, 20, 1 | Single point calibrator |
| Linear Range | 4.2 EU/mL – 160 EU/mL | Not specified |
| Limit of Detection | 4.2 EU/mL | Not specified |

ImmuLisa™ RNP Antibody ELISA

| Similarities | | |
|---------------------|--|--|
| Item | ImmuLisa™ RNP | Predicate Device |
| Intended Use | Enzyme linked immunoassay (ELISA) for the qualitative and semi-quantitative detection of RNP IgG antibodies in human serum as an aid in diagnosis of Systemic Lupus Erythematosus (SLE) and Mixed Connective Tissue Disease (MCTD) in conjunction with clinical findings and other laboratory tests. | QUANTA Lite™ RNP is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of RNP antibodies in human serum. The presence of RNP antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of Systemic Lupus Erythematosus (SLE) and related connective tissue diseases. |
| Assay Type | ELISA | Same |
| Quantitation | Semi-quantitative and qualitative | Semi-quantitative |
| Conjugate | HRP conjugated anti-human | Same |

| Similarities | | |
|---------------------|--|-------------------------|
| Item | ImmuLisa™ RNP | Predicate Device |
| | IgG | |
| Substrate | TMB | Same |
| Capture Antigen | Purified RNP | Same |
| Traceability | International Reference Preparation is not available. Results are traceable to in-house standards. | Same |
| Sample Type | Serum | Same |
| Screening Dilution | 1:101 | Same |
| Cut-off | 20 EU/mL | 20 units |
| Conjugate | HRP conjugated anti-human IgG | Same |
| Instrumentation | Spectrophotometer 450 nm | Same |
| Signal | Optical density | Same |

| Differences | | |
|--------------------|--|-------------------------|
| Item | ImmuLisa™ RNP | Predicate Device |
| Calibrators | Set of 5; values in EU/mL: 160, 80 40, 20, 1 | Single point calibrator |
| Linear Range | 3.0 EU/mL – 160 EU/mL | Not specified |
| Limit of Detection | 3.0 EU/mL | Not specified |

K. Standard/Guidance Document Referenced:

1. Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (CLSI EP6-A)
2. Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition (CLSI EP07-A2)
3. User Protocol for Evaluation of Qualitative Test Performance (CLSI EP12-A2)
4. Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline (CLSI EP17-A)

L. Test Principle:

The test is performed as a solid phase immunoassay. Controls, calibrators and patient sera are incubated in the antigen coated wells to allow specific antibodies present in the serum to bind to the antigen. Unbound antibodies and other serum proteins are removed by washing the microwells. Bound antibodies are detected by adding an enzyme labeled anti-human IgG conjugate to the microwells. Unbound conjugate is removed by washing. Enzyme substrate (TMB) is then added to the wells and the presence of antibodies is detected by a color change produced by the conversion of TMB substrate to a colored reaction product. The reaction is stopped and the intensity of the color change, which is proportional to the concentration of antibody, is read by a spectrophotometer at 450 nm. Results are expressed in ELISA units per milliliter (EU/mL) and reported as positive or negative.

Semi-quantitative results are determined from a series of five calibrators (160 EU/mL, 80 EU/mL, 40 EU/mL, 20 EU/mL, and 1 EU/mL). Values less than 20 EU/mL are considered negative results while values greater than 25 EU/mL are considered positive; results between 20 EU/mL and 25 EU/mL are considered 'indeterminate'. The sponsor recommends that indeterminate results should be retested and evaluated along with other laboratory methods. Qualitative results are determined using a ratio of the absorbance of the sample to the absorbance of the cut-off calibrator (20 EU/mL). The ratio is multiplied by the concentration of the cut-off calibrator to give a numerical value. Values greater than 20 EU/mL are considered positive.

Qualitative results are calculated by comparing the EU/mL values of the sample to that of Calibrator D. Specimens with an optical density (OD) greater than Calibrator D are considered positive and specimens with an OD less than Calibrator D are considered negative.

M. Performance Characteristics:

1. Analytical performance:

a. Precision/Reproducibility:

Semi-Quantitative Precision:

Native sera from the Intended Use population, were selected to cover the analytical measuring range and included samples in the negative range, ~20% below cut-off, around the cut-off, ~20% above cut-off and in the moderate positive range of the assays. All specimens were assayed 12 times on one day to determine intra-assay repeatability. The same specimens were also assayed across 13 runs with 6 repetitions in each to assess reproducibility (total replicates = 90). Assays were performed by two operators using different equipment sets. The first operator performed the assay manually with a multichannel pipettor, microplate washer and microplate reader. The second operator used an automatic system with a liquid handling system including a microplate reader. One assay lot was used for the study. The manufacturer's pre-determined acceptance criteria of CV < 15% was met for all measures.

SS-A Semi-Quantitative Precision:

| | EU/mL Mean | Repeatability | | Between Days | | Total | |
|---|---------------|---------------|-------|--------------|-------|-------|-------|
| | | SD | CV | SD | CV | SD | CV |
| 1 | 9.6 | 1.2 | 12.3% | 1.2 | 12.3% | 1.2 | 12.4% |
| 2 | 17.2 | 1.2 | 7.0% | 1.6 | 9.1% | 1.5 | 8.9% |
| 3 | 20.3 | 1.4 | 6.6% | 1.2 | 6.0% | 1.2 | 6.1% |
| 4 | 23.2 | 1.6 | 6.8% | 1.8 | 7.9% | 1.8 | 7.7% |
| 5 | 85.2 | 5.5 | 6.3% | 6.0 | 7.1% | 6.0 | 7.0% |
| 6 | 116.4 | 5.4 | 4.5% | 7.2 | 6.2% | 7.1 | 6.1% |
| 7 | 137.2 | 8.2 | 5.7% | 13.4 | 9.9% | 13.2 | 9.6% |

| | EU/mL | Manual/ Operator #1 | | Automated/ Operator # 2 | | Total Imprecision |
|---|-------|------------------------|-------|----------------------------|-------|----------------------|
| | | SD | CV | SD | CV | CV |
| 1 | 9.6 | 1.4 | 14.3% | 1.0 | 10.9% | 12.4% |
| 2 | 17.2 | 1.7 | 9.9% | 1.4 | 8.4% | 8.9% |
| 3 | 20.3 | 1.3 | 6.3% | 1.2 | 6.0% | 6.1% |
| 4 | 23.2 | 2.0 | 8.5% | 1.7 | 7.2% | 7.7% |
| 5 | 85.2 | 7.2 | 8.5% | 5.3 | 6.3% | 7.0% |
| 6 | 116.4 | 6.1 | 5.0% | 5.8 | 5.1% | 6.1% |
| 7 | 137.2 | 10.8 | 7.3% | 11.3 | 8.5% | 9.6% |

SS-B Semi-Quantitative Precision:

| | EU/mL Mean | Repeatability | | Between Days | | Total Imprecision | |
|---|---------------|---------------|------|--------------|-------|-------------------|------|
| | | SD | CV | SD | CV | SD | CV |
| 1 | 11.9 | 0.8 | 7.1% | 0.9 | 7.9% | 1.0 | 8.1% |
| 2 | 15.2 | 1.1 | 7.7% | 1.2 | 8.0% | 1.2 | 8.0% |
| 3 | 19.8 | 1.1 | 5.7% | 1.7 | 8.6% | 1.7 | 8.4% |
| 4 | 25.2 | 1.7 | 6.9% | 1.9 | 7.4% | 1.9 | 7.6% |
| 5 | 51.4 | 3.6 | 6.9% | 4.8 | 9.4% | 4.7 | 9.1% |
| 6 | 80.4 | 5.8 | 7.0% | 7.9 | 9.9% | 7.7 | 9.5% |
| 7 | 152.1 | 6.7 | 4.4% | 10.6 | 70.1% | 10.1 | 6.7% |

| | EU/mL | Manual/ Operator #1 | | Automated/ Operator # 2 | | Total Imprecision |
|---|-------|------------------------|-------|----------------------------|------|----------------------|
| | | SD | CV | SD | CV | CV |
| 1 | 11.9 | 1.0 | 8.3% | 0.9 | 8.0% | 8.1% |
| 2 | 15.2 | 1.3 | 8.8% | 1.2 | 7.6% | 8.0% |
| 3 | 19.8 | 2.0 | 10.4% | 1.4 | 6.9% | 8.4% |
| 4 | 25.2 | 2.0 | 8.0% | 1.9 | 7.4% | 7.6% |
| 5 | 51.4 | 4.5 | 8.9% | 4.8 | 9.3% | 7.1% |
| 6 | 80.4 | 7.3 | 9.0% | 7.9 | 9.8% | 7.5% |
| 7 | 152.1 | 12.5 | 8.5% | 7.7 | 5.0% | 6.7% |

Sm Semi-Quantitative Precision:

| | EU/mL Mean | Repeatability | | Between Days | | Total Imprecision | |
|---|---------------|---------------|-------|--------------|-------|-------------------|-------|
| | | SD | CV | SD | CV | SD | CV |
| 1 | 7.0 | 0.9 | 14.0% | 0.9 | 13.4% | 1.0 | 13.6% |
| 2 | 16.5 | 0.9 | 5.0% | 1.5 | 9.3% | 1.5 | 8.9% |
| 3 | 18.6 | 1.3 | 6.9% | 1.5 | 8.0% | 1.5 | 7.9% |
| 4 | 27.0 | 1.1 | 4.1% | 2.0 | 7.3% | 1.9 | 7.0% |
| 5 | 34.4 | 2.5 | 6.0% | 2.8 | 6.4% | 2.7 | 6.4% |
| 6 | 77.8 | 3.5 | 4.5% | 4.3 | 5.5% | 4.2 | 5.4% |
| 7 | 142.7 | 4.9 | 3.3% | 9.8 | 6.9% | 9.5 | 6.6% |

| | EU/mL | Manual/ Operator #1 | | Automated/ Operator # 2 | | Total Imprecision |
|---|-------|------------------------|-------|----------------------------|-------|----------------------|
| | | SD | CV | SD | CV | CV |
| 1 | 7.0 | 1.1 | 15.6% | 0.9 | 12.6% | 13.6% |
| 2 | 16.5 | 1.6 | 9.9% | 1.4 | 8.6% | 8.9% |
| 3 | 18.6 | 1.4 | 7.8% | 1.5 | 7.9% | 7.9% |
| 4 | 27.0 | 2.4 | 8.9% | 1.4 | 5.2% | 7.0% |
| 5 | 34.4 | 2.4 | 7.0% | 2.7 | 7.6% | 7.4% |
| 6 | 43.1 | 2.3 | 5.4% | 2.8 | 6.5% | 6.4% |
| 7 | 77.8 | 3.3 | 4.5% | 2.9 | 3.7% | 5.4% |

RNP Semi-Quantitative Precision:

| | EU/mL Mean | Repeatability | | Between Days | | Total Imprecision | |
|---|---------------|---------------|------|--------------|------|-------------------|------|
| | | SD | CV | SD | CV | SD | CV |
| 1 | 9.9 | 0.6 | 6.5% | 0.9 | 9.3% | 0.9 | 9.0% |
| 2 | 16.2 | 0.7 | 4.5% | 1.1 | 6.6% | 1.0 | 6.4% |
| 3 | 20.6 | 1.4 | 6.7% | 1.4 | 6.7% | 1.4 | 6.7% |
| 4 | 22.3 | 1.3 | 6.1% | 1.5 | 6.8% | 1.5 | 6.7% |
| 5 | 48.9 | 2.5 | 5.2% | 3.1 | 6.4% | 3.1 | 6.3% |
| 6 | 96.1 | 6.1 | 6.1% | 7.8 | 8.2% | 7.8 | 8.1% |
| 7 | 137.7 | 2.3 | 1.6% | 4.7 | 3.4% | 4.6 | 3.4% |

| | EU/mL | Manual/ Operator #1 | | Automated/ Operator # 2 | | Total Imprecision |
|---|-------|------------------------|------|----------------------------|------|----------------------|
| | | SD | CV | SD | CV | CV |
| 1 | 9.9 | 0.9 | 8.4% | 0.9 | 9.1% | 9.0% |
| 2 | 16.2 | 0.9 | 5.5% | 1.1 | 6.8% | 6.4% |
| 3 | 20.6 | 1.6 | 7.7% | 1.2 | 5.9% | 6.7% |
| 4 | 22.3 | 1.4 | 6.1% | 1.5 | 6.9% | 6.7% |
| 5 | 48.9 | 2.7 | 5.4% | 3.3 | 6.7% | 6.3% |
| 6 | 96.1 | 7.2 | 8.0% | 6.3 | 6.3% | 8.1% |
| 7 | 137.7 | 5.0 | 3.7% | 4.5 | 3.2% | 3.4% |

Qualitative Reproducibility:

Studies were performed under the guidance of CLSI EP12-A2 “User Protocol for Evaluation of Qualitative Test Performance.” Ninety (90) replicates each of native sera in the negative range, ~20% below cut-off, at ~cut-off, ~20% above cut-off and in the moderate positive range of the assays were performed to qualitative reproducibility. The samples were identical to those in the precision studies above; however, results were calculated using single-point (qualitative) analysis as indicated in the product insert. The manufacturer’s pre-determined acceptance criteria was >95% qualitative agreement for specimens \pm 20% the cut-off and 100% for negative and moderate positive specimens. Results are summarized below.

SS-A Qualitative Precision:

| Sample | Mean EU | % Negative | % Positive |
|--------------|---------|------------|------------|
| Low Negative | 10.3 | 100% | 0% |
| Cut-off -20% | 17.2 | 97.8% | 2.2% |
| Cut-off | 20.1 | 51.1% | 48.9% |

| Sample | Mean EU | % Negative | % Positive |
|-------------------|----------------|-------------------|-------------------|
| Cut-off +20% | 22.7 | 2.2% | 97.8% |
| Moderate Positive | 70.7 | 0% | 100% |

SS-B Qualitative Precision:

| Sample | Mean EU | % Negative | % Positive |
|-------------------|----------------|-------------------|-------------------|
| Low Negative | 13.3 | 100% | 0 |
| Cut-off -20% | 15.9 | 100% | 0% |
| Cut-off | 19.9 | 53.3% | 46.7% |
| Cut-off +20% | 24.2 | 0% | 100% |
| Moderate Positive | 45.4 | 0% | 100% |

Sm Qualitative Precision:

| Sample | Mean EU | % Negative | % Positive |
|-------------------|----------------|-------------------|-------------------|
| Low Negative | 6.3 | 100% | 0% |
| Cut-off -20% | 16.4 | 100% | 0% |
| Cut-off -26.7 | 18.35 | 83.3% | 16.7% |
| Cut off +20% | 26.7 | 0% | 100% |
| Moderate Positive | 33.7 | 0% | 100% |

RNP Qualitative Precision:

| Sample | Mean EU | % Negative | % Positive |
|-------------------|----------------|-------------------|-------------------|
| Low Negative | 10.1 | 100% | 0% |
| Cut-off -20% | 16.3 | 100% | 0% |
| Cut-off | 20.9 | 32.2% | 67.8% |
| Cut-off +20% | 22.8 | 2.2% | 97.8 |
| Moderate Positive | 44.5 | 0% | 100% |

Lot-to-Lot Reproducibility:

Inter-lot reproducibility was tested by using samples spanning the assay range. Three lots of material were used in the study. Assays were performed by two operators using different equipment sets. The first operator performed the assay manually with

a multichannel pipettor, microplate washer and microplate reader. The second operator used an automatic system with a liquid handling system including a microplate reader. One assay lot was used for the study. Each sample was tested in three runs of three replicates over three days on each lot. The manufacturer's pre-determined acceptance criteria were met for all measures.

SS-A Lot-to-Lot Reproducibility:

| Sample | EU/mL | Lot 1 CV | Lot 2 CV | Lot 3 CV | Between Lots CV |
|--------|-------|----------|----------|----------|-----------------|
| 1 | 9.6 | 3.0% | 6.7% | 3.1% | 10.1% |
| 2 | 16.3 | 7.3% | 7.8% | 6.6% | 9.3% |
| 3 | 20.1 | 7.4% | 4.0% | 6.9% | 7.6% |
| 4 | 23.6 | 3.5% | 7.9% | 3.8% | 6.5% |
| 5 | 85.6 | 2.4% | 2.5% | 6.0% | 6.5% |
| 6 | 114.6 | 5.6% | 3.9% | 6.4% | 5.1% |
| 7 | 133.5 | 8.8% | 4.2% | 8.5% | 6.6% |

SS-B Lot-to-Lot Reproducibility:

| Sample | EU/mL | Lot 1 CV | Lot 2 CV | Lot 3 CV | Between Lots CV |
|--------|-------|----------|----------|----------|-----------------|
| 1 | 10.8 | 6.5% | 15.8% | 10.9% | 13.4% |
| 2 | 15.3 | 3.6% | 10.5% | 12.2% | 8.4% |
| 3 | 20.0 | 12.7% | 4.6% | 6.8% | 8.7% |
| 4 | 24.7 | 9.6% | 4.8% | 9.3% | 8.0% |
| 5 | 50.5 | 14.8% | 4.6% | 9.7% | 10.5% |
| 6 | 71.0 | 1.3% | 6.7% | 5.1% | 5.4% |
| 7 | 147.6 | 3.6% | 6.4% | 6.1% | 5.3% |

Sm Lot-to-Lot Reproducibility:

| Sample | EU/mL | Lot 1 CV | Lot 2 CV | Lot 3 CV | Between Lots CV |
|--------|-------|----------|----------|----------|-----------------|
| 1 | 7.7 | 7.6% | 14.5% | 3.3% | 14.3% |
| 2 | 15.3 | 7.5% | 8.6% | 5.2% | 11.5% |
| 3 | 18.5 | 9.8% | 2.4% | 10.5% | 7.9% |
| 4 | 26.4 | 4.8% | 5.1% | 5.0% | 4.9% |
| 5 | 42 | 10.1% | 6.0% | 3.3% | 6.5% |
| 6 | 78.7 | 5.2% | 2.9% | 2.9% | 7.2% |
| 7 | 143.4 | 8.0% | 3.3% | 4.1% | 7.4% |

RNP Lot-to-Lot Reproducibility:

| Sample | EU/mL | Lot 1 CV | Lot 2 CV | Lot 3 CV | Between Lots CV |
|--------|-------|----------|----------|----------|-----------------|
| 1 | 9.2 | 6.4% | 15.0% | 11.7% | 12.7% |
| 2 | 16.0 | 2.5% | 3.9% | 8.2% | 6.8% |
| 3 | 20.0 | 3.6% | 4.0% | 4.0% | 3.9% |
| 4 | 22.2 | 4.3% | 4.7% | 6.1% | 5.0% |
| 5 | 46.0 | 6.3% | 2.1% | 4.4% | 4.7% |
| 6 | 98.7 | 7.5% | 3.3% | 4.3% | 6.3% |
| 7 | 135.7 | 2.2% | 1.6% | 2.5% | 5.8% |

b. Linearity/assay reportable range:

Positive samples with values throughout the calibrator ranges were selected and assayed in duplicate, at equidistant dilutions to determine linear range of the assays. The acceptance limit for linearity is $r^2 > 0.95$. The sponsor's pre-determined acceptance limit for recovery was 80% - 120%.

SS-A:

| Sample | Dilution Range (EU/mL) | Slope (95% CI) | Y-Intercept (95% CI) | R ² | % Recovery |
|--------|------------------------|---------------------|----------------------|----------------|--------------|
| 1 | 2.6 to 35.8 | 0.96 (0.89 to 1.03) | 0.76 (-0.7 to 2.2) | 0.995 | 88% to 104% |
| 2 | 3.0 to 143.4 | 0.97 (0.84 to 1.09) | 3.31 (-7.9 to 14.5) | 0.988 | 86% to 108% |
| 3 | 18.2 to 156.0 | 0.99 (0.92 to 1.07) | -1.32 (-8.5 to 5.8) | 0.995 | 94% to 110% |
| 4 | 30.3 to 147.5 | 0.78 (0.60 to 1.95) | 8.4 (-8.8 to 25.6) | 0.995 | 100% to 121% |
| 5 | 5.2 to 21.0 | 1.06 (0.99 to 1.13) | -1.11 (-2.1 to -0.1) | 0.997 | 98% to 115% |

SS-B:

| Sample | Dilution Range (EU/mL) | Slope (95% CI) | Y-Intercept (95% CI) | R ² | % Recovery |
|--------|------------------------|---------------------|-----------------------|----------------|--------------|
| 1 | 2.8 to 22.2 | 0.98 (0.89 to 1.07) | 0.26 (-1.0 to 1.6) | 0.991 | 92% to 106% |
| 2 | 8.7 to 81.8 | 0.88 (0.76 to 1.01) | 2.61 (-4.2 to 9.4) | 0.981 | 84% to 116% |
| 3 | 8.7 to 159.3 | 0.90 (0.79 to 1.01) | 3.92 (-6.6 to 14.5) | 0.989 | 94% to 117% |
| 4 | 6.4 to 147.4 | 0.92 (0.79 to 1.05) | -1.29 (-13.9 to 11.4) | 0.990 | 100% to 119% |
| 5 | 3.5 to 13.7 | 1.13 (0.95 to 1.32) | -1.27 (-3.0 to 0.4) | 0.979 | 92% to 122% |

Sm:

| Sample | Dilution Range (EU/mL) | Slope (95% CI) | Y-Intercept (95% CI) | R ² | % Recovery |
|--------|------------------------|---------------------|----------------------|----------------|--------------|
| 1 | 7.3 to 28.2 | 1.13 (0.89 to 1.37) | -2.2 (-6.7 to 2.2) | 0.967 | 89% to 121% |
| 2 | 4.2 to 75.7 | 1.07 (0.93 to 1.22) | -1.5 (-7.9 to 4.9) | 0.982 | 85% to 118% |
| 3 | 4.5 to 129.8 | 0.89 (0.71 to 1.1) | 1.9 (-12.1 to 15.8) | 0.988 | 100% to 116% |

RNP:

| Sample | Dilution Range (EU/mL) | Slope (95% CI) | Y-Intercept (95% CI) | R ² | % Recovery |
|--------|------------------------|---------------------|----------------------|----------------|-------------|
| 1 | 8.0 to 40.5 | 0.96 (0.87 to 1.05) | 1.73 (-0.5 to 4.0) | 0.994 | 84% to 104% |
| 2 | 5.1 to 158.4 | 0.91 (0.83 to 0.99) | 1.14 (-5.0 to 7.3) | 0.998 | 98% to 113% |
| 3 | 3.6 to 146.4 | 0.99 (0.94 to 1.04) | -1.45 (-5.8 to 2.9) | 0.998 | 95% to 112% |

The data indicate that for the claimed analytical measuring ranges of 2.0 EU/mL – 160 EU/mL for SS-A, 1.6 EU/mL – 160 EU/mL for SS-B, 4.2 EU/mL – 160 EU/mL for Sm, and 3.0 EU/mL – 160 EU/mL for RNP, all specimens met the manufacturer’s pre-determined linearity and dilutional recovery acceptance criteria (<20% deviation for specimens within the AMR, and <25% deviation for specimens outside AMR).

High dose hook effect: High concentrations specimens were serially diluted to assess the presence of artifactual decrease in assay signal associated with antigen excess (hook effect). A hook effect was not seen.

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

i) Traceability:

There are currently no recognized international standards for the measurement of anti-SS-A, SS-B, Sm or RNP antibodies. Calibrator and Control values are directly traceable to in-house standards.

ii) Value Assignment:

Calibrators and Positive Controls are dilutions of pooled SS-A (Ro) (52 kD and 60 kD), SS-B (La), Sm, or RNP antibody positive sera. IMMCO formulates new calibrator and control lots from an array of antibody positive sera obtained from various commercial plasma centers. The calibrators and controls are taken from different pooled sera. All source sera has been tested and found negative for infectious disease as stated in the product insert. Manufactured calibrator sets are stored in aliquots frozen at -70°C. As new lots of calibrators are developed, comparison studies are performed to calibrate values against original calibrators.

iii) Stability:

Shelf life stability:

Accelerated and open kit studies for each device were performed on three lots of components/reagents. Accelerated studies were conducted with materials incubated at 37°C. In these conditions, one day is considered equivalent to one month stored at 2°-8°C. Materials are removed from the incubator for testing at three-day intervals for a minimum of 21 days.

Real time stability of three kit lots was tested using 5 specimens with reactivities across the analytical measuring range. The acceptance criteria are as follows:

- Positive and negative controls must be in the range stated within product labeling
- Maximum deviation of positive and negative sample results from timepoint 0 is 25%
- R^2 value of the calibrators is greater than 0.95.
- Average % deviation of calibrators A-D from timepoint 0 is less than 20%
- Maximum deviation of calibrators A-D from timepoint 0 is 25%

Conclusion: Data from accelerated and real time stability studies support an 18 month shelf life stability claim.

Open Kit Stability:

For open kit stability studies, materials are opened and stored as required for bench-top usage, then assayed at 15, 45 and 90 day intervals. Acceptance criteria are as listed above for accelerated and real time stability studies. Open vial stability studies demonstrate opened reagents are stable at 45 days, but the sponsor chose a more restrictive one month open kit stability claim.

d. Detection limit:

Limit of Blank:

The Limit of Blank (LoB) and the Limit of Detection (LoD) were determined by following CLSI EP17-A. Sixty samples of diluent were run as blank samples and six different normal human sera were each assayed 10 times. Summary data are presented in the table below.

| Assay | LoB | LoD |
|--------------|-----|-----|
| SS-A (Ro) Ab | 1.5 | 2.0 |
| SS-B (La) Ab | 2.0 | 1.6 |
| Sm (Ab) | 2.0 | 4.2 |
| RNP Ab | 2.5 | 3.0 |

e. Analytical specificity:

Comparison to Reference Sera:

Reference sera were obtained from the Centers for Disease Control and Prevention (CDC), Atlanta, GA. These sera have known reactivity for a number of autoantibodies that include SS-B/La, SS-A/Ro, Sm and RNP. Both semi-quantitative and qualitative analyses were performed. Qualitative values were concordant with the semi-quantitative determinations and are provided in the following table:

| CDC Panel # and Expected Pattern | SS-A Qual Result | Expected Result | SS-B Qual Result | Expected Result | Sm Qual Result | Expected Result | RNP Qual Result | Expected Result |
|----------------------------------|------------------|------------------|------------------|------------------|----------------|------------------|-----------------|------------------|
| 1 ANA homogeneous | Neg | Neg | Neg | Neg | Pos | Neg ¹ | Ind | Neg ¹ |
| 2 Speckled - SS-B /La | Pos | Pos | Pos | Pos ² | Neg | Neg | Neg | Neg |
| 3 Speckled - SS-B /La | Pos | Pos | Pos | Pos ² | Neg | Neg | Neg | Neg |
| 4 RNP | Neg | Neg | Neg | Neg | Neg | Neg | Pos | Pos |
| 5 Sm | Neg | Neg | Neg | Neg | Pos | Pos | Pos | Pos |
| 6 Nucleolar - U3 RNP | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg |
| 7 SS-A2/ Ro | Pos | Pos | Neg | Neg | Neg | Neg | Neg | Neg |
| 8 Centromere | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg |
| 9 Scl-70 | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg |
| 10 Jo-1 | Pos | Neg ³ | Neg | Neg | Neg | Neg | Neg | Neg |
| 11 PM-Scl | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg |
| 12 Ribosomal P | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg |

¹ Insert indicate antibodies not included, however, positive reaction with confirmation on alternate methods

² Not reported in insert and not excluded, but strong positive and often occurring with Ro

³ Not reported on insert and not excluded, but strong positive with confirmation on alternate methods

Interference:

The studies were performed according to CLSI EP07-A2, *Interference Testing in Clinical Chemistry, Approved Guideline- Second Edition*. Interference was studied by mixing sera with known SS-A, SS-B, RNP and Sm antibody levels with potentially interfering serum samples and studying deviation from expected results. Known levels of antibodies included negative, around the cut-off and low positive (i.e. 2-5 times the cut-off). No significant interference (defined as >25% deviation from expected) was demonstrated in the SSA, SS-B Ab, Sm Ab or RNP-Ab assays for the following substances at the levels indicated: Hemoglobin (2 g/L), Bilirubin (342 µmol/L), Rheumatoid Factor (100 EU/mL), Triglycerides (37 mmol/L) and Cholesterol (130 mmol/L).

2. Comparison studies:

a. *Method comparison with predicate device:*

SS-A (Ro) Antibody ELISA:

A method comparison study was performed using 229 disease associated samples [143 Systemic Lupus Erythematosus (SLE) and 86 Sjögren’s Syndrome (SS)] and 394 disease controls. Sample values were between the LoD and the highest level calibrator. Performance relative to the predicate was determined by calculating agreement where borderline values were considered positive or negative.

| Borderline values considered positive | | Quanta Lite SS-A (Ro) Antibody ELISA | | |
|---------------------------------------|----------|--------------------------------------|----------|-------|
| | | Positive | Negative | Total |
| IMMCO SS-A (Ro) Ab ELISA | Positive | 138 | 24 | 162 |
| | Negative | 9 | 452 | 461 |
| | Total | 147 | 476 | 623 |

Positive Percent Agreement: 93.9% (95% CI 88.4 – 97.0%)
 Negative Percent Agreement: 95.0% (95% CI 92.5 – 96.7%)
 Total Agreement: 94.7% (95% CI 92.6 – 96.3%)

| Borderline values considered negative | | Quanta Lite SS-A (Ro) Antibody ELISA | | |
|---------------------------------------|----------|--------------------------------------|----------|-------|
| | | Positive | Negative | Total |
| IMMCO SS-A (Ro) Ab ELISA | Positive | 133 | 16 | 149 |
| | Negative | 14 | 460 | 474 |
| | Total | 147 | 476 | 623 |

Positive Percent Agreement: 90.5% (95% CI 84.2 – 94.5%)
 Negative Percent Agreement: 96.6% (95% CI 94.5 – 98.0%)
 Total Agreement: 95.2% (95% CI 93.1 – 96.7%)

SS-B (La) Antibody ELISA:

A method comparison study was performed using 219 disease associated samples (151 SLE and 68 SS) and 354 disease controls. Sample values were between the LoD and the highest level calibrator. Performance relative to the predicate was determined by calculating agreement where borderline values were considered positive or negative.

| Borderline values considered positive | | Quanta Lite SS-B (La) Antibody ELISA | | |
|---------------------------------------|----------|--------------------------------------|----------|-------|
| | | Positive | Negative | Total |
| IMMCO SS-B (La) Ab ELISA | Positive | 62 | 8 | 70 |
| | Negative | 5 | 498 | 503 |
| | Total | 67 | 506 | 573 |

Positive Percent Agreement: 92.5% (95% CI 82.7 – 97.2%)
 Negative Percent Agreement: 98.4% (95% CI 96.8 – 99.3%)
 Total Agreement: 97.7% (95% CI 96.1 – 98.7%)

| Borderline values considered negative | | Quanta Lite SS-B (La) Antibody ELISA | | |
|---------------------------------------|----------|--------------------------------------|----------|-------|
| | | Positive | Negative | Total |
| IMMCO SS-B (La) Ab ELISA | Positive | 59 | 7 | 66 |
| | Negative | 8 | 499 | 507 |
| | Total | 67 | 506 | 573 |

Positive Percent Agreement: 88.1% (95% CI 77.3 – 94.3%)
 Negative Percent Agreement: 98.6% (95% CI 97.0 – 99.4%)
 Total Agreement: 97.4% (95% CI 95.6 – 98.5%)

Sm Antibody ELISA:

A method comparison study was performed using 167 disease associated samples (SLE) and 215 disease controls. Sample values were between the LoD and the highest level calibrator. Performance relative to the predicate was determined by calculating agreement where borderline values were considered positive or negative.

| Borderline values considered positive | | Quanta Lite Sm Antibody ELISA | | |
|---------------------------------------|----------|-------------------------------|----------|-------|
| | | Positive | Negative | Total |
| IMMCO Sm Ab ELISA | Positive | 35 | 9 | 44 |
| | Negative | 4 | 334 | 338 |
| | Total | 39 | 343 | 382 |

Positive Percent Agreement: 89.7% (95% CI 74.8 – 96.7%)
 Negative Percent Agreement: 97.4% (95% CI 94.9 – 98.7%)
 Total Agreement: 96.6% (95% CI 94.1 – 98.1%)

| Borderline values considered negative | | Quanta Lite Sm Antibody ELISA | | |
|---------------------------------------|----------|-------------------------------|----------|-------|
| | | Positive | Negative | Total |
| IMMCO Sm Ab ELISA | Positive | 32 | 4 | 36 |
| | Negative | 7 | 339 | 346 |
| | Total | 39 | 343 | 382 |

Positive Percent Agreement: 82.1% (95% CI 65.9 – 91.9%)
 Negative Percent Agreement: 98.8% (95% CI 96.8 – 99.6%)
 Total Agreement: 97.1% (95% CI 94.8 – 98.5%)

RNP Antibody ELISA:

A method comparison study was performed using 153 disease associated samples (28 MCTD and 125 SLE) and 244 disease controls. Sample values were between the LOD and the highest level calibrator. Performance relative to the predicate was determined by calculating agreement where borderline values were considered positive or negative.

| Borderline values considered positive | | Quanta Lite RNP Antibody ELISA | | |
|---------------------------------------|----------|--------------------------------|----------|-------|
| | | Positive | Negative | Total |
| IMMCO RNP Ab ELISA | Positive | 58 | 8 | 66 |
| | Negative | 3 | 334 | 337 |
| | Total | 61 | 342 | 403 |

Positive Percent Agreement: 95.1% (95% CI 85.4 – 98.7%)
 Negative Percent Agreement: 97.7% (95% CI 95.3 – 98.9%)
 Total Agreement: 97.3% (95% CI 95.0 – 98.6%)

| Borderline values considered negative | | Quanta Lite RNP Antibody ELISA | | |
|---------------------------------------|----------|--------------------------------|----------|-------|
| | | Positive | Negative | Total |
| IMMCO RNP Ab ELISA | Positive | 54 | 6 | 60 |
| | Negative | 7 | 336 | 343 |
| | Total | 61 | 342 | 403 |

Positive Percent Agreement: 88.5% (95% CI 77.2 – 94.9%)
 Negative Percent Agreement: 98.2% (95% CI 96.0 – 99.3%)
 Total Agreement: 96.8% (95% CI 94.4 – 98.2%)

b. *Matrix comparison:*

Not applicable since human serum is the only claimed specimen matrix.

3. Clinical studies:

a. *Clinical Sensitivity and Specificity:*

Serum samples from cohorts of patients with the target condition(s) or other autoimmune and infectious conditions expected to be found in the intended use population were tested and each IMMCO assay performance was assessed against the clinical diagnosis. Borderline results were considered positive and negative for these analyses.

SS-A (Ro) Ab ELISA:

| Borderline values considered positive | | Diagnosis - SLE | | |
|---------------------------------------|----------|-----------------|-------------------------|-------|
| | | SLE | Controls (excluding SS) | Total |
| IMMCO SS-A(Ro) Ab ELISA | Positive | 69 | 34 | 103 |
| | Negative | 106 | 367 | 473 |
| | Total | 175 | 401 | 576 |

Sensitivity = 39.4% (95% C.I. = 32.2 – 47.1%)

Specificity = 91.5% (95% C.I. = 88.2 – 94.0%)

| Borderline values considered negative | | Diagnosis - SLE | | |
|---------------------------------------|----------|-----------------|-------------------------|-------|
| | | SLE | Controls (excluding SS) | Total |
| IMMCO SS-A(Ro) Ab ELISA | Positive | 67 | 27 | 94 |
| | Negative | 108 | 374 | 482 |
| | Total | 175 | 401 | 576 |

Sensitivity = 38.3% (95% C.I.= 31.1 – 46.0%)

Specificity = 93.3% (95% C.I. = 90.2 – 95.4%)

| Borderline values considered positive | | Diagnosis - SS | | |
|---------------------------------------|----------|----------------|--------------------------|-------|
| | | SS | Controls (excluding SLE) | Total |
| IMMCO SS-A(Ro) Ab ELISA | Positive | 81 | 34 | 115 |
| | Negative | 23 | 367 | 390 |
| | Total | 104 | 401 | 505 |

Sensitivity = 77.9% (95% C.I. = 70.6 – 86.3%)

Specificity = 91.5% (95% C.I. = 88.2 – 94.0%)

| Borderline values considered negative | | Diagnosis - SS | | |
|---------------------------------------|----------|----------------|--------------------------|-------|
| | | SS | Controls (excluding SLE) | Total |
| IMMCO SS-A(Ro) Ab ELISA | Positive | 75 | 27 | 102 |
| | Negative | 29 | 374 | 403 |
| | Total | 104 | 401 | 505 |

Sensitivity = 72.1% (95% C.I. = 62.3 – 80.2%)

Specificity = 93.3% (95% C.I. = 90.2 – 95.4%)

The distribution of the SS-A positivity rate in the cohort is in the Table below:

| SS-A Cohort | n | # Pos | % Pos |
|---------------------------------|-----|-------|-------|
| SLE | 175 | 69 | 39.4% |
| Sjögren's Syndrome | 104 | 81 | 77.9% |
| Systemic Sclerosis | 154 | 30 | 19.5% |
| Mixed Connective Tissue Disease | 17 | 0 | 0.0% |
| Rheumatoid Arthritis | 33 | 1 | 3.0% |
| Celiac Disease | 26 | 0 | 0.0% |
| Dermatitis Herpetiformis | 10 | 0 | 0.0% |
| Wegener's Granulomatosis | 8 | 0 | 0.0% |
| Churg-Strauss Syndrome | 10 | 0 | 0.0% |
| Hashimoto's Thyroiditis | 8 | 0 | 0.0% |
| Grave's Disease | 10 | 0 | 0.0% |
| Antiphospholipid Syndrome | 15 | 0 | 0.0% |
| Syphilis | 30 | 1 | 3.3% |
| Hepatitis C | 10 | 1 | 10.0% |
| Toxoplasmosis | 10 | 0 | 0.0% |
| Cytomegalovirus | 10 | 0 | 0.0% |
| Rubella | 10 | 0 | 0.0% |

| SS-A Cohort | n | # Pos | % Pos |
|------------------------------|----------|--------------|--------------|
| Herpes Simplex | 20 | 0 | 0.0% |
| Osteoarthritis | 10 | 0 | 0.0% |
| Thrombocytopenia | 10 | 1 | 10.0% |
| Total Non SLE or SS Controls | 401 | 34 | 8.5% |

SS-B (La) Ab ELISA:

| Borderline values considered positive | | Diagnosis - SLE | | |
|---------------------------------------|----------|-----------------|-------------------------|-------|
| | | SLE | Controls (excluding SS) | Total |
| IMMCO SS-B (La) Ab ELISA | Positive | 22 | 8 | 30 |
| | Negative | 129 | 346 | 475 |
| | Total | 151 | 354 | 505 |

Sensitivity = 14.6% (95% C.I. = 9.6 – 21.4%)

Specificity = 97.7% (95% C.I. = 95.4 – 98.9%)

| Borderline values considered negative | | Diagnosis - SLE | | |
|---------------------------------------|----------|-----------------|-------------------------|-------|
| | | SLE | Controls (excluding SS) | Total |
| IMMCO SS-B(La) Ab ELISA | Positive | 22 | 5 | 27 |
| | Negative | 129 | 349 | 478 |
| | Total | 151 | 354 | 505 |

Sensitivity = 14.6% (95% C.I. = 9.6 – 21.4%)

Specificity = 98.6% (95% C.I. = 95.5 – 99.5%)

| Borderline values considered positive | | Diagnosis - SS | | |
|---------------------------------------|----------|----------------|--------------------------|-------|
| | | SS | Controls (excluding SLE) | Total |
| IMMCO SS-B(La) Ab ELISA | Positive | 41 | 8 | 49 |
| | Negative | 27 | 346 | 373 |
| | Total | 68 | 354 | 442 |

Sensitivity = 60.3% (95% C.I. = 41.2 – 71.7%)

Specificity = 97.7% (95% C.I. = 95.8 – 99.1%)

| Borderline values considered negative | | Diagnosis - SS | | |
|---------------------------------------|----------|----------------|--------------------------|-------|
| | | SS | Controls (excluding SLE) | Total |
| IMMCO SS-B(La) Ab ELISA | Positive | 39 | 5 | 44 |
| | Negative | 29 | 349 | 378 |
| | Total | 68 | 354 | 422 |

Sensitivity = 57.4% (95% C.I. = 44.8 – 69.1%)

Specificity = 98.6% (95% C.I. = 96.5 – 99.5%)

The distribution of the SS-B positivity rate in the cohort is in the table below:

| SS-B Cohort | n | # Pos | % Pos |
|---------------------------------|-----|-------|-------|
| SLE | 151 | 22 | 14.6% |
| Sjögren's Syndrome | 68 | 41 | 60.3% |
| Systemic Sclerosis | 70 | 3 | 4.3% |
| Myositis | 48 | 1 | 2.1% |
| Mixed Connective Tissue Disease | 10 | 0 | 0.0% |
| Rheumatoid Arthritis | 33 | 1 | 3.0% |
| Celiac Disease | 25 | 0 | 0.0% |
| Dermatitis Herpetiformis | 10 | 0 | 0.0% |
| Wegener's Granulomatosis | 8 | 0 | 0.0% |
| Churg-Strauss Syndrome | 10 | 0 | 0.0% |
| Hashimoto's Thyroiditis | 8 | 0 | 0.0% |
| Grave's Disease | 10 | 0 | 0.0% |
| Antiphospholipid Syndrome | 12 | 0 | 0.0% |
| Syphilis | 30 | 1 | 3.3% |
| Hepatitis C | 10 | 0 | 0.0% |
| Toxoplasmosis | 10 | 0 | 0.0% |
| Cytomegalovirus | 10 | 0 | 0.0% |
| Rubella | 10 | 0 | 0.0% |
| Herpes Simplex | 20 | 1 | 5.0% |
| Osteoarthritis | 10 | 0 | 0.0% |
| Thrombocytopenia | 10 | 1 | 10.0% |
| Total Non SLE Controls | 354 | 8 | 2.3% |

Sm Ab ELISA:

| Borderline values considered positive | | Diagnosis - SLE | | |
|---------------------------------------|----------|-----------------|----------|-------|
| | | SLE | Controls | Total |
| IMMCO Sm Ab ELISA | Positive | 44 | 2 | 46 |
| | Negative | 154 | 399 | 553 |
| | Total | 198 | 401 | 599 |

Sensitivity = 22.2% (95% C.I. = 16.8 – 28.8%)

Specificity = 99.5% (95% C.I. = 98.0 – 99.9%)

| Borderline values considered negative | | Diagnosis - SLE | | |
|---------------------------------------|----------|-----------------|----------|-------|
| | | SLE | Controls | Total |
| IMMCO Sm Ab ELISA | Positive | 39 | 0 | 39 |
| | Negative | 159 | 401 | 560 |
| | Total | 198 | 401 | 599 |

Sensitivity = 19.7% (95% C.I. = 14.5 – 26.1%)

Specificity = 100.0% (95% C.I. = 98.8 – 100%)

The distribution of Sm positivity rate in the tested cohort is in the table below:

| Sm Cohort | n | # Pos | % Pos |
|---------------------------------|-----|-------|-------|
| SLE | 198 | 44 | 22.2% |
| Sjögren's Syndrome | 21 | 0 | 0.0% |
| Systemic Sclerosis | 44 | 0 | 0.0% |
| Polymyositis | 51 | 0 | 0.0% |
| Dermatomyositis | 20 | 0 | 0.0% |
| Mixed Connective Tissue Disease | 33 | 0 | 0.0% |
| Rheumatoid Arthritis | 33 | 0 | 0.0% |
| Celiac Disease | 28 | 0 | 0.0% |
| Dermatitis Herpetiformis | 10 | 0 | 0.0% |
| Wegener's Granulomatosis | 8 | 0 | 0.0% |
| Autoimmune Thyroiditis | 18 | 1 | 5.6% |
| Syphilis | 30 | 0 | 0.0% |
| Hepatitis C | 10 | 0 | 10.0% |

| Sm Cohort | n | # Pos | % Pos |
|---------------------------|----------|--------------|--------------|
| Toxoplasmosis | 10 | 0 | 0.0% |
| Cytomegalovirus | 10 | 0 | 0.0% |
| Rubella | 10 | 0 | 0.0% |
| Herpes Simplex | 20 | 0 | 0.0% |
| Antiphospholipid syndrome | 15 | 1 | 6.7% |
| Osteoarthritis | 10 | 0 | 0.0% |
| Thrombocytopenia | 10 | 0 | 0.0% |
| Ulcerative colitis | 10 | 0 | 0.0% |
| Total Non SLE Controls | 401 | 2 | 0.5% |

RNP Ab ELISA:

| Borderline values considered positive | | Diagnosis - SLE | | |
|---------------------------------------|----------|-----------------|---------------------------|-------|
| | | SLE | Controls (excluding MCTD) | Total |
| IMMCO RNP Ab ELISA | Positive | 41 | 7 | 48 |
| | Negative | 110 | 328 | 438 |
| | Total | 151 | 335 | 486 |

Sensitivity = 27.2% (95% C.I. = 20.4 – 35.1%)

Specificity = 97.9% (95% C.I. = 95.6 – 99.1%)

| Borderline values considered negative | | Diagnosis - SLE | | |
|---------------------------------------|----------|-----------------|---------------------------|-------|
| | | SLE | Controls (excluding MCTD) | Total |
| IMMCO RNP Ab ELISA | Positive | 38 | 4 | 42 |
| | Negative | 113 | 331 | 444 |
| | Total | 151 | 335 | 486 |

Sensitivity = 25.2% (95% C.I. = 18.6 – 33.0%)

Specificity = 98.8% (95% C.I. = 96.8 – 99.6%)

| Borderline values considered positive | | Diagnosis - MCTD | | |
|---------------------------------------|----------|------------------|--------------------------|-------|
| | | MCTD | Controls (excluding SLE) | Total |
| IMMCO RNP Ab ELISA | Positive | 32 | 7 | 39 |
| | Negative | 3 | 328 | 331 |
| | Total | 35 | 335 | 370 |

Sensitivity = 91.4% (95% C.I. = 75.8 – 97.8%)

Specificity = 97.9% (95% C.I. = 95.6 – 99.1%)

| Borderline values considered negative | | Diagnosis - MCTD | | |
|---------------------------------------|----------|------------------|--------------------------|-------|
| | | MCTD | Controls (excluding SLE) | Total |
| IMMCO RNP Ab ELISA | Positive | 32 | 4 | 36 |
| | Negative | 3 | 331 | 334 |
| | Total | 35 | 335 | 370 |

Sensitivity = 91.4% (95% C.I. = 75.8 – 97.8%)

Specificity = 98.8% (95% C.I. = 96.8 – 99.6%)

The distribution of the RNP positivity rate in the tested cohort is in the Table below:

| RNP Cohort | n | # Pos | % Pos |
|---------------------------------|-----|-------|-------|
| SLE | 151 | 41 | 27.2% |
| Mixed Connective Tissue Disease | 35 | 32 | 91.4% |
| Sjögren's Syndrome | 18 | 0 | 0.0% |
| Systemic Sclerosis | 21 | 0 | 0.0% |
| Polymyositis | 40 | 0 | 0.0% |
| Dermatomyositis | 24 | 0 | 0.0% |
| Rheumatoid Arthritis | 33 | 1 | 3.0% |
| Celiac Disease | 28 | 0 | 0.0% |
| Dermatitis Herpetiformis | 10 | 0 | 0.0% |
| Wegener's Granulomatosis | 8 | 0 | 0.0% |
| Autoimmune Thyroiditis | 18 | 0 | 0.0% |
| Syphilis | 30 | 0 | 0.0% |
| Hepatitis C | 10 | 0 | 10.0% |
| Toxoplasmosis | 10 | 0 | 0.0% |
| Cytomegalovirus | 10 | 0 | 0.0% |

| | | | |
|---------------------------|-----|---|-------|
| Rubella | 10 | 0 | 0.0% |
| Herpes Simplex | 20 | 0 | 0.0% |
| Antiphospholipid syndrome | 15 | 2 | 13.3% |
| Osteoarthritis | 10 | 0 | 0.0% |
| Thrombocytopenia | 10 | 0 | 0.0% |
| Ulcerative colitis | 10 | 0 | 0.0% |
| Total Non SLE Controls | 335 | 3 | 0.9% |

Clinical cut-off:

Not applicable

5. Expected values/Reference range:

Test results in a normal population are expected to be negative. A study of 142 normal, apparently disease-free samples tested with the anti-SS-A assay yielded one borderline result (0.7%) and three positive results (2.1%). A study of 133 normal, apparently disease-free samples tested with the anti-SS-A assay yielded three borderline results (2.3%). A study of 80 normal, apparently disease-free samples tested with the anti-Sm assay did not yield any borderline or positive results. A study of 80 normal, apparently disease-free samples tested with the anti-RNP assay yielded two indeterminate results (2.5%).

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.