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

A. 510(k) Number:
k033528

B. Analyte:
Anti-Jo-1 antibody

C. Type of Test:
Semi-quantitative ELISA

D. Applicant:
RhiGene, Inc.

E. Proprietary and Established Names:
MESACUP-2 Test Jo-1

F. Regulatory Information:

1. Regulation section:
   21 CFR §866.5100 Antinuclear Antibody Immunological Test System
2. Classification:
   Class II
3. Product Code:
   LLL
4. Panel:
   IM 82

G. Intended Use:

1. Intended use(s):
   The MESACUP-2 Test Jo-1 is a semi-quantitative enzyme-linked immunosorbent assay (ELISA) for the detection of anti-Jo-1 antibodies in human serum as an aid in the diagnosis of polymyositis and/or dermatomyositis, or other related connective tissue diseases.
2. Indication(s) for use:
   The MESACUP-2 Test Jo-1 is a semi-quantitative enzyme-linked immunosorbent assay (ELISA) for the detection of anti-Jo-1 antibodies in human serum as an aid in the diagnosis of polymyositis and/or dermatomyositis, or other related connective tissue diseases. The MESACUP-2 Test Jo-1 is intended to be used by clinical (hospital and reference) laboratories.
3. Special condition for use statement(s):
   For prescription use only.
4. Special instrument Requirements:
   None

H. Device Description:

The device is an enzyme-linked immunosorbent assay (ELISA) using microtiter plates as the solid phase. The plate wells are coated with recombinant Jo-1 antigen

which captures Jo-1 autoantibodies present in the patient sample. The conjugate is polyclonal goat anti-human IgG, IgM and IgA (heavy chain specific) horseradish peroxidase (HRP) which uses 3,3'5,5' tetramethylbenzidine dihydrochloride/hydrogen peroxide (TMB/H_2O_2) as substrate. The kit contains 2 levels of calibrators (0 U/mL and 100 U/mL) for interpretation of results. A positive and a negative control are included with the kit. The kit also contains sample diluent, wash buffer concentrate and stop solution.

I. Substantial Equivalence Information:

1. Predicate device name(s):
   Quanta Lite Jo-1 ELISA from INOVA Diagnostics
2. Predicate K number(s):
   K926562
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
Indications for Use	For detection of anti-mitochondrial antibodies as an aid in the diagnosis of polymyositis and/or dermatomyositis	Same
Assay principle	ELISA	Same
Sample matrix	Serum	Same
Substrate	TMB	Same
Differences		
Item	**Device**	**Predicate**
Cut-off	18 U/mL	20 Units
Detection range	0-300 U/mL	Not given
Assay time	150 minutes	90 minutes
Conjugate	HRP-goat anti-human IgG/IgM/IgA	HRP-goat anti-human IgG

J. Standard/Guidance Document Referenced (if applicable):
Not applicable

K. Test Principle:
Enzyme-linked immunosorbent assay (ELISA) technology is a well established methodology.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. *Precision/Reproducibility:*
      Three lots of the MESACUP-2 Test Mitochondria M2 were tested to determine the assay's intra-assay, inter-assay and inter-lot value precision.

Intra-assay

Intra-assay precision (%CV) was determined by running 3 serum samples (low, moderate and high positive) with 8 dilutions each on 3 different coated plates from 3 different plate lots. The 3 separate plates employed were randomly selected from each plate-coating run (kit-lot). The mean intra-assay precision for the 3 samples tested on 3 plates from each lot was 2.3% ranging from 1.3-3.2%.

Inter-assay, intra-lot

To determine the amount of variability between plates of the same lot, 3 samples in duplicate were tested on 6 separate assays employing 6 different plates randomly selected from the same plate lot. This study was performed on 3 separate plate lots. The mean %CV for inter-assay, intra-lot precision was 3.9% with a range of 1.0-4.3%.

Inter-assay, inter-lot

The precision between lots was determined by comparing the values recovered for 3 different samples on 3 different pilot lots. Each of the 3 samples was tested in duplicate and by 2 operators in each assay. The mean inter-assay, inter-lot %CV was 5.9%.

b. *Linearity/assay reportable range:*
The reportable range is 5-300 U/mL.

c. *Traceability (controls, calibrators, or method):*
An international reference material for anti-Jo-1 antibodies is not available. The assay is calibrated in relative arbitrary units.

d. *Detection limit:*
Not furnished but not relevant for this assay.

e. *Analytical specificity:*
Hemoglobin (up to 480 mg/dL), bilirubin C (up to 20.4 mg/dL), Bilirubin F (up to 18.7 mg/dL), chyle (up to 2780 units as Formazine) and Rheumatoid Factor (up to 520 IU/mL) do not interfere with the assay.

f. *Assay cut-off:*
A healthy population consisting of 265 unselected human serum samples was tested for anti-Jo-1 antibodies. All samples were tested in duplicate. Of 265 samples, 264 tested negative (99.6%). The same 265 healthy donor specimens were also tested on a double immunodiffusion (DID) method for anti-Jo-1 antibodies. All 265 specimens were negative by DID. In addition, a population of 730 collagen disease specimens were tested on both the ELISA and the DID. Forty (40) of these specimens were positive by both DID and the new assay. To determine the assay's cut-off value, the values of both populations were compared to the DID results for relative sensitivity and specificity. A cut-off value was established as 18

U/mL where the recovery of both specificity and sensitivity resulted in the best overall accuracy when compared to DID. There is no equivocal (gray) zone for this assay.

2. Comparison studies:
   a. *Method comparison with predicate device:*
      Comparison studies for 80 subjects (60 healthy blood donors and 20 patients with polymyositis or dermatomyositis) showed an overall agreement of 96%.
   b. *Matrix comparison:*
      Not applicable.
3. Clinical studies:
   a. *Clinical sensitivity:*
      Clinical sensitivity for the new assay was determined by testing a population of polymyositis and dermatomyositis patient serum specimens (n=26). Using a cut-off of 18 U/mL, 9 of the 26 samples (35%) were positive for anti-Jo-1 antibodies. This value is similar to the 25-30% positive rate in these populations found in the literature.
   b. *Clinical specificity:*
      A total of 575 samples were tested. Serum samples from 265 consecutive healthy blood donors were used as a normal population. Serum samples from various autoimmune disease groups were tested to further determine clinical specificity of the test. The groups included Rheumatoid Arthritis (n=47), Sjogren's Syndrome (n=47), Systemic Lupus Erythematosus (n=103), Systemic Sclerosis (n=23), Mixed Connective Tissue Disease (n=59) and CREST Syndrome (n=31). Five hundred seventy two samples were found negative in these populations demonstrating a specificity of 99.5% for the 18 U/mL cut-off value.
   c. *Other clinical supportive data (when a and b are not applicable):*

4. Clinical cut-off:
   See assay cut-off.
5. Expected values/Reference range:
   The expected value in the normal population is negative. According to published literature, the incidence in the PM/DM group is around 25-30%.

M. Conclusion:

The MESACUP-2 Test Jo-1 is substantially equivalent to other devices regulated under 21 CFR §866.5100, product code LLL, Class II.