510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k083188

B. Purpose for Submission:
Device modification [Replacement of SmD full-length peptide antigen with a 16 amino acid peptide fragment of the human SmD protein (k050625)]

C. Measurand:
dsDNA, U1RNP (RNP70, A, C), Sm, SS-A/Ro (52 kDa, 60 kDa), SS-B/La, Scl-70, CENP-B and Jo-1 antibodies

D. Type of Test:
Qualitative Enzyme Immunoassay (EIA)

E. Applicant:
Phadia US, Inc.

F. Proprietary and Established Names:
Varelisa® ReCombi ANA Screen

G. Regulatory Information:

1. Regulation section:
   21 CFR§ 866.5100, Antinuclear Antibody Immunological Test System
2. Classification:
   Class II
3. Product code:
   LJM, Antinuclear antibody (enzyme-labeled), Antigen and Controls
4. Panel:
   Immunology (82)

H. Intended Use:

1. Intended use(s):
   See below.
2. Indication(s) for use:
   The Varelisa ReCombi ANA Screen EIA kit is designed for the qualitative determination of eight antinuclear antibodies in human serum or plasma to aid in the diagnosis of systemic rheumatic diseases such as SLE (systemic lupus erythematosus), scleroderma (progressive systemic sclerosis), MCTD (mixed connective tissue disease), SS (Sjögren's syndrome) and polymyositis/ dermatomyositis. The Varelisa ReCombi ANA Screen detects antibodies against dsDNA, U1RNP (RNP70, A, C), Sm, SS-A/Ro (52 kDa, 60 kDa), SS-B/La, Scl-70, CENP-B and Jo-1 in a single microwell.
3. Special conditions for use statement(s):
   The device is for prescription use only.
4. Special instrument requirements:
   Microplate reader capable of measuring OD at 450 nm.

I. Device Description:
The assay contains the following: microplate strips coated with nuclear antigens: dsDNA, U1RNP (RNP70, A, C), Sm, SS-A/Ro (52 kDa, 60 kDa), SS-B/La, Scl-70,

CENP-B and Jo-1, calibrator, negative control, wash buffer concentrate, sample diluent, horseradish peroxidase conjugated anti-human IgG, 3,3',5,5 tetramethylbenzidine (TMB) substrate, and H_2SO4 stop solution.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Varelisa ReCombi ANA Screen
2. Predicate K number(s):
   k050625
3. Comparison with predicate:
   The new assay is similar to the predicate assay as follows: same indications for use, specimen type, assay principle, conjugate, and reagents. The new assay differs from the predicate assay by replacing the previous Sm antigen with a synthetic human Sm peptide and the elimination of a pre-washing step.

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

CLSI H18-A3: Procedures for the Handling and Processing of Blood Specimens; Approved Guideline—Third Edition

L. Test Principle:

The Varelisa ReCombi ANA Screen is an indirect noncompetitive enzyme immunoassay for the qualitative determination of dsDNA, U1RNP (RNP 70, A, C), Sm[D], SS-A/Ro (52 kDa, 60 kDa), SS-B/La, Scl-70, CENP-B and Jo-1 antibodies in serum and plasma. If antibodies specific for the nuclear antigens are present in a patient sample they bind to these nuclear antigens coated to the assay wells. In the second step an enzyme labeled secondary antibody (conjugate) binds to the antigen-antibody complex. The enzyme labeled antigen-antibody complex converts the substrate to form a colored solution. The results are read spectrophotometrically and are interpreted as positive, equivocal, or negative by comparison to a cut-off calibrator.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. *Precision/Reproducibility:*
      Three samples (high negative, equivocal and high positive) per parameter were analyzed in 5 runs, with 8 replicates per run. Each sample contained all eight antigens. The runs were carried out by one operator within one day.

Sample		**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean Ratio**	**Variance**	
								intra	inter
High Negative	Mean(Ratio)	**0.9**	**0.9**	**0.8**	**0.9**	**0.8**	**0.9**	**3.2**	**4.4**
	CV%	3.5	1.2	2.1	2.3	5.3			
Equivocal	Mean(Ratio)	**1.2**	**1.0**	**1.0**	**1.0**	**1.0**	**1.0**	**3.5**	**6.6**
	CV%	5.0	2.1	2.7	3.2	3.2			
High positive	Mean(Ratio)	**3.4**	**3.1**	**3.1**	**3.2**	**3.2**	**3.2**	**2.2**	**3.9**
	CV%	1.5	1.5	1.6	3.3	2.5			

   b. *Linearity/assay reportable range:*
      Not applicable.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
The consistency of calibration material is ensured by a testing procedure comparing the new calibrators to corresponding master calibration material demonstrating that the internal target values are met. No international standards have been established for these analytes. The performance of the bulk production of positive and negative control sera is adjusted until the release criteria are met. Values have to be found in accordance to an established acceptance range.

Based on accelerated stability testing, unopened kits were projected to be stable for 12 months when stored at 2 – 8°C. Sample stability claims are based on the recommendations in CLSI H18-A3.

d. *Detection limit:*
This study was intended to verify the previously established detection limit (k050625). Sample diluent was measured 90 times per run on two different lots. Analytical sensitivity (detection limit) was calculated as the mean of the optical densities (OD) of the sample diluent plus three times the standard deviations (SD) then converted to a ratio. The analytical sensitivity for the assay was 0.1.

e. *Analytical specificity:*
Ten CDC ANA human reference sera from the Centers for Disease Control and Prevention and 10 sera of the AMLI Consensus Reference Panel 2002, were diluted and tested twice by two different operators on two different lots of the new device and one lot of the predicate device. Both the new assay and the predicate assay gave a positive result (> 1.4 mean ratio) for CDC 1, 2, 3, 4, 5, 7, 8, 9, and 10 which all contain antigens recognized by the Recombi ANA Screen. All other samples in the CDC panel were negative (CDC 6, 11, and 12). Both the new assay and the predicate assay gave a positive result for AMLI panel members AMLI 2, 3, 4, 5, 6, 7, 8, and 9 which contain target antigens recognized by the test. AMLI 1 and 10 were negative as expected.

Endogenous interferences:
The following interfering substances (up to the listed concentration) did not adversely affect the test results (i.e. change the outcome): Bilirubin C (21.6 mg/dL), Bilirubin F (19.1 mg/dL), Chyle (1590 FTU), Hemoglobin (494 mg/dL), and Rheumatoid Factor (550 IU/mL).

f. *Assay cut-off:*
The equivocal range and cut-off of the assay were determined in k05625. The sponsor presented a study of 460 healthy Caucasian blood donors evenly distributed by age and sex that confirmed the established cut-offs.

2. <u>Comparison studies:</u>

a. *Method comparison with predicate device:*
The performance of the new device was compared to the performance of the old device using 253 samples from patients with known clinical background (50 infectious disease, 50 non-connective tissue disease (CTD), 103 CTD, and

50 Rheumatoid Arthritis) and 92 blood donor samples. Equivocal results were regarded as negative:

		Predicate ReCombi ANA Screen		
		Positive	Negative	Total
New ReCombi ANA Screen	Positive	102	5	107
	Negative	1	145	146
	Total	103	150	253

Positive Percent Agreement: 99.0% (102/103) 95% CI: 94.7 – 100%
Negative Percent Agreement: 96.7% (145/150) 95% CI: 92.4 – 98.9%
Total Percent Agreement: 97.6% (247/253) 95% CI: 94.9 – 99.1%

b. Matrix comparison:
26 negative samples from healthy donors and 13 samples spiked with different amounts of sera from ANA+ donors each available as serum, citrate plasma, EDTA plasma, and heparin plasma were run in duplicate. Performance was acceptable in citrate plasma and EDTA plasma however heparin plasma samples interfered with correct test results. The labeling contains a warning not to use heparin plasma.

3. Clinical studies:
   a. Clinical Sensitivity:
   Not applicable.
   b. Clinical specificity:
   Not applicable.
   c. Other clinical supportive data (when a. and b. are not applicable):
   Not applicable.
4. Clinical cut-off:
   See Assay Cut-off above.
5. Expected values/Reference range:
   See Assay Cut-off above.

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.