510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k050967

B. Purpose for Submission:
New Device

C. Measurand:
dsDNA, RNP, (RNP 70, A, C), Sm (B, B', D) SS-A/Ro (52 kDa, 60 kDa), SS-B/La, Scl-70, CENP-B, Histone, Ribosomal P protein and Jo-1

D. Type of Test:
Qualitative, ELISA

E. Applicant:
Sweden Diagnostics(Germany) GmbH

F. Proprietary and Established Names:
Varelisa®ReCombi CTD Screen EIA kit

G. Regulatory Information:

1. Regulation section:
   21CFR 866.5100, antinuclear antibodies, immunological test system
2. Classification:
   Class II
3. Product code:
   LJM, Antinuclear antibody (enzyme-labeled), antigen, control
4. Panel:
   Immunology (82)

H. Intended Use:

1. Intended use(s):
   The Varelisa ReCombi CTD Screen EIA kit is for qualitative determination of 10 antinuclear antibodies in human serum or plasma to aid in the diagnosis of systemic rheumatic diseases such as SLE (systemic lupus erythematosus), drug induced lupus, scleroderma (progressive systemic sclerosis), MCTD (mixed connective tissue disease), SS (Sjogren's syndrome) and Polymyositis/dermatomyositis. The Varelisa ReCombi CTD Screen detects antibodies against dsDNA, RNP, (RNP 70, A, C), Sm (B, B', D) SS-A/Ro (52 kDa, 60 kDa), SS-B/La, Scl-70, CENP-B, Histone, Ribosomal P protein and Jo-1 in a single microwell.

2. Indication(s) for use:
   Same as intended use

3. Special conditions for use statement(s):
   Prescription use only

4. Special instrument requirements:
   Microtiter plate photometer SLT Spectra II (type F039002).

I. Device Description:
The kit contains: (a) Microplate strips (12 strips x 8 wells) coated with nuclear antigens (dsDNA, RNP, Sm, SS-A/Ro, SS-B/La, Scl-70, CENP-B, Histone, PM-Scl,

Rib-P and Jo-1); (b) Calibrator-BSA buffer, sodium azide and human serum - ready to use; (c) Positive and negative control; (d) Wash buffer; (e) Sample diluent; (f) IgG horseradish peroxidase (HRP) conjugate - ready to use; (g) Substrate Tetramethyl-benzedine and (h) stop solution.

J. Substantial Equivalence Information:
1. Predicate device name(s):
   Varelisa ReCombi ANA screen
2. Predicate 510(k) number(s):
   k993108
1. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended use	Qualitative test for determination of ANA in serum and plasma to aid in the diagnosis of systemic rheumatic diseases.	Same
Antigens used	SS-A/Ro, SS-B, RNP, Scl-70, CENP-B, Jo-1, dsDNA	Same
Matrix	Serum and plasma	Same
Assay principle	Indirect non competitive Enzyme immunoassay	Same
Type	Qualitative	Same
Sample dilution	1:101	Same
Conjugate	Anti-human IgG (rabbit) horseradish peroxidase	Same
Reagents	Calibrator, control, sample diluent, stop solution	Same

Differences		
Item	Device	Predicate
Sm antigen	Synthetic human SmD peptide plus recombinant SmBB	Sm antigen from calf thymus
Additional antigen	Histone and Rib-P	
Calibrator, control	1.0 ml	1.5 ml
Wash buffer-vol used	75 ml	50ml
Sample diluent	100ml ready to use	20 ml 5x concentrate
Conjugate	20ml	15 ml
Substrate	20 ml	15 ml
Stop solution	20 ml	10 ml
Incubation time	30 minutes	10 min

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

L. Test Principle:
Varelisa CTD screen test is an indirect non-competitive EIA. The microtiter plate wells are coated with human recombinant and native purified nuclear antigens and dsDNA. Antibodies specific for the nuclear antigens present in the patient sample binds to these nuclear antigens. In the second step the conjugate binds to the complex and converts the added substrate to form the colored solution. The amount of color produced is proportional to the concentration of the respective antibodies in the patient sample. Test result is expressed as ratio (OD patient sample/OD of cut off).

M. Performance Characteristics (if/when applicable):
1. Analytical performance:
    a. *Precision/Reproducibility:*
    To determine reproducibility, variation was estimated within-run and between-runs using 4 samples (negative, low positive, moderate and high positive) with 8 replicates per run for 6 runs. Within one day one operator carried out the analyses. The intra-assay variability and inter-assay variability target values for positive samples (ratio 1.0-4.0) were <12% and <9% respectively. No target value was specified for the negative samples. For all samples tested, the specifications were met. Results are summarized below.

Sample		Run 1	Run2	Run3	Run4	Run5	Run6	Overall Mean Ratio	%CV Intra	%CV Inter
Negative	Mean ratio	0.9	0.9	0.9	0.8	0.8	0.9	0.9	8.1	0.5
	%CV	14.3	4.4	7.5	4.5	6.5	6.1			
Low positive	Mean ratio	1.2	1.2	1.1	1.1	1.1	1.2	1.2	3.4	2.4
	%CV	4.1	2.2	3.3	4.3	2.9	3.4			
Moderate positive	Mean ratio	1.9	2.0	1.9	1.9	1.9	2.1	1.9	4.7	3.1
	%CV	2.9	1.7	2.0	2.2	3.3	9.5			
High positive	Mean ratio	4.3	4.3	4.2	4.2	4.0	4.1	4.2	2.2	2.4
	%CV	2.8	2.5	2.4	1.8	1.5	2.0			

    b. Linearity/assay reportable range:
    Not applicable.
    c. Traceability, Stability, Expected values (controls, calibrators, or methods):
    No information provided.
    d. *Detection limit:*
    The purpose of this study was to determine the detection limit. The ready-to-use sample diluent was measured 80 times per run (2 runs per lot). Analytical sensitivity was calculated as the mean of the optical density (OD) of the sample diluent plus 3SD (expressed as ratios). The specification for detection limit is <0.3. For the first run, the (mean OD + 3SD) = [0.02 +3(0.009)] = 0.047 and ratio 0.1 and for the second run, the (mean OD + 3SD) = [0.017 + 3(0.010)] = 0.046 and ratio 0.1. The results met the specifications.
    e. *Analytical specificity:*
    Diluted samples were spiked and tested to determine interference from 4 different concentrations each of Bilirubin F (4.6-18.5mg/dL), Bilirubin C (5.4-21.6 mg/dL), Chyle (530-2120 units), Hemoglobin (116.8-467 mg/dL) and

rheumatoid factor (100-500 IU/mL). The target value for the deviation of the value of the spiked sample with the interfering substance should be ± 20% of the value of the sample spiked with a buffer blank. The results obtained were within the specification. The above noted substances did not adversely affect the results.

f. *Assay cut-off:*

The assay cut-off is set to 1.0 based on the results of normal population study (400 samples from Caucasian subjects), the reference sera and correlation study. The specification was that the 95th percentile should lie below the ratio 1.0. Results are considered negative if the ratio <1.0, low positive if the ratio is between 1.0 and 1.4, moderate to high positive if the ratio >1.4.

2. Comparison studies:

a. *Method comparison with predicate device:*

Substantial equivalence to the predicate was determined by testing 183 CTD samples and 100 disease control samples. Equivocal results of predicate ANA assay is considered as negative and CTD low and moderate positive as positive. Analysis of agreement between the current and predicate assay is shown below

			Varelisa ANA screen			
			Positive	Equivocal	Negative	Total
Varelisa CTD	CTD Samples	>1.4	144	1	4	149
		1.0-1.4	9	2	0	11
		<1.0	3	5	15	23
		Total	156	8	19	183
	Control Samples	>1.4	11	2	0	13
		1.0-1.4	3	1	4	8
		<1.0	2	5	72	79
		Total	16	8	76	100

	Varelisa CTD		Varelisa ANA screen		
	% agreement	95% CI	% agreement	95% CI	Difference
Positive (CTD)	87.4	81.7-91.9%	85.2	79.3-90.0%	2.2 %
Negative (Control)	79.0	69.7-86. 5%	84.0	75.3-90.6%	-5.0 %

The new device was also compared to the predicate device using the ANA reference panel from CDC, reference panel 2002 from AMLI and WHO International Standard. Samples were analyzed in duplicate. The specificities of the CDC, AMLI and WHO reference samples and the predicate/dew device comparative results are listed below.

CDC Reference Panel			AMLI Reference Panel		
Target	New Device (Ratio)	Predicate (Ratio)	Target	New Device (Ratio)	Predicate (Ratio)
dsDNA & weak Sm	2.7	4.7	Negative	0.8	1.1
SSB & week SSA	5.5	8.0	Sm	4.4	5.3

CDC Reference Panel			AMLI Reference Panel		
Target	New Device (Ratio)	Predicate (Ratio)	Target	New Device (Ratio)	Predicate (Ratio)
Weak Sm, U1RNP, SSA, SSB	6.1	7.7	RNP	5.7	6.9
U1-RNP	5.3	5.3	SSA	3.3	5.1
Sm, histone	5.2	5.0	SSB	5.6	7.9
Nucleolar pattern	0.3	0.3	Scl-70	3.0	2.8
SSA	3.3	5.3	Jo-1	3.3	4.6
Cenp	4.6	7.1	Centromere	2.4	3.6
Scl-70	4.5	4.9	dsDNA	4.8	6.9
Jo-1	3.7	5.4	Negative	0.2	0.2
PM-Scl	1.5	0.9	WHO		
Ribosomal P	1.1	0.5	dsDNA	2.8	3.7

     b. Matrix comparison:
To demonstrate that serum and plasma (EDTA, heparin and citrate) samples gave same results, four samples for the different anticoagulants were collected from each of 10 blood donors and assayed in duplicates. The samples were then spiked with 10 different ANA antibody positive samples. All spiked samples were run in four replicates. The specification was set that the difference between the serum and plasma results should be lower than ± 20 % for positive samples. Negative samples should be negative. For EDTA and citrate plasmas, the specifications were met. For heparin plasma, a significant difference between serum and plasma was found. Heparin plasma samples are not recommended for this assay and the limitation is stated in the package insert.

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:
Same as assay cut-off.
5. Expected values/Reference range:
Expected value in the normal population was determined by measuring samples from 400 healthy blood donors with equal numbers of both genders and equal distribution in the defined age categories (<30 years to >60 years). The mean ratio for the female cohort is higher than that of the male. The overall mean and median ratios for both cohorts were 0.44 and 0.37 respectively. The mean ratio and 2 SD was 1.08 and the 95th Percentile was 0.83.

N. Proposed Labeling:
The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

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