

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

A. 510(k) Number:

k050625

B. Purpose for Submission:

Modification of the manufacturer's existing cleared device (Varelisa® ReCombi ANA Profile) by replacing the Sm antigen with the Sm[D] antigen (k042629)

C. Measurand:

Anti-Sm[D] antibodies

D. Type of Test:

Qualitative Enzyme Immunoassay (EIA)

E. Applicant:

Sweden Diagnostics (Germany) GmbH

F. Proprietary and Established Names:

Varelisa® ReCombi ANA Profile

G. Regulatory Information:

1. Regulation section:
21 CFR 866.5100 Antinuclear Antibodies Immunological
2. Classification:
II
3. Product code:
LJM, Antinuclear antibody, Antigen and Control
4. Panel:
Immunology 82

H. Intended Use:

1. Intended use(s):
The Varelisa ® ReCombi ANA Profile EIA kit is designed for the qualitative determination of eight antinuclear antibodies in human serum or plasma to aid in the diagnosis of SLE (systemic lupus erythematosus), scleroderma (progressive systemic sclerosis and CREST syndrome), MCTD (mixed connective tissue disease), SS (Sjogren's syndrome) and polymyositis/dermatomyositis. The Varelisa ReCombi ANA Profile individually detects antibodies against dsDNA, U1RNP (RNP 70, A, C), SmD, SS-A/Ro (52 kDa, 60 kDa), SS-B/La, Scl-70, CENP-B and Jo-1. For *in vitro* diagnostic use only.
2. Indication(s) for use:
Same as intended use.
3. Special conditions for use statement(s):
For prescription use only.
4. Special instrument requirements:
Microplate reader capable of measuring OD at 450nm.

I. Device Description:

For detection of Sm[D], the modified assay contains microplate wells coated with synthetic peptides (Sm[D]) in place of Sm. All other reagents are the same as the previously cleared device.

J. Substantial Equivalence Information:

1. Predicate device name(s):
Varelisa® ReCombi ANA Profile (No. 12996) and Varelisa® Sm Antibodies
2. Predicate 510(k) number(s):
k993109 and k042629
3. Comparison with predicate:

Similarities		
Item	New Device	Predicate Device
Indications for use	To aid in the diagnosis of SLE (systemic lupus erythematosus), scleroderma (progressive systemic sclerosis and CREST syndrome), MCTD (mixed connective tissue disease), SS (Sjogrens syndrome), and polymyositis/dermatomyositis.	Same
Technology	ELISA	Same
Assay Format	Qualitative	Same
Sample dilution	1:101 dilution	Same
Enzyme-Conjugate	Rabbit anti-human IgG Horseradish Peroxidase	Same
Substrate, wash buffer and stop solution	Same	Same
Incubation times	30, 30 and 10 minutes	Same
Platform	96 well microtitre plates	Same
Result interpretation (ratio compared to cut-off control)	Negative: <1.0 Equivocal: 1.0 – 1.4 Positive: >1.4	Same
Matrix	Serum and plasma (EDTA, citrate)	Same
Antigens	dsDNA, U1RNP (RNP 70, A, C), SS-A/Ro (52 kDa, 60 kDa), SS-B/La, Scl-70, CENP-B and Jo-1	Same
Differences		
Item	Device	Predicate
Antigen	Synthetic human Sm[D] peptide.	Sm antigen purified from calf thymus.
Sample diluent	20mL 5X Concentrate	100 mL ready to use

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

None referenced.

L. Test Principle:

The Varelisa ReCombi ANA Profile is an indirect noncompetitive enzyme

immunoassay for the individual 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. The wells of a microplate are coated with human recombinant nuclear antigens, synthetic peptides (SmD) or plasmid DNA. Antibodies specific for the nuclear antigens are present in a patient sample bind to these nuclear antigens. In a second step the enzyme labeled second antibody (conjugate) binds to the antigen-antibody complex which leads to the formation of an enzyme labeled conjugate-antibody-antigen complex. The enzyme labeled antigen-antibody complex converts the added substrate to form a colored solution. The rate of color formation from the chromogen is a function of the amount of conjugate complexed with the bound antibody and is proportional to the initial concentration of the respective antibodies in the patient sample. The results are read spectrophotometrically and are interpreted by comparison to a cut-off calibrator.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

Data presented are for all eight analytes.

a. *Precision/Reproducibility:*

- i. Study design: Three samples (equivocal, low positive, high positive) per analyte were analyzed in 5 runs, with 5 replicates per run. Calibrator and control were analyzed in triplicates. Within one day one operator carried out the analyses.
- ii. Results/Acceptance criteria: Target values for the study were set at variance intra assay = <12% and inter assay = <8%. Intra-assay variation ranged from 1.8% to 7.9% and inter-assay variation ranged from 1.0% to 7.2% and all were within the target values.

Analyte	Sample	Mean (Ratio)	Variability (CV %)	
			Intra-assay	Inter-assay
dsDNA	equivocal	1.2	2.4	3.9
	low positive	2.3	3.4	4.3
	high positive	3.0	2.4	5.9
U1RNP	equivocal	1.4	3.3	2.5
	low positive	2.0	3.6	3.8
	high positive	3.4	2.1	7.2
Sm[D]	equivocal	1.1	2.3	2.3
	low positive	1.9	3.7	5.4
	high positive	3.3	1.8	3.2
SS-A/Ro	equivocal	1.1	3.9	1.9
	low positive	2.7	3.0	1.3
	high positive	4.5	3.4	3.3
SS-B/La	equivocal	1.3	2.7	2.5
	low positive	1.9	2.2	5.6
	high positive	3.1	1.9	2.9
Scl-70	equivocal	1.2	3.9	4.5
	low positive	2.5	4.1	5.6
	high positive	3.9	3.1	4.5
CENP	equivocal	1.1	2.8	1.0
	low positive	1.8	3.2	3.4
	high positive	3.3	3.2	4.0
Jo-1	equivocal	1.3	2.2	2.8
	low positive	2.1	7.9	3.1
	high positive	2.7	3.8	5.0

b. *Linearity/assay reportable range:*

Not applicable.

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

Not applicable.

d. *Detection limit:*

The sample diluent (ready to use) was measured 30 times per analyte (10 modules of 3 different solid phase batches). Calibrator and Control were run in singlicate. The value for the 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) (expressed in Ratios). Specifications were: the mean plus 3 SD of the OD of the Sample Diluent should be lower than or equal to 0.3 for each analyte. The analytical sensitivity for the eight analytes ranged from 0.0 to 0.1. The new device met the specifications.

Analyte	Mean OD (n=30)	Standard deviation (SD)	Analytical Sensitivity	
			OD	Ratio
dsDNA	0.005	0.005	0.021	0.1
U1RNP	0.016	0.007	0.038	0.0
Sm[D]	0.010	0.006	0.027	0.0
SS-A/Ro	0.010	0.007	0.030	0.1
SS-B/La	0.006	0.004	0.018	0.0
Scl-70	0.005	0.005	0.021	0.0
CENP	0.007	0.004	0.020	0.0
Jo-1	0.008	0.007	0.029	0.1

e. *Analytical specificity:*

Interference Study: Interference study data were referred to the two cleared devices: Varelisa ReCombi ANA Profile (k993109) for the 7 antigens and Varelisa Sm Antibodies (k042629) for the Sm[D] antigen. Data showed heparin interfered with the measurement of Sm antibodies and lipemic, hemolyzed or microbially contaminated samples could give poor results.

Crossreactivity to other Autoantibodies: Ten CDC International ANA Human Reference Sera were analyzed in singlicate together with the Calibrator and Control. The results are depicted in the table below and are comparable to the predicate device. The new device detected the expected targets except for sera CDC5 and CDC 10. CDC5 was found to react with in addition to Sm, The false positive reaction with U1RNP was due to the presence of high titers of antibodies directed against the RNP 70, A, and C in CDC 5. Western blot analysis confirmed that CDC5 did not react with U1RNP. CDC 10 reacted with SS-A/Ro in addition to Jo-1. The false positive result was due to the presence of antibodies to the SS-A 52 kDa protein. The co-occurrence of antibodies to SS-A 52 in sera of patients with idiopathic inflammatory myopathy was described in Rutjes et al., 1997.

Panel Table: Results for the International ANA Human Reference Panel from the Center of Disease Control (CDC)

Sample	Target	New device (U/ml)							
		ds	U1	Sm	Ro	La	Scl	Cen	Jo
CDC	dsDNA & weak Sm	7.1	0.5	<i>1.0</i>	0.2	0.0	0.1	0.2	0.1
CDC	SS-B/La	0.2	0.2	0.2	2.6	3.3	0.1	0.1	0.1
CDC 3	speckled pattern, U1RNP, SS-A/Ro,	0.2	3.4	0.2	3.0	2.8	0.1	0.1	0.1
CDC 4	U1-RNP	0.2	3.3	0.2	0.1	0.0	0.1	0.1	0.1
CDC 5	Sm	0.5	2.8	4.5	0.2	0.0	0.2	0.2	0.1
CDC 6	nucleolar pattern	0.2	0.3	0.4	0.3	0.2	0.3	0.2	0.1
CDC 7	SS-A/Ro	0.6	0.1	0.2	3.5	0.1	0.1	0.1	0.1
CDC 8	CenP	0.2	0.1	0.1	0.1	0.0	0.1	4.7	0.1
CDC 9	Scl-70	0.4	0.2	0.2	0.1	0.0	3.8	0.2	0.1
CDC	Jo-1	0.1	0.1	0.1	1.8	0.0	0.1	0.1	4.2

Positive results are in bold letters, disagreements with the target specificity are shaded in gray.

¹ reported as weak Sm positive by Tan E.M. et al. (1999)

* SS-B/La usually does not occur without SS-A/Ro

f. Assay cut-off:

The equivocal range and the cut-off of the new device were determined by analyzing 100 serum samples from apparently healthy Caucasian blood donors (50 males and 50 females). The serum samples were analyzed in singlicate together with Calibrator and Control. The specification for the study was that the 95th percentile should lie below the lower limit of the equivocal range for the parameter. The equivocal range of the new device was 1.0 to 1.4. The results are depicted in the tables and histogram below. The 95th percentile of 100 normal healthy controls ranged from 0.1 to 0.8 which met the specifications.

Statistical evaluation for n=100 Samples per analyte (*[Ratio])

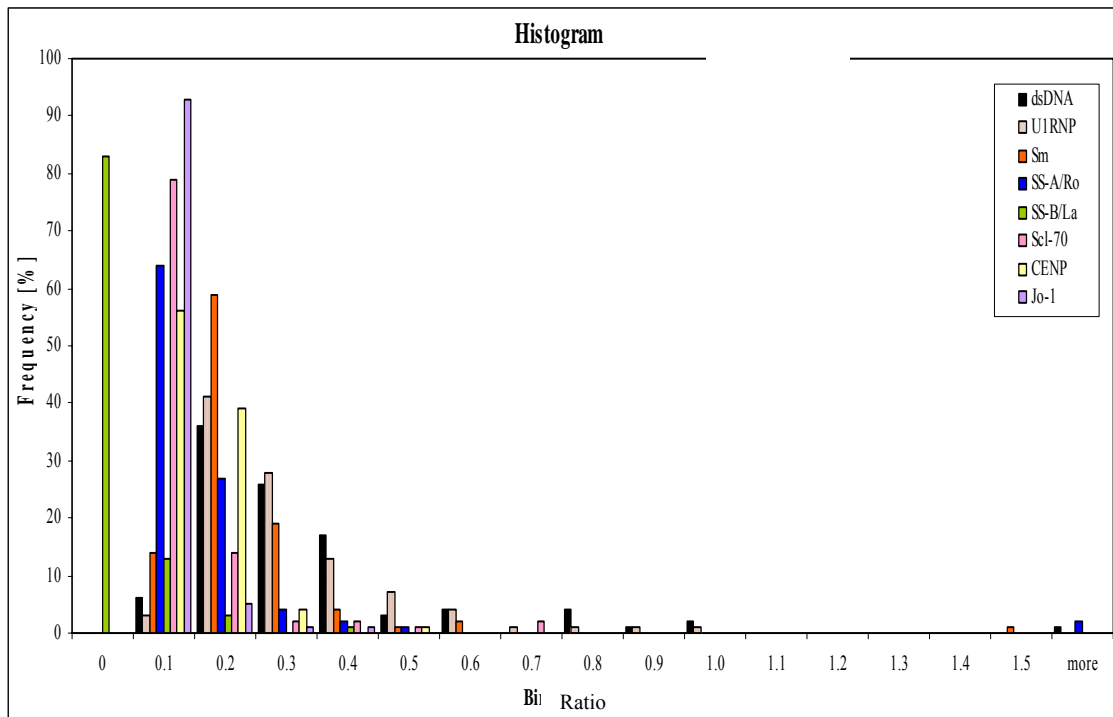
Analyter	dsDNA	U1RNP	Sm	SS-A/Ro	SS-B/La	Scl-70	CENP	Jo-1
n	100	100	100	100	100	100	100	100
Median*	0.3	0.3	0.2	0.1	0.0	0.1	0.1	0.1
Mean*	0.3	0.3	0.2	0.2	0.0	0.1	0.2	0.1
SD*	0.2	0.2	0.2	0.6	0.1	0.1	0.1	0.0
Mean + 2 SD*	0.8	0.6	0.6	1.4	0.1	0.4	0.3	0.2
95% Percentile*	0.8	0.6	0.4	0.3	0.1	0.3	0.3	0.2
98% Percentile*	1.1	0.8	0.6	0.5	0.2	0.5	0.3	0.2

Statistical evaluation for samples separated depending on age and gender

Gender	male					female				
	≤ 30	31 - 40	41 - 50	51 - 60	≥ 60	≤ 30	31 - 40	41 - 50	51 - 60	≥ 60
n	10	10	10	10	10	10	10	10	10	10
Parameter	dsDNA									
Median	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.2	0.3
Mean	0.3	0.3	0.4	0.2	0.3	0.4	0.5	0.3	0.3	0.4
SD	0.2	0.1	0.2	0.1	0.1	0.3	0.5	0.1	0.2	0.3
Mean + 2 SD	0.8	0.4	0.7	0.4	0.5	1.0	1.5	0.5	0.8	1.0

Gender	male					female				
Age	≤ 30	31 - 40	41 - 50	51 - 60	≥ 60	≤ 30	31 - 40	41 - 50	51 - 60	≥ 60
n	10	10	10	10	10	10	10	10	10	10
Median	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Mean	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1
SD	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0
Mean + 2 SD	0.4	0.3	0.2	0.2	0.3	0.3	0.3	0.2	0.2	0.2
95% Percentile	0.3	0.3	0.2	0.2	0.2	0.3	0.3	0.2	0.2	0.2
98% Percentile	0.4	0.3	0.2	0.2	0.2	0.3	0.3	0.2	0.2	0.2
Parameter	Jo-1									
Median	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Mean	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
SD	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
Mean + 2 SD	0.3	0.1	0.2	0.2	0.1	0.3	0.2	0.2	0.1	0.2
95% Percentile	0.3	0.1	0.1	0.2	0.1	0.2	0.2	0.1	0.1	0.2
99% Percentile	0.4	0.1	0.1	0.2	0.1	0.3	0.2	0.2	0.1	0.2

Histogram



2. Comparison studies:

Data presented are for SmD only.

a. Method comparison with predicate device:

- Study design: Refer to the method comparison data in Varelisa ReCombi ANA Profile (k993109) and Varelisa Sm Antibodies

(k042629). Since the major difference between the new and predicate device is Sm, a comparative study was performed between the SmD of the new device and the Sm (B, B', D) of the predicate device. The new test was also compared to a semiquantitative test (k042629). One hundred and eighty samples positive for at least one autoantibody and 20 samples from blood donors were analyzed in single determinations. Calibrators and Controls were analyzed in duplicates. Results of both comparisons are depicted in the tables below. Equivocal results were regarded as negative.

Correlation of New and Predicate device

<i>Sm</i>		Predicate device			Σ
		Positive	Equivocal	Negative	
New device	Positive	16	3	8	27
	Equivocal	1	2	5	8
	Negative	5	1	159	165
	Σ	22	6	172	200

Positive Agreement 72.7% (16/22)

Negative Agreement 93.8% (167/178)

Total Agreement 91.5% (183/200)

Correlation of New Device and Semiquantitative test (K042629)

<i>Sm</i>		Semiquantitative test			Σ
		Positive	Equivocal	Negative	
New device	Positive	23	3	1	27
	Equivocal	0	2	6	8
	Negative	0	1	164	165
	Σ	23	6	171	200

Positive Agreement 100% (23/23)

Negative Agreement 97.7% (173/177)

Total Agreement 98.0% (196/200)

- b. Matrix comparison:*
The Sm[D] study included the use of serum, heparin plasma, citrate plasma and EDTA plasma. The conclusion of the study is that the use of heparin interfered with the Sm antigen.
3. Clinical studies:
 - a. Clinical Sensitivity:*
Not given.
 - b. Clinical specificity:*
Not given.
 - c. Other clinical supportive data (when a. and b. are not applicable):*
Not Applicable.
4. Clinical cut-off:
Refer to Assay cut-off.
5. Expected values/Reference range:
Refer to Assay cut-off.

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.