

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

A. 510(k) Number:

k042629

B. Purpose for Submission:

New device by modification of the manufacturer's existing device

C. Measurand:

Anti-Sm[D] antibodies

D. Type of Test:

Semiquantitative and qualitative enzyme-linked immunosorbent assay (ELISA)

E. Applicant:

Sweden Diagnostics (Germany) GmbH

F. Proprietary and Established Names:

Varelisa® Sm Antibodies

G. Regulatory Information:

1. Regulation section:
21 CFR 866.5100 Antinuclear Antibodies Immunological Test System
2. Classification:
Class II
3. Product code:
LKP Anti-Sm Antibody, Antigen and Control
4. Panel:
Immunology 82

H. Intended Use:

1. Intended use(s):
The Varelisa Sm Antibodies EIA kit is designed for the semiquantitative and qualitative determination of SmD antibodies in serum or plasma to aid in the diagnosis of systemic lupus erythematosus (SLE).
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 450 nm.

I. Device Description:

The assay contains the following: microplate strips coated with a synthetic SmD peptide antigen, six levels of calibrators (0, 3, 7, 16, 40 and 100 U/mL), positive and negative controls (human serum), wash buffer concentrate, sample diluent, rabbit anti-human IgG horseradish peroxidase conjugate, 3,3',5,5' tetramethylbenzidine (TMB) substrate, and H₂SO₄ stop solution.

J. Substantial Equivalence Information:

1. Predicate device name(s):
Pharmacia Varelisa Sm antibodies
2. Predicate 510(k) number(s):
k000312
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Indications for Use	To aid in the diagnosis of systemic lupus erythematosus (SLE)	To aid in the diagnosis of systemic lupus erythematosus (SLE)
Calibrators	6 levels: 0, 3, 7, 16, 40 and 100 U/ml	Same
Conjugate	Rabbit anti-human IgG horseradish peroxidase	Same
Substrate	TMB	Same
Assay principle	Indirect noncompetitive enzyme immunoassay	Same
Sample dilution	1:101	Same
Result interpretation (ratio compared to cut-off control)	Negative = <1.0 Equivocal = 1.0 – 1.4 Positive = > 1.4	Same
Result interpretation (semiquantitative)	Negative: <10 U/mL Equivocal: 10-15 U/mL Positive: >15	Same
Matrix	Serum and plasma	Same
Differences		
Item	Device	Predicate
Antigen	Synthetic human SmD3 peptide	Sm antigen purified from calf thymus
Prewashing of microtiter plates	Not required	Required

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

None referenced

L. Test Principle:

The Varelista Sm Antibodies is an indirect noncompetitive enzyme immunoassay for the semiquantitative and qualitative determination of SmD3 antibodies in human serum or plasma. The wells of a microplate are coated with a synthetic SmD3 peptide. Antibodies specific for SmD3 present in the patient sample bind to the antigen. 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 (qualitative) or a standard curve (semiquantitative).

M. Performance Characteristics (if/when applicable):**1. Analytical performance:****a. *Precision/Reproducibility:***

Three samples (one of each: negative near equivocal, equivocal near positive and positive) were used in a standard 1:100 dilution and were analyzed in 5 runs, with 4 replicates per run. Calibrator and controls were analyzed in triplicate. Within one day one operator carried out the analyses. Target values for the study were set at within run %CV $\leq 12\%$ and between run $\leq 8\%$. Within-run %CV ranged from 2.6% to 4.0% and between run ranged from 3.9% to 4.0% and were all within the target values.

Sample:	Within run %CV	Between runs %CV
Negative	2.6	3.5
Equivocal	3.1	1.8
Positive	4.0	3.9

b. *Linearity/assay reportable range:*

Three positive sera were diluted beginning with a standard dilution of 1:101 followed by dilutions of 1:1, 2:3, 1:2, 1:4 to 1:32 using sample diluent. Calibrators, controls and each dilution step were analyzed in triplicate. The specifications for the study were: observed/expected (O/E %) units should be within $\pm 20\%$ for at least 3 successive dilution steps of each sample tested. All dilutions met the specifications through at least the 1:8 dilution step.

Sample ID	Dilution	U/mL	O/E %
1	1:1	69.9	100.0
	2:3	42.9	92.1
	1:2	29.8	85.2
	1:4	14.8	84.7
	1:8	7.0	80.5

Sample ID	Dilution	U/mL	O/E %
2	1:16	3.5	79.4
	1:32	1.6	74.8
	1:1	91.9	100.0
	2:3	54.2	88.4
	1:2	40.0	87.1
	1:4	18.5	80.7
	1:8	9.2	80.1
	1:16	4.2	73.1
3	1:32	2.0	70.8
	1:1	>100	---
	2:3	62.3	100.0
	1:2	43.7	93.4
	1:4	20.2	86.3
	1:8	9.9	84.4
	1:16	5.0	85.6
	1:32	2.5	86.7
Calibrator	1:1	93.1	---
	2:3	64.5	100.0
	1:2	44.6	92.1
	1:4	20.8	85.9
	1:8	10.5	86.8
	1:16	5.3	87.1
	1:32	2.7	89.3

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Not applicable

d. *Detection limit:*

The sample diluent was diluted according to the directions for use and measured 56 times on one plate. Calibrators and controls were analyzed in four replicates. The value for the analytical sensitivity (detection limit) was calculated as the mean of the optical densities of the sample diluent plus 3 times the standard deviation by using a spline approximation procedure. Specifications were: the mean plus 3 standard deviations (SD) of the OD of the sample diluent should be lower than the calibrator point S2 and the discrimination value should be >2.0. The analytical sensitivity was 0.03 U/mL and the discrimination value for Calibrator 2 was 13.2. The new device met the specifications.

e. *Analytical specificity:*

Interference study:

The purpose of this study was to investigate whether high concentrations of potentially interfering substances in serum, like bilirubin, hemoglobin, chyle, and rheumatoid factor, adversely affect the results of the new device. Three samples (one negative, equivocal and positive) were diluted 1:101 in sample diluent. The samples were spiked with different amounts of interfering substances or their respective blank solutions and analyzed in triplicate. The calibrators and controls were analyzed in duplicate. Data reduction was done using a spline approximation procedure. Specifications for the study were: the

deviation of the value of the sample spiked with the interfering substance should be less than $\pm 20\%$ of the value of the sample spiked with a buffer blank. The percent of deviation ranged from -8.9% to 15.0%.

Crossreactivity to other autoantibodies:

Ten CDC ANA human reference sera from the Centers for Disease Control and Prevention, 10 sera of the AMLI Consensus Reference Panel 2002, and ten sera of the AMLI Consensus Reference Panel 2001 were diluted 1:101 and if necessary 1:201 and 1:401 in sample diluent. The samples were analyzed in triplicate. The calibrators and controls were analyzed in duplicate. The assay gave a positive result (>100 U/mL) for CDC 5 as expected and all other samples were negative. The AMLI 2001 sample, AMLI-I, tested positive (>100 U/mL) as expected but sample AMLI-J, the dsDNA positive sample also gave a positive result of 22.3 U/mL. The manufacturer cited literature where this dsDNA sample tested positive for Sm antibodies by immunoblotting. AMLI 2002 Sm antibody positive sample SLR-2 tested negative for Sm antibodies with the new assay. Upon analysis by Western Blot, the sample was found to be negative for the 16-18 kDa bands representing the SmD proteins and thus could not be detected by the new assay.

f. Assay cut-off:

The purpose of the normal sera studies was to confirm the defined negative and equivocal ranges of the new device by measuring 432 apparently healthy blood donor samples, equally distributed by gender and age, as normal controls. Additionally data from 198 patients from the correlation study were used to calculate the diagnostic values to further support the equivocal range decision. The samples were diluted 1:101 in sample diluent and analyzed in duplicate. The calibrators and controls were also analyzed in duplicate. The specification for this study was that the 98th percentile should lie below the lower limit of the equivocal range. The statistical evaluation for the 432 samples showed:

	U/mL
Mean	1.8
SD	2.8
Mean + 2SD	7.4
Mean + 3SD	10.3
Median	1.4
95 th percentile	3.7
98 th percentile	5.1

In the analysis of the diagnostic values for the 198 sera, different possible equivocal ranges for the new device were considered. At an equivocal range of 10 – 15 U/mL, the diagnostic specificity was 93.9% and the diagnostic sensitivity was 23.0%.

2. Comparison studies:

a. *Method comparison with predicate device:*

The comparison involved a total of 198 samples: 100 in the SLE target population and 98 disease controls (36 mixed connective tissue disease, 20 Sjogren's syndrome, 5 myositis, 17 primary biliary cirrhosis, 15 bacterial infections and 5 patients with viral infections). Analysis was performed according to the instructions for use. Agreements were calculated using the data for the new device compared to the predicate device. Diagnostic values were calculated using the data for the new device or predicate device and the diagnostic information. Equivocal results were regarded as negative.

		Varelsa Sm Assay (predicate)		
		Positive	Equivocal	Negative
Varelsa Sm[D]	Positive	19	1	9
	Equivocal	4	2	8
	Negative	14	4	137

Positive percent agreement 54.1% (19/37)

Negative percent agreement 93.8% (151/161)

Overall agreement 85.9% (170/198)

b. *Matrix comparison:*

Ten Sm antibody negative samples, each available as serum, heparin plasma, citrate plasma and EDTA plasma and 10 Sm antibody positive sera were run according to the directions for use. The 10 Sm antibody negative samples were run in duplicate together with calibrators and controls. Then they were spiked with the 10 different positive sera. All spiked samples were run in four replicates and calibrators and controls were run in duplicate. The specifications for this study were: the differences between serum and plasma results should not be higher than $\pm 20\%$ for positive or equivocal samples. Negative samples should be negative as serum or plasma samples.

The percent differences ranged from -14.7% to 7.5% for citrate plasma and EDTA plasma. Percent difference for the heparin plasma samples ranged from -53.7% to 29.9% with a warning regarding heparin plasma in the labeling.

3. Clinical studies:

a. *Clinical Sensitivity:*

The same samples from the method comparison study were used for determination of clinical sensitivity and specificity. Results are tabulated below.

New device	Predicate device	Total patients	Disease positive	Disease negative
+	+	19	17	2
eq	+	4	1	3
-	+	14	1	13

New device	Predicate device	Total patients	Disease positive	Disease negative
+	eq	1	1	0
+	-	9	5	4
-	eq	4	2	2
eq	-	8	5	3
eq	eq	2	2	0
-	-	137	66	71
Total		198	100	98

	<u>New</u>	<u>Predicate</u>
Clinical sensitivity:	23.0% (23/100)	19.0% (19/100)
Clinical specificity:	93.9% (92/98)	81.6% (80/98)

- b. *Clinical specificity:*
See section M.3.a.
- c. Other clinical supportive data (when a. and b. are not applicable):
Not applicable.
4. Clinical cut-off:
See assay cut-off.
5. Expected values/Reference range:
The expected value in the normal population is negative. However, apparently healthy asymptomatic individuals may test positive for Sm autoantibodies. The incidence of these antibodies increases with age. The presence of Sm autoantibodies is regarded as a marker for SLE with the overall prevalence ranging from 20 to 30%. Expected values may vary depending on the population tested. The frequency distribution for Sm antibodies in a group of 432 apparently healthy subjects (Caucasian individuals equally distributed by age and gender tested in Freiburg, Germany) showed a mean of 1.8 U/mL, mean plus 2 SD of 7.4 U/mL, a median of 1.4 U/mL and a 95 percentile of 3.7 U/mL.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10. Test components containing human source materials were tested by methods that are in accordance with current European legislation (Directive 98/79/EC) and found to be negative for hepatitis B surface antigen, antibodies to HIV1, HIV2 and hepatitis C virus.

O. Conclusion:

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