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

A. 510(k) Number:
k040451

B. Purpose of Submission:
New device

C. Analyte:
β2-Glycoprotein I IgM antibodies

D. Type of Test:
Semiquantitative and qualitative ELISA

E. Applicant:
Pharmacia Deutschland GmbH/Sweden Diagnostics (Germany) GmbH

F. Proprietary and Established Names:
Varelisa B2-Glycoprotein I IgM Antibodies EIA Kit

G. Regulatory Information:
1. Regulation section:
   21 CFR 866.5660, Multiple Autoantibodies Immunological Test System
2. Classification:
   Class II
3. Product Code:
   MSV, System, Test, Antibodies, β2-Glycoprotein I (β2-GPI)
4. Panel:
   Immunology (82)

H. Intended Use:
1. Intended use(s):
   The Varelisa B2-Glycoprotein I IgM Antibodies Kit is designed for the semiquantitative and qualitative determination of β2-glycoprotein I IgM antibodies in serum or plasma.
2. Indication(s) for use:
   The presence of β2-glycoprotein I antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of thrombotic disorders related to the primary Antiphospholipid Syndrome or occurring secondary to systemic lupus erythematosis (SLE) or other autoimmune diseases.
3. Special condition for use statement(s):
   Prescription use only.

4. <u>Special instrument Requirements:</u>
   None.

I. Device Description:

The Varelisa B2-Glycoprotein I IgM Antibodies kit consists of an ELISA strip wells (12 strips X 8 wells) coated with purified human β2-glycoprotein I antigen. It also contains ancillary reagents such as calibrators, positive and negative controls, wash buffer, sample diluent, antibody-chromogen conjugate, enzyme substrate, stop solution for enzyme reaction, and a frame to hold ELISA strip wells. Also included are a package insert and a control certificate.

J. Substantial Equivalence Information:

1. <u>Predicate device name(s):</u>
   INOVA QUANTA Lite B2 GPI IgM
2. <u>Predicate K number(s):</u>
   k973014
3. <u>Comparison with predicate:</u>

Similarities		
Item	**Device**	**Predicate**
Intended Use	Semiquantitative and qualitative determination of β2-glycoprotein I IgM antibodies in serum or plasma for use in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of thrombotic disorders related to primary Antiphospholipid Syndrome or occurring secondary to systemic lupus erythematosis (SLE) or other autoimmune diseases.	Qualitative detection of β2-GPI IgM antibodies in serum for use in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of certain autoimmune disease thrombotic disorders, such as those secondary to systemic lupus erythematosis (SLE) or other lupus-like thrombotic diseases.
Antigen	Human affinity-purified β2-glycoprotein I	β2-glycoprotein I, unknown source
Conjugate	Anti-human IgM Antibody Conjugate, Horseradish peroxidase	Same
Assay Principle	Indirect noncompetitive enzyme immunoassay	Same
Sample dilution	1:101	Same

Differences		
Item	**Device**	**Predicate**
Specimen	Serum and plasma	Serum
Calibrators	Six prediluted calibrators	Five prediluted calibrators
Assay evaluation	OD cut-off	Decision point

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

L. Test Principle:
The Varelisa B2-Glycoprotein I IgM Antibodies EIA Kit is an indirect noncompetitive enzyme immunoassay. The wells of the microplate are coated with human purified β2-glycoprotein I antigen. Antibodies specific for β2-glycoprotein I present in the applied patient sample bind to the antigen and are detected by an enzyme labeled secondary 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 (substrate) is a function of the amount of conjugate complexed with the bound antibody and thus is proportional to the initial concentration of the respective antibodies in the patient sample. The antibody concentration is determined by comparison to a calibration curve run with the assay and is reported in U/mL.

M. Performance Characteristics (if/when applicable):
1. Analytical performance:
   a. *Precision/Reproducibility:*
      Five serum samples (low, medium, high, equivocal, negative) were selected from a serum bank. The samples were diluted 1:101 and measured in 5 runs, with 4 replicates per run. Standards and controls were analyzed in triplicate. Specified target values were: variance "within" <12%; "between" <10%; "total" < 15%. The specifications were met for each sample, with the following variances:

Sample ID		Run 1	Run 2	Run 3	Run 4	Run 5	Mean (U/mL)	Variance		
								Within	Between	total
ZSB 7483	Mean (u/mL)	53.0	55.8	54.2	52.4	52.5	53.6	4.26	1.61	4.56
	CV%	5.11	2.89	0.85	7.16	2.60				
ZSB 7170	Mean (u/mL)	34.4	35.2	32.3	32.7	29.6	32.8	3.08	6.42	7.12
	CV%	0.74	3.82	1.62	4.87	2.25				
ZSB 6910	Mean (u/mL)	16.7	17.3	16.4	16.8	16.2	16.7	2.36	2.23	3.25
	CV%	2.31	1.93	3.13	2.78	1.10				
negative	Mean (u/mL)	5.3	3.6	3.6	3.5	3.3	3.9	11.0	19.6	22.5
	CV%	11.4	1.4	4.7	19.3	7.9				
equiv.	Mean (u/mL)	16.7	17.3	16.4	16.8	16.2	16.7	2.4	2.2	3.3

	CV%	2.31	1.93	3.13	2.78	1.10			

b. *Linearity/assay reportable range:*
Three samples selected from a serum bank were diluted 1:1, 2:3, 1:2, 1:4, 1:8, 1:16, and 1:32 in Sample Diluent. Standards, Controls and each dilution were measured in triplicate. Specifications were that observed/expected units ratio would not differ by more than $\pm$ 20% for at least 3 successive dilutions of each tested sample.
The dilutions met the criteria and were considered linear, although some of the higher dilutions were >20% over expected.
Recovery of analyte was assayed using two samples from a serum bank. Each sample was tested to determine pre-existing antibodies. Each sample was then diluted 1:101 and spiked with 1/10 volume of Standards S1 to S6 and measured in duplicate. Acceptance criteria were that recovery would be $100 \pm 20\%$. In all cases for both samples, the acceptance criteria were met.

c. *Traceability (controls, calibrators, or method):*
Not traceable to external sources. The consistency of the calibration material is given by a testing procedure comprising several internal target values that must be met. Each new production of calibration material is adapted to the corresponding master calibration material.

d. *Detection limit:*
Sample Buffer was diluted according to Directions for Use and measured 56 times on one plate. Standards and Controls were analyzed in four replicates. Two dilutions of the first standard point, S2 (1:2, 1:4) were run in four replicates. Analytical sensitivity was calculated as the mean of the optical densities of the sample buffer plus 3SD and expressed in U/mL. The discrimination value D, proves the ability to discriminate between the lowest standard point and the background, was performed as follows:

$$D = \frac{\eta_B - \eta_A}{\sqrt{(\sigma_B{}^2 - \sigma_A{}^2)}}$$

Where: A is Sample Buffer; B is Standard S2; $\acute{\eta}_A$, $\acute{\eta}_B$ are mean OD's; σ_A, σ_B are SD's.

Specifications were that analytical sensitivity should be below 1 U/mL, the mean + 3SD of sample buffer should be < standard point S2, and the discrimination value should be > 2.0.

The mean +3SD was 0.021 OD, which corresponded to analytical sensitivity of 0.07 U/mL. The other criteria were met.

e. *Analytical specificity:*
Interference was tested against potentially interfering substances found in blood: bilirubin, hemoglobin, chyle, and rheumatoid factor.

Three samples from a serum bank were diluted 1:101. Samples were spiked with different super-normal amounts of interferent. Spiked samples were analyzed in triplicate. Acceptance criteria were that spiked samples should show no more than 20% variation from unspiked sample.

Additives (final sample concentration)	I	II	III	IV	V
Bilirubin F (mg/dL)	0.0	4.8	9.5	14.3	19.0
Bilirubin C (mg/dL)	0.0	5.5	11.0	16.5	22.0
Chyle (Units)	0.0	357.5	715.0	1072.5	1430.0
Hemoglobin (mg/dL)	0.0	122.5	245.0	367.5	490.0
RF (IU/mL)	0.0	98.0	294.0	490.0	-

All samples met acceptance criteria and showed no significant influence on the test results. Additional interference tests on negative and equivocal sera with the same interferents demonstrated a negligible effect of the interferents on the negative samples, and a more pronounced effect on the equivocal samples. The negative samples, however, remained negative, so the interference was judged to be not important.

f. *Assay cut-off:*
The semi-quantitative cut-points were determined by measuring 432 samples from apparently healthy Caucasian donors, equally distributed by sex and age. Diluted samples, standards, and controls were analyzed in duplicate. Specifications called for the mean +2SD to be smaller than the lower limit of the equivocal range, and the mean +3SD to be smaller than the upper limit of the equivocal range. The following values were selected for negative, equivocal, and positive:

<10 U/mL = negative
10-15 U/mL = equivocal
>15 U/mL = positive

The specifications were fulfilled and no difference was detected between sexes or ages.

2. Comparison studies:
    a. *Method comparison with predicate device:*
    The following samples were used in comparing the new device with 42 samples from a serum bank at Pharmacia, representing SLE, lupus-like, AIDS, APS, unknown clinical diagnosis, and healthy individuals. The results were compared semiquantitatively (Varelisa)

vs qualitatively (QUANTA Lite) in a 6 field analysis. 38 of 42 sera agreed qualitatively (pos/neg) for overall agreement of 90.5%. No sera were equivocal or positive by the Varelisa assay and negative by the predicate. Four sera were negative by Varelisa assay and positive by the qualitative predicate.

Varelisa B2-glycoprotein I IgM Antibodies Assay		INOVA QUANTA Lite	
	n = 42	Positive	Negative
	positive	25	0
	equivocal	0	0
	negative	4	13

    b. Matrix comparison:
Twenty blood donor samples in the positive and negative range of the assay were tested in serum, citrate plasma, heparin plasma, and EDTA plasma, in the negative range of the assay. No significant differences were noted for any of the matrices.

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:
Not applicable
5. Expected values/Reference range:
Expected value for normal healthy individuals is negative, or <10 U/mL. In a study of 432 healthy Caucasians, the following values were obtained:
N: 432
Mean: 1.1 U/mL
Mean +2SD   5.6 U/mL
Median: 0. 6 U/mL
95th percentile   3.4 U/mL

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