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

A. 510(k) Number:
k040810

B. Purpose for Submission:
New device

C. Analyte:
Histone antibodies

D. Type of Test:
Qualitative and semi-quantitative enzyme immunoassay

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

F. Proprietary and Established Names:
Varelisa® Histone Antibodies

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, Controls
4. Panel:
   Immunology 82

H. Intended Use:
1. Intended use(s):
   The Varelisa Histone Antibodies EIA kit is designed for the semiquantitative and qualitative determination of IgG and IgM antibodies to histone in serum or plasma to aid in the diagnosis of systemic lupus erythematosus (SLE) or drug-induced lupus erythematosus (DIL).
2. Indication(s) for use:
   The Varelisa Histone Antibodies EIA kit is designed for the semiquantitative and qualitative determination of IgG and IgM antibodies to histone in serum or plasma to aid in the diagnosis of systemic lupus erythematosus (SLE) or drug-induced lupus erythematosus (DIL).
3. Special condition 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 components include human histone antigen coated microplate strips, 6 levels of calibrator: 0, 5, 12, 30, 80 and 200 U/mL, positive and negative control materials, wash buffer concentrate, sample diluent concentrate, anti-human IgG/IgM

horseradish peroxidase conjugate, 3,3',5,5' tetramethylbenzidine (TMB) substrate, and 0.5 M H_2SO_4 stop solution.

J. Substantial Equivalence Information:
1. Predicate device name(s):
   INOVA Quanta Lite Histone IgG
2. Predicate K number(s):
   k934053
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
Indications for Use	To aid in the diagnosis of systemic lupus erythematosus (SLE) or drug-induced systemic lupus erythematosus (DIL)	To aid in the diagnosis of systemic lupus erythematosus (SLE), drug- induced SLE and related connective tissue diseases
Antigen	Purified human histone	Same
Substrate	TMB	Same
Assay principle	Indirect noncompetitive enzyme immunoassay	Same
Sample dilution	1:101	Same
Result interpretation	Negative = <1.0 Equivocal = 1.0 – 1.4 Positive = > 1.4	Negative <1.0 Units Weak positive 1.0-1.5 Units Moderate positive 1.6-2.5 Units Strong positive >2.5 Units
Differences		
Item	**Device**	**Predicate**
Specimen matrix	Serum and non-heparinized plasma	Serum
Conjugate	Anti-human IgG and IgM	Anti-human IgG
Calibrators	Set of 6 prediluted calibrators: 0, 5, 12, 30, 80, 200 U/mL	None provided
Controls	Positive and negative	Negative, low positive and high positive
Result interpretation (units)	Negative <15 U/mL Equivocal 15-30 U/mL Positive >30 U/mL	No calibrators for Unit value interpretation

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

L. Test Principle:
The wells of a microtiter plate are coated with human histone antigen. Antibodies specific for histone present in the patient samples 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 thus is proportional to the initial concentration of antibodies in the patient sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*
   The purpose of the precision study was to investigate variation within and between runs. The samples (low, medium, and high) were used in a standard 1:101 dilution and were analyzed in 5 runs, with 4 replicates per run. Calibrator and controls were analyzed in triplicate. One operator carried out the analyses within one day. The target values set for the studies were: within and between run variance should be <10%. The within run % CV ranged from 4.1 to 4.6% and between run ranged from 5.5 to 7.2 % thus meeting the target values.

   b. *Linearity/assay reportable range:*
   Dilution linearity:
   The reportable range for the assay is 1 – 200 U/mL. The purpose of the dilution linearity study was to demonstrate linearity of the assay over the measuring range. Beginning with a standard dilution of 1:101, 4 samples were further diluted 1:1, 2:3, 1:2, 1:4 then doubling dilutions through 1:64. Calibrators, controls and each dilution step were analyzed in duplicate. Specifications set for the study were: the observed/expected values should be within ± 20% for at least 3 successive dilution steps of each tested sample. Measured values ranged from 4.9 to >200 U/mL. Percent recovery for all four samples through the 1:8 dilution, ranged from 82 to 114%, thus meeting the specifications. For dilutions up to 1:16 recovery ranged from 74 to 127%.
   Recovery:
   The purpose of this study was to demonstrate that the assay detects added amounts of histone antibodies. Two positive samples were used in a 1:101 dilution. The sample dilutions were spiked with 1:10 volume of calibrators S1 – S6. The spiked samples, calibrators and controls were analyzed in duplicate. The specifications for this study were: the percent recovery (observed/expected x 100) should be within ± 20% of the expected values. Recoveries ranged from 95.7 to 113.9% and met the study specifications.

   c. *Traceability (controls, calibrators, or method):*
   There is no recognized reference material for histone antibodies. Results are reported in arbitrary units.

   d. *Detection limit:*
   The analytical sensitivity study was performed to verify the detection limit and to prove the ability of the assay to differentiate between the

background and the first calibrator point. The sample diluent was diluted according to the directions for use and measured 56 times on one plate. Calibrators and controls were analyzed in 4 replicates. For curve control below calibrator point S2, 2 dilutions of Calibrator S2 were performed (1:2 and 1:4) and also run in 4 replicates. The value for the analytical sensitivity (detection limit) was calculated as the mean of the optical densities (OD) of the Sample Diluent plus 3 times the standard deviation (SD), expressed in U/mL. The specifications for the study were: the mean plus 3 SD of the OD of the sample diluent should be lower than the Calibrator S2; the detection limit should be equal or below 1 U/mL; and the discrimination value should be >2.0. The mean plus 3SD of the sample diluent was 0.066 U/mL (lower than Calibrator S2 and lower than 1.0 U/mL). The discrimination value was above 2 U/mL and diluted Calibrator S2 could be discriminated from the background. Thus specifications for the study were met.

e. *Analytical specificity:*

The purpose of the study was to investigate whether high concentrations of potentially interfering substances in serum including bilirubin (F and C), hemoglobin, chyle, and rheumatoid factor (RF) adversely affect results of the assay. Three serum samples were diluted 1:101 in sample diluent and spiked with different amounts of interfering substances or their respective blank solutions and analyzed in triplicate. The calibrators and controls were run in duplicate. The specification to be met for this study was: the deviation of the values of the sample spiked with the interfering substance should be less than ± 20% of the value of the sample spiked with a buffer blank. The spiking of high concentrations of the potentially interfering substances showed no significant influence on the test results (deviations ranged from -9.4 to 18.1%). The specification for this study was met.

f. *Assay cut-off:*

A study was performed to establish and confirm the defined cut-off by measuring 432 apparently healthy blood donor samples, equally distributed by gender and age. Specifications for the study were: 95% of the normal population should be negative. Therefore the 95th percentile should lie below the lower limit of the equivocal range. The cut-offs were set as <15 U/mL is negative, 15-30 U/mL is equivocal and >30 U/mL is considered positive. The 95th percentile was 12.6 U/mL and thus below the negative cut-off so, the specifications were met. The results were independent of gender and age.

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

a. *Method comparison with predicate device:*

The comparison was made by testing 120 sera (histone antibody positive and negative). Analyses and calculations were performed

according to assay procedures. Correlations and Four Field Analysis were performed. Because and equivocal range was not defined in the predicate assay, Varelisa equivocal samples (n=2) were judged as negative. The comparison showed an overall agreement of 96.7% (116/120) (95% CI 93.5% to 99.9%). The positive agreement was 90.7% (39/43) (95% CI 82.0% to 99.4%) and negative agreement was 100%

b. *Matrix comparison:*
The predicate device uses serum only. The new device recommends use of both serum and plasma. A study was performed to demonstrate that the new assay gives the same results for serum, heparin plasma, citrate plasma and EDTA plasma collected from the same specimen. Nine histone antibody negative samples and 9 histone positive samples, each available as serum, heparin plasma, citrate plasma and EDTA plasma were assayed. The 9 histone antibody negative samples were run in duplicate together with calibrators and controls. Then they were spiked with the 9 different histone antibody positive sera. All spiked samples were run in duplicate together with calibrators and controls. Specifications for this study were: the percent deviation between serum and plasma results for positive samples should not be higher than ± 20% and negative samples should be negative as serum or plasma. The data showed no difference greater than ± 20% (deviations ranged from -12.5% to 16.5%) for citrate or EDTA plasma and no negative sample changed from negative to positive. Thus the specifications were met for citrate and EDTA plasma. However, for heparin plasma, a significant difference between serum and plasma values was noted. The following limitation appears in the package insert, "The use of plasma preparation with heparin is not recommended because heparin interferes with the measurement of histone (IgG, IgM) antibodies".

3. Clinical studies:
    a. *Clinical sensitivity:*
    Not provided
    b. *Clinical specificity:*
    Not provided
    c. *Other clinical supportive data (when a and b are not applicable)*

4. Clinical cut-off:
    See assay cut-off.
5. Expected values/Reference range:
    Histone antibodies are found in up to 80% of patients with the active form of SLE and, depending on the inducing drug, in up to 95% of patients with drug-induced LE. On the other hand, ≥ 95% of the normal population is expected to be negative.

N. Conclusion:

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