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

A. 510(k) Number:
K032240

B. Analyte:
Anti-Phosphatidylserine IgA, IgG, IgM

C. Type of Test:
Semi-quantitative ELISA

D. Applicant:
The Binding Site Ltd.

E. Proprietary and Established Names:
BINDAZYME® Human Anti-Phosphatidylserine IgA, IgG, and IgM EIA kit

F. Regulatory Information:
1. Regulation section:
   21CFR 866.5660 Multiple Autoantibodies Immunological Test System

2. Classification:
   Class II
3. Product Code:
   MID   System, Test, Anti-cardiolipin Immunological
4. Panel:
   Immunology

G. Intended Use:

1. Indication(s) for use:

   - **Bindazyme Human Anti-Phosphatidylserine IgA Enzyme Immunoassay kit**
     This assay is intended for the *in vitro* measurement of IgA anti-phosphatidylserine antibodies in human serum, as an aid in the diagnosis of anti-phospholipid syndrome (APS)**.**
   - **Bindazyme Human Anti-Phosphatidylserine IgG Enzyme Immunoassay kit**
     This assay is intended for the *in vitro* measurement of IgG anti-phosphatidylserine antibodies in human serum, as an aid in the diagnosis of anti-phospholipid syndrome (APS)**.**
   - **Bindazyme Human Anti-Phosphatidylserine IgM Enzyme Immunoassay kit**

This assay is intended for the *in vitro* measurement of IgM anti-phosphatidylserine antibodies in human serum, as an aid in the diagnosis of anti-phospholipid syndrome (APS).

2. Special condition for use statement(s):

3. Special instrument Requirements:
   Microplate reader capable of measuring optical densities at 450 nm referenced on air.

H. Device Description:
The device is an enzyme-linked immunosorbent assay (ELISA) for the measurement of IgA, IgG or IgM anti-phosphatidylserine in human serum.
Sufficient materials are supplied to allow a maximum of 41 samples to be tested in duplicate or 89 in single, with a calibration curve and a positive and negative control.

I. Substantial Equivalence Information:
1. Predicate device name(s):
   REAADS IgA Anti-Phosphatidylserine Semi-Quantitative Test kit
   REAADS IgG/IgM Anti- Phosphatidylserine
2. Predicate K number(s):
   K013018
   K024196
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
Intended Use	Measurement of IgA, IgG, IgM anti-phosphatidylserine antibodies in human serum	Same
Methodology	ELISA	Same
Sample	Serum	Same
Source of antigen	bovine	Same
Differences		
Item	**Device**	**Predicate**
Conjugate	Rabbit anti-human HRP	Goat anti-human HRP
Sample dilution	1:100	1:150
Calculation of Results	OD reading from a calibration curve	OD values multiplied by a conversion factor

J. Standard/Guidance Document Referenced (if applicable):
Not applicable

K. Test Principle:
Microwells are pre-coated with phosphatidylserine and cofactor. Calibrators, controls and patient samples are added to the wells and autoantibodies recognizing phosphatidylserine bind during the first incubation. After washing the wells to remove all unbound proteins, peroxidase labeled rabbit anti-human antibody (heavy

chain specific) conjugate is added. The conjugate binds to the captured human antibody and the excess unbound conjugate is removed by further wash step. The bound conjugate is visualized with TMB substrate which gives a blue reaction product, the intensity of which is proportional to the concentration of the autoantibody in the sample. Phosphoric acid is added to each well to stop the reaction. This produces a yellow end point color, which is read at 450 nm.

L. Performance Characteristics (if/when applicable):
    1. <u>Analytical performance:</u>
        a. Precision/Reproducibility:
        The intra and inter-assay precision was measured using three samples within the range of the calibration curve.

Inter-assay Precision:

	IgA		IgG		IgM	
n=3	Conc. APS U/mL	%CV	Conc GPS U/mL	% CV	Conc MPS U/mL	%CV
Sample 4	9.8	11.3	16.7	2.9	17.6	11.5
Sample 5	20	3.9	22.4	10.6	42.1	7.1
Sample 6	31.2	7.8	34.2	4.8	75.4	9.2

Intra-assay Precision

	IgA		IgG		IgM	
n=16	Conc. APS U/mL	%CV	Conc GPS U/mL	% CV	Conc MPS U/mL	%CV
Sample 1	10.1	5.0	12.7	7.3	21.6	3.2
Sample 2	35	7.2	25.7	6.6	54.2	4.8
Sample 3	50.1	8.1	75.4	6.9	88.6	5.1

        b. Linearity/assay reportable range:
        1.23 - 100 APS, GPS or MPS U/mL

        c. Traceability (controls, calibrators, or method):
        Standards are calibrated against the Louisville reference LAPL-GM-200.
        The positive control material is obtained from a US commercial supplier positive for cardiolipin (either G, A or M) since the presence of both antibodies to cardiolipin and anti-phosphatidylserine are indicative of APS. Upon receipt, the sera were tested in the Binding Site assay and positives confirmed in the Corgenix kits.

        d. Detection limit:

Sensitivity was determined as the mean concentration + 2 SD given by 20 determinations of the sample diluent.

IgA = 0.35 APS U/mL
IgG = 0.21 GPS U/mL
IgM = 0.28 MPS U/mL

e. *Analytical specificity:*
A range of interfering substances has been spiked into an anti-phosphatidylserine high and low sample, which have been subsequently assayed. The method used to check these substances was based on the Interference Check A plus™ Kokusai Shiyaku, Japan.

Substance	Concentration
Bilirubin F (Free)	18.3 mg/dL
Bilirubin C (Conjugate)	19.0 mg/dL
Hemolyzed hemoglobin	490.0 mg/dL
Chyle	1930.0 Units

f. *Assay cut-off:*
Not Applicable

2. Comparison studies:
   a. *Method comparison with predicate device:*
   The device was compared with Corgenix REAADS Anti-Phosphatidylserine IgA, IgG and IgM Semi-quantitative test kit. Correlation was done in two ways, both using the same data. One was performed by a scratch plot which groups the data into positive and negative samples using the cut-off of each assay. The other was done by a regression analysis to obtain correlation coefficient. Normal samples were supplied by the Blood transfusion unit, Queen Elizabeth Hospital, Birmingham UK. Clinical samples were from patients with antibodies to phosphatidylserine from the following sources:
   Department of Clinical Immunology, Queen Elizabeth Hospital, UK
   Biomedical Resources Hatboro, PA, USA
   SeraCare Life Sciences Inc, Oceanside, CA, USA.

	IgA	IgG	IgM
Relative sensitivity	81.8%	100.0%	92.6%
Relative specificity	84.4%	64.3%	83.3%
Relative agreement	83.9%	81.8%	87.3%

The data shows excellent sensitivity at 100% but lower specificity for IgG. The possible reasons for this are:
   1. Cut off is higher for the Corgenix kit.

2. Corgenix assay is not accurately calibrated to the Louisville reference, the correlation indicates a 60% mismatch.
3. There is a reported correlation between the presence of anti-phosphatidylserine and anti-cardiolipin antibodies in patient samples. Samples with negative results in the alternative Corgenix assay were tested in an anti-cardiolipin assay. Seven of the nine IgA, 8 of the 10 IgG, 8of the 8 IgM were confirmed positive.

IgA PS vs Corgenix correlation
$y = 0.5138x + 4.7965$
$R^2 = 0.8885$
IgG PS vs Corgenix correlation
$y = 0.4036x + 4.2123$
$R^2 = 0.9251$
IgM PS vs Corgenix correlation
$y = 0.3535x + 5.2721$
$R^2 = 0.7578$
The low gradient of regression line may be due to the difference in calibration between the 2 assays (The Binding Site assay has a standard curve while the Corgenix has a single point).

b. *Matrix comparison:*
Both assay use serum.
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:
   The normal range was determined on serum from 200 normal adult blood donors.

	IgA	IgG	IgM
Negative result	<20 APS U/mL	<11 GPS U/mL	<25 MPS U/mL
Positive result	≥ 20 APS U/mL	≥11 GPS U/mL	≥25 MPS U/mL

M. Conclusion:
The Bindazyme® Human anti-Phosphatidylserine IgA, IgG, IgM EIA kits appears to be substantially equivalent to devices regulated under 21 CFR 866.5660, Multiple Autoantibodies Immunological Test System, Product Code MID, Class II.