510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K042152

B. Purpose for Submission:

New device

C. Measurand:

IgG subclasses 1, 2, 3, and 4

D. Type of Test:

Semi-quantitative turbidimetry

E. Applicant:

The Binding Site, Ltd.

F. Proprietary and Established Names:

Binding Site Human IgG Subclass Kit for use on the Olympus AU Series of Instruments.

G. Regulatory Information:

1. Regulation section:
   21 CFR 866.5510, Immunoglobulins A, G, M, D, E Immunological Test Systems

2. Classification:
   Class II

3. Product Code:
   CFN, Method, Nephelometric, Immunoglobulins (G, A, M)

4. Panel:
   Immunology (82)

H. Intended Use:

1. Intended use(s):
   This kit is intended for quantifying human IgG subclasses 1, 2, 3, and 4 immunoglobulins in serum on the Olympus™ AU series analyzers. Measurement of these immunoglobulins aids in the diagnosis of abnormal protein metabolism and the body's lack of ability to resist infectious agents.

2. Indication(s) for use:
   Same as Intended Use

3. Special condition for use statement(s):
   For prescription use only

4. Special instrument Requirements:
   Olympus™ AU series analyzers: AU400, AU640, AU2700 and AU5400

I. Device Description:

The set of reagents includes: Human IgG1/IgG2 antisera - sheep IgG monospecific for the relevant subclass supplied in liquid form; Human IgG3/IgG4 reagent - latex coated with sheep IgG monospecific for the relevant subclass and supplied in liquid form; Human IgG1/2 Olympus Calibrators 1-6; Human IgG3/4 Olympus Calibrators 1-6; Reaction Accelerators (reaction accelerator to the appropriate subclass); and Human IgG Subclass Low and High Controls.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Binding Site IgG subclass kit for use on the Behring BNII analyzer

2. Predicate K number(s):
   K012291

3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
Indications for use	Measurement of human IgG subclasses 1,2,3 and 4 immunoglobulins in serum as an aid in the diagnosis of abnormal protein metabolism and the body's lack of ability to resist infectious agents.	Same
Solid phase for capture of IgG3 and IgG4	Latex	Same
Controls	Low and high	Same
Reaction accelerator	Included	Included but called supplementary reagent
Differences		
Item	**Device**	**Predicate**
Instrument	Olympus AU series	Behring BNII
Principle	Turbidimetry	Nephelometry
Number of calibrators for IgG1 and 2	Six	Single calibrator diluted to 6 points
Number of calibrators for IgG3 and 4	Six	Single calibrator diluted to 5 points
Measuring range in mg/L	IgG1: 1500 - 40000 IgG2: 486 - 13125 IgG3: 78 - 1250 IgG4: 20 - 750	IgG1: 2625 - 84000 IgG2: 613 - 19600 IgG3: 55 - 875 IgG4: 38 - 613

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

None referenced

L. Test Principle:

IgG subclasses are measured by turbidimetry. This involves the addition of the diluted test sample to a solution containing human IgG 1, 2, 3 or 4 antisera in a reaction cuvette. A beam of light is passed through the cuvette and as the antigen-antibody reaction proceeds, the light passing through the cuvette is scattered increasingly as insoluble immune complexes are formed. The antibody in the cuvette is in excess so the amount of immune complex formed is proportional to the antigen concentration. In turbidity, light scatter is monitored by measuring the decrease in intensity of the incident beam of light. A series of six calibrators are assayed initially to produce a calibration curve of measured light scatter versus antigen concentration. Controls and patient samples are then assayed and the results read from the

calibration curve. For measurement of IgG3 and IgG4, the antisera are coated onto latex particles to make the antigen-antibody complex sufficiently large to be detected.

M. Performance Characteristics (if/when applicable):

All studies were performed on the Olympus AU400. Other instruments in the AU series utilize the same software and the same parameters are used. The instruments contain the same hardware units but differ in sample capacity and speed. Some instruments contain more reaction cells.

1. Analytical performance:
   a. Precision/Reproducibility:
   Within run reproducibility was demonstrated by running three different concentration samples ten times each. The mean and %CV was calculated for each set of ten results. For IgG1, %CV for the three samples ranged from 2.6 to 3.1%; for IgG2 from 1.8 to 5.5%; for IgG3 1.2 to 1.3%; and for IgG4 %CV ranged from 1.3 to 2.7%.

Within run			
IgG1	Sample 1	Sample 2	Sample 3
Mean in mg/L	2241.1	8136.9	21889.5
% CV	2.6	3.1	3.1
IgG2			
Mean in mg/L	695	2975.6	8397
% CV	5.5	2.2	1.8
IgG3			
Mean in mg/L	225.1	457.6	777.4
% CV	1.2	1.2	1.3
IgG4			
Mean in mg/L	111	361.8	671.7
% CV	2.5	1.3	2.7

Between run reproducibility was demonstrated by running three different concentration samples in singlicate on ten separate days. The mean and %CV was calculated. For IgG1, %CV for the three samples ranged from 1.6 to 3.2%; for IgG2 from 1.2 to 5.1%; for IgG3 3.0 to 3.5%; and for IgG4 %CV ranged from 3.1 to 4.8%.

Between run			
IgG1	Sample 1	Sample 2	Sample 3
Mean in mg/L	2230.7	7991.1	22162.2
% CV	3.2	1.6	2.4
IgG2			
Mean in mg/L	714.3	3006	8339
% CV	5.1	1.2	1.8
IgG3			

Between run			
Mean in mg/L	238.3	480.2	781.2
% CV	3.0	3.2	3.5
IgG4			
Mean in mg/L	110.1	361.9	683.7
% CV	3.1	3.5	4.8

b. Linearity/assay reportable range:
The absolute measuring ranges for the assays are: IgG1: 150-80000 mg/L; IgG2: 97-52500 mg/L; IgG3: 15.6-5000 mg/L; and IgG4: 4-3000 mg/L. To confirm the linearity of the assays, two-fold serial dilutions of clinical serum samples with high concentrations of the relevant IgG subclass were tested. Each dilution was analyzed in triplicate. Linear regression analyses were performed comparing the actual results versus the calculated values. The following equations were obtained:
IgG1: $y = 0.985x + 415.52$, $r^2 = 0.9997$
IgG2: $y = 0.9955x + 29.67$, $r^2 = 0.9999$
IgG3: $y = 0.9971x + 2.217$, $r^2 = 0.9999$
IgG4: $y = 1.006x + 2.423$, $r^2 = 0.9993$

Additional studies performed to demonstrate linearity at the lower end of the measuring ranges showed:
IgG1: $y = 1.0068x + 131.88$, $r^2 = 0.9986$
IgG2: $y = 0.9993x + 69.23$, $r^2 = 0.9996$
IgG3: $y = 1.0023x + 4.6866$, $r^2 = 0.9993$
IgG4: $y = 1.0057x + 4.1172$, $r^2 = 0.9998$

c. Traceability (controls, calibrators, or method):
The calibrators and controls were not standardized against a recognized reference material.

d. Detection limit:
Not determined.

e. Analytical specificity:
Potentially interfering substances (bilirubin = 0.3 g/L, hemoglobin = 5 g/L, and chyle = 1930 turbidity units) were spiked into normal sera with water spiked in the same way to act as control to see if the interfering substance had any effect on the assay value. No significant effect on assay results was noted by addition of these substances and the % differences compared to the control ranged from -3.5 to 1.6%.

f. Assay cut-off:
See Expected Values/Reference Range

2. Comparison studies:
    a. *Method comparison with predicate device:*
    Fifty clinical serum samples and 50 normal serum samples were used for the comparison. The concentrations for IgG1 ranged from 2531 to 11154 mg/L; IgG2 ranged from 1044 to 11693 mg/L, IgG3 ranged from 146 yo 1178 mg/L and IgG4 ranged from 13 to 720 mg/L. Linear regression analyses yield the following results:
    IgG1: $y = 0.9761x + 365.12$, $r^2 = 0.98$
    IgG2: $y = 1.0391x + 41.96$, $r^2 = 0.9813$
    IgG3: $y = 0.9416x + 37.64$, $r^2 = 0.9833$
    IgG4: $y = 1.0548x + 11.175$, $r^2 = 0.9889$

    b. *Matrix comparison:*
    Serum is the recommended matrix for the new assays as well as the predicate device assays.

3. Clinical studies:
    a. *Clinical sensitivity:*
    Not determined

    b. *Clinical specificity:*
    Not determined

    c. *Other clinical supportive data (when a and b are not applicable)*
    Not applicable

4. Clinical cut-off:
    Not determined

5. Expected values/Reference range:

    The pediatric and adult ranges shown in section 11.1 and 11.2 of the labeling (package insert) were obtained by measuring the total IgG and/or IgG subclasses using the Binding Site radial immunodiffusion (RID) assay. A validation study was performed to show correlation between the BNII nephelometric method and RID and the same calibrator material and antibody specificities are applied across the Binding Site IgG subclass ranges irrespective of the methodology used. The ranges listed in the labeling are in line with those found in Tietz Fundamentals of Clinical Chemistry, 4th edition, 1997. The manufacturer recommends that users generate ranges for their own patient populations.

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.