510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k072889

B. Purpose for Submission:
New submission

C. Measurand:
IgG and IgG subclasses 1, 2, 3, and 4

D. Type of Test:
Semi-quantitative Nephelometry

E. Applicant:
The Binding Site, Ltd.

F. Proprietary and Established Names:
Binding Site Human IgG and IgG Subclass Liquid Reagent Kits for use on the SPA$_{PLUS}$ Analyzer

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 and IgG subclasses 1, 2, 3, and 4 immunoglobulins in serum using the SPA$_{PLUS}$ Analyzer. 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 conditions for use statement(s):
   For prescription use only
4. Special instrument requirements:
   SPA$_{PLUS}$ Analyzer (k062372)

I. Device Description:
The device consists of these reagents: Polyclonal monospecific sheep antisera in liquid form for IgG1 and IgG2; polystyrene latex coated with polyclonal monospecific sheep antisera for IgG3 and IgG4; total IgG antiserum in liquid form; Calibrators 1-6; Low and High controls (pooled human sera) in liquid form; and supplementary reagent.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Binding Site Human IgG Subclass kit for use on the Behring II Analyzer
   Dade Behring N Antisera to Human Immunoglobulins

2. Predicate K number(s):
   k012292 (IgG subclass)
   k042735 (IgG)

3. Comparison with predicate:

Similarities		
Item	Device	Predicates
Intended Use	Aid in the diagnosis of abnormal protein metabolism and the body's lack of ability to resist infectious agents.	Same
Number of calibrators	Six	Same
Detection Method	Nephelometry	Same

Differences		
Item	Device	Predicate
Sample Matrix	Serum	Serum, plasma, urine and CSF
Instrument	The Binding Site SPA$_{PLUS}$™	Dade Behring II Analyzer

K. Standard/Guidance Document Referenced (if applicable):
   CLSI document EP5-A: Evaluation of Precision Performance of Clinical Chemistry;

L. Test Principle:
   Nephelometry involves the measurement of light scattered by particles in solution. This can be applied to the measurement of soluble antigen since, in the presence of the appropriate antibody, insoluble immune complexes are formed, which are light scattering. When the antibody is in excess, the light scatter is directly proportional to the concentration of antigen. Concentrations are automatically calculated by reference to a standard curve stored within the instrument.

   Latex-enhanced Antibodies: Some antibody-antigen reactions do not form sufficiently large immune complexes to be detected nephelometrically. If the antibody is coated onto latex particles of a suitable size, the light scattering ability of the immune complexes formed with antigen is enhanced sufficiently to enable turbidimetric detection.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. *Precision/Reproducibility:*

   A precision study was performed according to CLSI document EP5-A: Evaluation of Precision Performance of Clinical Chemistry Approved Guideline. Three different serum samples representing the low, medium and high levels of the measuring range were tested over 21 days, 2 runs per day, in duplicate. One user assessed three different samples using three different lots. Acceptance criteria ±15% were met

IgG		Within run		Between-run		Between-day		Total Precision	
Level	Mean (g/L)	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low	2.43	0.05	2.1	0.03	1.4	0.18	7.6	0.19	8.0
Medium	6.0	0.09	1.5	0.07	1.1	0.29	4.8	0.31	5.1
High	33.0	0.81	2.5	0.0	0.0	0.61	1.91	1.02	3.1

IgG 1		Within run		Between-run		Between-day		Total Precision	
Level	Mean (g/L)	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low	2.55	0.05	2.1	0.08	3.2	0.22	8.7	0.24	9.5
Medium	3.83	0.10	2.7	0.09	2.3	0.30	7.8	0.33	8.5
High	31.86	0.70	2.2	0.46	1.5	1.65	5.3	1.85	5.9

IgG 2		Within run		Between-run		Between-day		Total Precision	
Level	Mean (g/L)	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low	0.245	0.01	3.6	0.02	7.3	0.01	4.9	0.02	9.6
Medium	2.349	0.06	2.4	0.07	3.0	0.07	3.1	0.12	4.9
High	4.145	0.03	0.8	015	3.5	0.07	1.6	0.16	3.9

IgG 3		Within run		Between-run		Between-day		Total Precision	
Level	Mean (g/L)	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low	0.081	0.0	2.8	0.00	2.8	0.01	8.3	0.01	9.2
Medium	0.227	0.01	3.3	0.00	0.0	0.01	3.8	0.01	5.1
High	0.844	0.03	3.3	0.00	0.0	0.03	3.3	0.04	4.6

IgG 4		Within run		Between-run		Between-day		Total Precision	
Level	Mean (g/L)	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low	0.049	0.0	5.3	0.00	0.0	0.00	8.3	0.00	9.8
Medium	0.066	0.0	4.0	0.00	2.3	0.01	7.8	0.01	9.0
High	0.769	0.01	1.9	0.01	0.9	0.04	5.1	0.04	5.5

   b. *Linearity/assay reportable range:*

   Three serum samples previously identified as containing high levels of IgG and IgG subclasses were serially diluted (off-line dilution, 1:2 to 1:128) and each dilution assayed using the SPA$_{PLUS}$, using three different lots. The assays

were run at the normal sample dilutions 1/10 and 1/1 (neat) to cover the measuring ranges as follows:

IgG   1.65 – 35.0 g/L
IgG1   1.50 - 36.0 g/L
IgG2   0.20 – 5.5 g/L
IgG3   0.055 – 1.00 g/L
IgG4   0.03 – 0.85 g/L

Each dilution was analyzed in duplicate. Linear regression analyses were performed comparing the actual results versus the calculated values. The following equations were obtained:

IgG $y=1.0082x - 0.0784, r^2=0.9970$
IgG1 $y=0.9945x - 0.2107, r^2=0.9992$
IgG2 $y=1.0091x -0.0046, r^2=0.9996$
IgG3 $y=1.0076x – 0.001, r^2=0.9998$
IgG4 $y=0.9993x – 0.0005, r^2 = 1.00$

Data confirms the linearity of the assay over the reportable measuring ranges.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Calibrator and corresponding control sera are sourced from different pools of sera. Calibrator and control value assignment is controlled during kit production using set target values for the initial fluid of pooled human serum. An internal reference standard (IR7955) was assigned by comparison with the CRM470 International Reference Material. Values of 10.081 g/L for IgG, 5.3691 g/L for IgG1, 3.597 g/L for IgG2, 0.464 g/L for IgG3 and 0.334 g/L for IgG4 were obtained. This IR was then used to validate curves and standardize kit calibration.

To demonstrate the stability of the Binding site's (TBS) IgG and IgG subclass 1-4 assays on the SPA$_{PLUS}$ Analyzer, three kit lots of IgG and each of the 4 IgG subclasses were tested according to the product insert. The first assays were run following 1 week of storage at 22°C for 1 week to simulate shipping conditions. The kits were tested at Time 0 and at 3 and 7 months. The kits were stored at recommended temperature of 2-8°C. At each stage a calibration curve was run together with the two kit controls and an internal reference (IR) standard.

The results obtained demonstrate that the IgG and IgG subclass 1-4 kits are stable for at least 7 months from the date of manufacture when stored at the recommended storage temperature of 2-8°C.

d. *Detection limit:*
Limit of blank was determined as the mean concentration + 2 SD given by 20 determinations of the sample diluent and results are summarized below:
IgG   0.165 g/L

IgG1   0.150 g/L
IgG2   0.02 g/L
IgG3   0.0055 g/L
IgG4   0.003 g/L

   e. *Analytical specificity:*
Susceptibility to interference was assessed by adding high concentrations of bilirubin, hemoglobin and chyle to test serum samples which contained a known concentration of IgG, IgG1, IgG2, IgG3, and IgG4. The test samples consisted of the respective low kit controls, which were diluted with saline. Percentage interference was calculated from comparison with (serum + saline) blank. Deviations less than or equal to ±10% of the blank value were considered to show no significant interference. Results demonstrated

- No significant assay interference by 200 mg/L bilirubin or 5g/L hemoglobin.
- No significant assay interference by 4395 formazine turbidity units (FTU) of chyle IgG1, IgG3 and IgG4.
- Slight assay interference (15%) with chyle (2210 FTU) but no significant interference was noted with chyle at 1670 FTU for IgG2.

   f. *Assay cut-off:*
Not provided.

2. Comparison studies:
   a. *Method comparison with predicate device:*
The IgG assay was compared to the Dade Behring assay (k042735) and the IgG subclass assays were compared to TBS IgG subclass BN assays (k012292). For these studies, 60 sera (30 normal and 30 clinical samples with elevated levels of IgG/IgG1-4) were tested. The following summation data were provided:

IgG: $y = 1.0779x - 0.9028$ $R^2 = 0.9787$
IgG1: $y = 1.0437x - 0.1612$ $R^2 = 0.9850$
IgG2: $y = 1.059x - 0.1619$ $R^2 = 0.9874$
IgG3: $y = 1.0062x - 0.0082$ $R^2 = 0.97831$
IgG4: $y = 0.9897x - 0.0052$ $R^2 = 0.9959$

Additional ten samples were assayed to cover the low end of the measuring ranges with the following results:

IgG: $y = 0.932x + 0.0433$ $R^2 = 0.9844$
IgG1: $y = 0.9145x - 0.0546$ $R^2 = 0.9985$
IgG2: $y = 0.9972x - 0.167$ $R^2 = 0.9883$
IgG3: $y = 0.948x - 0.0015$ $R^2 = 0.9944$
IgG4: $y = 0.9237x - 0.0501$ $R^2 = 0.9987$

   b. *Matrix comparison:*

Serum is the only recommended sample for this assay.

3. Clinical studies:
   a. *Clinical Sensitivity:*
   None provided.

   b. *Clinical specificity:*
   None provided.

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

4. Clinical cut-off:
   Not provided

5. Expected values/Reference range:
   Adult normal range was assessed using 120 normal sera for IgG and 30 normal sera for the subclasses obtained from healthy adult blood donors. All samples were stored at $-20^{o}C$ prior to the assay. A non-parametric distribution of IgG results was seen that gave a 95 percentile reference interval of 6.103-16.16 g/L with a mean of 10.926 g/L. These results are very similar to that of the predicate device (Dade Behring).

IgG Subclass	Number (n)	Mean (g/L)	Median (g/L)	95th Percentile range (g/L)
Total IgG	120	10.926	10.807	6.103-16.16
IgG1	30	6.33	6.085	3.824-9.286
IgG2	30	4.528	4.541	2.418-7.003
IgG3	30	0.7907	0.7064	0.2182-1.7606
IgG4	30	0.28	0.2153	0.0392-0.864

Pediatric normal ranges shown in the package insert (section 11.2) were obtained using Binding Site radial immunodiffusion (RID) assay. In order to justify the inclusion of pediatric data and to demonstrate correlation between RID and the Binding Site Human IgG Subclass Liquid Reagent Kits for use on the SPA$_{PLUS}$ Analyzer, 14 panel samples were tested and the following results were obtained:

IgG: $y= 1.1112x – 1.3378$ $R^2 = 0.9932$
IgG1: $y= 0.9898x – 0.1586$ $R^2 = 0.9828$
IgG2: $y= 0.9094x – 0.0039$ $R^2 = 0.9489$
IgG3: $y= 1.2158x – 0.1089$ $R^2 = 0.9489$
IgG4: $y= 0.8967x – 0.0123$ $R^2 = 0.9832$

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.