510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k090920

B. Purpose for Submission:
Device modification to the IgG3 assay: to increase the assay measuring range

C. Measurand:
IgG3 antibodies

D. Type of Test:
Quantitative turbidimetric assay

E. Applicant:
The Binding Site, Ltd.

F. Proprietary and Established Names:
Human IgG Subclass Liquid Reagent Kit

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):
   Human IgG Subclass Liquid Reagent kit is intended as an in vitro diagnostic test for the quantification of Human IgG Subclasses in serum. Measurement of these immunoglobulins aids in the diagnosis of abnormal protein metabolism and the body's lack of ability to resist infectious agents and should be used in conjunction with other clinical information.
2. Indication(s) for use:
   Same as Intended Use
3. Special conditions for use statement(s):
   For Prescription Use only
4. Special instrument requirements:
   Roche Hitachi Modular P (k953239)

I. Device Description:
The device consists of these reagents: monospecific sheep anti-human IgG3 Latex Reagent (Latex bound to IgG3) supplied in stabilized liquid form; Reaction buffer; IgG3 latex buffer; Single calibrator; Low and high controls.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Human IgG3 Subclass Liquid Reagent Kits (latexed) (for use on Behring BNII analyzer)

2. Predicate 510(k) number(s):
   k012292
3. Comparison with predicate:

Similarities		
Item	Device Human IgG3 Subclass Liquid Reagent Kit for use on the Hitachi Modular P	Predicate Human IgG3 Subclass Liquid Reagent Kit for use on the Behring BNII Analyser (k012292)
Intended Use	Quantification of IgG subclasses in human serum. Measurement of immunoglobulins aids in the diagnosis of abnormal protein metabolism and the body's lack of ability to resist infectious agents and should be used in conjunction with other clinical information.	Same
Sample type	Human serum	Same
Reference intervals	218.2-1760.6mg/L Additional ranges for children	Same
Antibodies	Sheep	Same
Calibrator	Single calibrator	Same
Traceability	Standardized against CRM 470	Same
Calibrator Matrix	Pool of de-lipidated human serum	Same
Controls	Normal and High level liquid ready-to-use controls made from stabilized de-lipidated human serum.	Same

Differences		
Item	Device	Predicate
Analyzers	Modular P	BNII
Method	Turbidimetry	Nephelometry
Measuring range	~52.5 mg/L-1312.5 mg/L	~55 mg/L-875mg/L
Sensitivity	~7mg/L	~2.7mg/L

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

CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline

CLSI EP6-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach

L. Test Principle:

The determination of soluble antigen (IgG3) concentration by turbidimetric methods involves the reaction with specific antiserum to form insoluble complexes. To enable turbidimetric detection of these antigen/antibody complexes, latex-enhanced antibodies are used in this assay to enhance the size of the complexes. When light is passed through the suspension, a portion of the light is transmitted and focused onto a photodiode by an optical lens system. The amount of transmitted light is indirectly proportional to the specific protein concentration in the test sample. Concentrations are automatically calculated by reference to a standard curve stored within the instrument.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Three serum samples representing the low, medium and high levels of the measuring range were tested in duplicate, 2 runs per day over 21 days using three different reagent lots (n=84). The results are summarized in the table below:

IgG		Within- run		Between-run		Between-day		Total Precision	
Level	Mean (mg/L)	SD (mg/L)	%CV	SD (mg/L)	%CV	SD (mg/L)	%CV	SD (mg/L)	%CV
Low	124.4	9.8	8.0	9.4	7.7	1.0	0.8	13.6	11.2
Medium	550.9	11.7	2.0	8.8	1.5	20.6	3.6	0.31	5.1
High	939.6	24.9	2.5	55.3	5.6	18.8	1.9	25.3	4.4

b. Linearity/assay reportable range:

Testing was performed according to CLSI EP6-A.

A serum sample of 1557 mg/L was diluted at regular intervals with a low level sample of 80 mg/L (100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, 7.5% and 5%). In order to cover the assay range, two additional samples with IgG3 concentrations of 123.3 and 122.8 mg/L were tested. These two latter samples were similarly diluted with sample diluent. Each dilution was run in triplicate on two different kit lots. Results are summarized below:

Mean Conc Range (mg/L)	% recovery	% CV	Linear fit
89.1 – 1325.05	99.8 – 118.8	0.1 - 12.7	1.0809x + 2.401 R^2 0.9844
9.7 - 123.3	94.2 - 111.1	0.1 - 18.1	0.9573x + 1.952 R^2 0.9573
8.0 – 122.8	96.2 - 118.4	1.1 - 14.1	0.9638x + 2.335 R^2 0.9638

The claimed assay measuring range is ~52.5 to 1312.5 mg/L.

High dose hook effect:
A check for antigen excess capacity was performed using the same kit components that were used for the linearity testing. A sample with a value of 4830 mg/L, which was three and a half times higher than the upper limit of the calibration curve (1335 mg/L), was tested at the standard 1/15 sample dilution. This resulted in a flagged value (LIMH) of greater than the upper limit and the Modular P analyzer automatically retested the sample at a higher dilution of 1/50. This result was also flagged showing that the assay has an antigen excess capacity of at least 4830 mg/L.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Calibrators and Controls: These are pooled human sera and are supplied in liquid stabilized form. The concentrations of IgG subclasses are obtained by comparison with the CRM470 international reference material.
Stability study: Two kit lots were tested at QC release and post expiry after storage at the recommended temperature of 2-8°C. At each stage calibration curves were run along with high control, low control and an internal reference material. The % difference between the QC release and post expiry date ranged 1.3-4.0 %. The kit is stable for at least 18 months when stored at the recommended storage temperature of 2-8°C.
Open Vial Stability: A curve was calibrated on day 0. The kit controls and internal reference standard for three lots were run against the curve. The kit controls were then stored at 2-8°C and retested after 1 month. The % difference from day 0 to 1 month later ranged between 0.3 – 11.3%. The reagents once opened are stable for 4 weeks at 2 - 8°C.

d. *Detection limit:*
Limit of blank (LoB) was determined by using the mean blank value of 21 replicates of the standard 1 [sample diluent (saline)] + 3 standard deviations. The LoB was estimated as 3.5 mg/L.
The lower limit of detection (LoD): LoD was determined as the LOB x2 (3.56 x 2 = 7.12 mg/L). LoD was estimated as 7 mg/L.

e. *Analytical specificity:*
Interference Study/Endogenous compounds: Two serum samples (613 mg/L and 84 mg/L) were spiked with the following interferents: bilirubin at 200 mg/L, hemoglobin at 5 g/L, and intralipid at 0.5 %. A comparison of the unspiked samples to the spiked samples indicates that the tested concentrations do not interfere with the IgG3 assay results.

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

2. Comparison studies:

a. *Method comparison with predicate device:*
The performance of The Binding Site Latex IgG3 Kit run on the Roche Modular P analyzer (New device) was compared with the IgG3 BNII kit run on the BNII analyzer (Predicate device). 51 samples ranging from 24.0 to 1240.1 mg/L were tested. They included: 22 quality control samples (patient samples with known values and used for quality control checks), 12 normal

samples; 13 clinical samples from patients with abnormal IgG and 4 samples with IgG3 concentrations well below the normal range (approximate concentrations 40, 64, 119 and 62 mg/L). A Passing Bablock linear regression analysis of all the samples gave the following result:
y=1.03x – 5.77 mg/L $R^2 = 0.964$
Intercept 95%CI -25.34 to 10.16
Slope 95% CI 0.98 to 1.09

b. Matrix comparison:
Not applicable

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 applicable
5. Expected values/Reference range:
   Adult normal ranges for IgG3 were obtained from a limited number of British samples and are intended for guidance purposes only.
   N=30 Mean = 790.7 mg/L 95 % range 218.2 - 1761 mg/L

   The sponsor recommends that each user generate their own local reference ranges.

   Pediatric ranges were obtained by measuring the total IgG and subclass content of pediatric serum samples from a Birmingham, UK hospital using Binding Site Radial Immunodiffusion products. All concentrations are in mg/L.

Age	N	Mean (mg/L)	95th percentile Range (mg/L)
0-2	39	445	186-853
2-4	36	396	173-676
4-6	49	531	99-1221
6-8	43	437	155-853
8-10	32	521	127-853
10-12	46	620	173-1730
12-14	54	583	283-1250
14-18	48	708	230-1960

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.