

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY**

**A. 510(k) Number:**

k121045

**B. Purpose for Submission:**

New device

**C. Measurand:**

Human Albumin

**D. Type of Test:**

Turbidometric assay

**E. Applicant:**

The Binding Site Group Ltd.

**F. Proprietary and Established Names:**

Human Albumin CSF kit on SPA<sub>PLUS</sub> model NK032.L.S

**G. Regulatory Information:**

1. Regulation section:

21 CFR 866.5040, Albumin immunological test system

2. Classification:

II

3. Product code:

DCF, Albumin, antigen, antiserum, control

4. Panel:

Immunology (82)

**H. Intended Use:**

1. Intended use(s):

Human Albumin CSF Kit for use on SPA<sub>PLUS</sub> is intended for the in-vitro quantification of human albumin in serum and cerebrospinal fluid (CSF) samples on the SPA<sub>PLUS</sub> analyser.

Measurement of albumin aids in the diagnosis of kidney and intestinal diseases in conjunction with other laboratory and clinical findings.

2. Indication(s) for use:

Same as Intended Use.

3. Special conditions for use statement(s):

Prescription use only

4. Special instrument requirements:

SPA<sub>PLUS</sub> analyzer - model NK032.L.S

**I. Device Description:**

The kit contains the following materials or reagents:

- Albumin CSF Antiserum: Antibody monospecific for albumin and is supplied in stabilized liquid form. It contains 0.099% sodium azide, 0.1% E-amino-n-caproic acid (EACA) and 0.01% benzamidine as preservatives.
- Calibrator and Controls: These consist of pooled human serum and are supplied in stabilized liquid form. They contain 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives. For the calibrators, there are 6 levels: 17, 34, 69, 138, 200 and 275 mg/L. For the control, a high and a low level with concentrations of 750 mg/L and 325 mg/L respectively.
- Reaction Buffer: Containing 0.099% sodium azide as a preservative.

Use of the device requires the SPA<sub>PLUS</sub> analyzer (instrument cleared under k040958) using software (cleared under k062372).

**J. Substantial Equivalence Information:**

1. Predicate device name(s):

Siemens N Antiserum to Human Albumin

2. Predicate 510(k) number(s):

k972929

3. Comparison with predicate:

| <b>Similarities</b>          |  |   |
|------------------------------|--|---|
| <b>Item</b>                  | <b>Device</b>  | <b>Predicate</b>  |
| Indication for use statement | Measurement of albumin aids in the diagnosis of kidney and intestinal diseases in conjunction with other laboratory and clinical findings. | an aid in the diagnosis of kidney and intestinal diseases |
| Analyte                      | Albumin  | Same  |

| <b>Differences</b>  |   |   |
|---------------------|---|---|
| <b>Item</b>         | <b>Device</b>   | <b>Predicate</b>  |
| Method              | Turbidometry  | Nephelometry  |
| Specimen type       | Human CSF and serum   | CSF, serum, heparinized and EDTA plasma and urine   |
| Instrument          | SPA <sub>PLUS</sub> analyzer  | BNII System   |
| Antibody            | Sheep anti-human albumin  | Rabbit anti-human albumin   |
| Measuring range     | CSF:<br>170- 2700 mg/L (1/10 dilution)<br>Serum (1/301 off line dilution):<br>5.1-81 g/L (1/1 dilution)<br>51-810 g/L (1/10 dilution) | 86 – 2750 mg/L (at 1/10 dilution of CSF specimen)<br>Range for serum not present in labeling              |
| Open vial stability | 3 months  | 4 weeks   |
| On-board stability  | 30 days   | 5 days at 8 hours each  |
| Calibrators         | 6 levels (17, 34, 69, 138, 200, 275 mg/mL)  | N Protein Standard SL (available separately). Levels and concentrations not available                     |
| Controls            | High control (750 mg/mL)<br>Low control (325 mg/mL)   | N/T Protein Controls SL/L, M and H (human), available separately. Levels and concentrations not available |

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

The following Standards documents are referenced in the submission:

1. Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition (EP5-A2)
2. Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical

**L. Test Principle:**

The determination of soluble antigen concentration by turbidimetric method involves the reaction of albumin with specific antiserum to form insoluble complexes. When light is passed through the suspension containing the insoluble complexes a portion of the light is transmitted and detected by a photodiode in an optical lens system. The amount of transmitted light is proportional to the concentration of insoluble complexes and thus proportional to the specific protein concentration in the test sample when compared with transmitted light for calibrators of known concentration. Concentrations are automatically calculated by reference to a calibration curve stored within the instrument.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

a. *Precision/Reproducibility:*

*Cerebrospinal fluid:* The precision evaluation was based upon CLSI guideline; “Evaluations of Precision Performance of Quantitative Measurement Methods EP5-A2”. The study was carried out using three pools of CSF to which albumin was added to give three concentrations; 1 low, 1 middle, and 1 high analyte concentration. Each precision sample was analyzed over 5 days with 2 runs per day (duplicate determinations in each run); giving a total of 20 measurements for each sample.

Three different samples were assessed using one instrument and one reagent lot. The target and observed albumin concentrations for the three concentration levels as follows:

| Sample   | Target concentration (mg/L) | Measured Concentration (mg/L) |
|----------|-----------------------------|-------------------------------|
| Sample 1 | 2200                        | 2172.7                        |
| Sample 2 | 350                         | 395.3                         |
| Sample 3 | 240                         | 301.2                         |

The kit controls were included in each assay run over the 5 day period of the study. All the control results were within the acceptance criteria ( $\pm 15\%$  of the assigned control value). For total precision, a %CV no greater than 10% was considered acceptable.

| Sample | N  | Mean (mg/L) | Within Run |     | Between run |     | Between day |     | Total |     |
|--------|----|-------------|------------|-----|-------------|-----|-------------|-----|-------|-----|
|        |    |             | SD         | %CV | SD          | %CV | SD          | %CV | SD    | %CV |
| High   | 20 | 2303.59     | 35.56      | 1.5 | 0.00        | 0.0 | 88.15       | 3.8 | 95.05 | 4.1 |
| Medium | 20 | 406.60      | 4.73       | 1.2 | 8.75        | 2.2 | 12.36       | 3.0 | 15.87 | 3.9 |
| Low    | 20 | 307.45      | 4.23       | 1.4 | 6.17        | 2.0 | 5.16        | 1.7 | 9.09  | 3.0 |

*Serum:* The study was carried out using three serum pools; 1 low, 1 middle, and 1 high analyte concentration. Analysis was carried out over 21 days with 2 runs per day (each of the two runs in duplicate) using three reagent lots and three instruments. Standard deviations and % CV were calculated for the different sources of variation. Acceptance criteria were: total precision < 10%.

| Sample | Mean (mg/L) | Within run |      | Between run |      | Between day |      | Total |      |
|--------|-------------|------------|------|-------------|------|-------------|------|-------|------|
|        |             | SD         | CV % | SD          | CV % | SD          | CV % | SD    | CV % |
| High   | 2158        | 42.9       | 2.0  | 45.1        | 2.1  | 163.0       | 7.6  | 174.5 | 8.1  |
| Medium | 415         | 7.5        | 1.8  | 15.0        | 3.6  | 32.9        | 7.9  | 37.0  | 8.9  |
| Low    | 252         | 5.8        | 2.3  | 5.7         | 2.3  | 23.4        | 9.3  | 24.8  | 9.8  |

*b. Linearity/assay reportable range:*

*Cerebrospinal fluid:* A linearity on dilution study was carried out based on CLSI EP6-A (Evaluation of the Linearity of Quantitative Measurement Procedures). The linear ranges were established by analysis of a dilution series of pooled CSF, and evaluation of results at each concentration against pre-defined goals for recovery and %CV for each of 3 lots. Regression equations and R<sup>2</sup> values were also generated to support the linearity on dilution claim. The allowable non-linearity goal was 15%CV. The assay was shown to give a linear response over the measuring range.

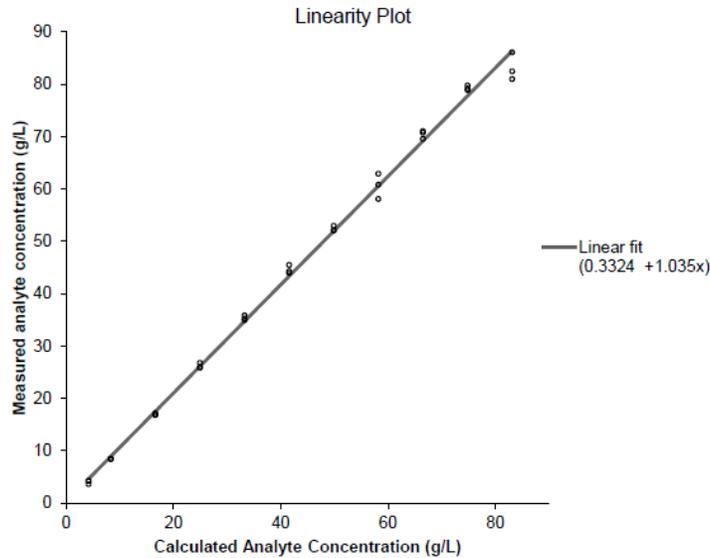
The range of reportable concentrations in the assay using the CSF specimen is 170 to 2700 mg/L.

*Serum:* A study was performed to evaluate the linearity of the assay on dilution using serum samples. A dilution series was prepared by blending a high pool with instrument diluent. The high pool was prepared from equal parts of 6 normal serum samples that were then diluted 1/130 with instrument diluent. The high pool concentration (83.076 g/L) was determined by triplicate analysis. The dilution series was prepared by blending the high pool with instrument diluent in varying percentages to give a dilution series of 11 samples. The expected analyte concentration was calculated from the percentage of the high pool in the sample. Each of the dilution samples were analyzed in triplicate and the mean observed result calculated. Linearity was evaluated by calculating the mean percentage recovery for each sample in the dilution series. The acceptance criteria for the study were;

- CV for the 3 replicates of <8%
- Mean recovery between 80 – 120%
- Mean recovery at the medical decision point (35g/L) 90 – 110%

A linear regression equation of  $y = 1.035x + 0.3324$  g/L with a correlation coefficient of 0.9965 was obtained. The slope was 1.035 (95% CI: 1.012 – 1.057). The intercept was 0.3324 (95% CI: 0.7670– 1.4318 g/L). The slope of the best fit linear regression line is not statistically equivalent with 1.0 (1.0 is outside the 95% confidence interval of the line, 1.012 – 1.057). While the non-equivalence of the slope with 1.0 could

indicate non-linearity, the deviation from non-linearity is not substantial (from 1.2% to 5.7%). The linear range on dilution using a serum specimen was 4.01 – 83.18 g/L. Evaluation of the graph of linearity also indicates substantial linearity as shown below:



#### *Antigen Excess*

The assay does not give results in antigen excess up to a concentration of 500 mg/L at the standard 1/10 sample dilution. In order for a sample to report a value in antigen excess (i.e. a false low result), the OD value of the assay would have to be less than the OD value of the highest calibrator.

#### *c. Traceability, Stability, Expected values (controls, calibrators, or methods):*

The calibrators and controls were standardized to the reference standard ERM-DA470k. A provisional value was assigned using an albumin assay. The provisional assigned value was used to prepare the bulk calibrator set to the target values. The final assignment was carried out by determining multiple calibration curves using four concentrations of DA470k. The final assigned values of the calibrator set must be within  $\pm 10\%$  of the target values.

Bulk fluid for controls was assigned a provisional value. The final value was assigned to the control using the calibrator set and reagents designated for a particular kit. The control was tested 5 times on 5 different calibration curves and the median result was the control final assigned value.

Stability studies indicate that the kits are stable for 3 months after opening when stored between 2-8°C and for 30 days on-board the SPA<sub>PLUS</sub> at 8-12°C. Real time stability studies are underway and results to date demonstrate that kits are stable at 10 months when stored at 2-8°C. The kit reagents are stable once opened for up to 3 months when stored between testing at 2-8°C.

#### *d. Detection limit:*

The following limits are claimed:

|                       |           |                                      |
|-----------------------|-----------|--------------------------------------|
| Limit of blank        | 0 mg/L    | Material tested – saline             |
| Limit of detection    | 10.6 mg/L | Material tested – CSF                |
| Limit of quantitation | 17 mg/L   | Material tested – calibrator 17 mg/L |

The limits were calculated from 60 replicates of instrument diluent (saline), or cerebrospinal fluid, or calibrator sample with 17 mg/L concentration

The distribution of optical density values from each claimed limit was calculated. The 95<sup>th</sup> percentile value of optical density value for the limit of blank was 0.041. The 5<sup>th</sup> percentile value of optical density value for the limit of detection was 0.055 (corresponding to a concentration of 10 mg/L).

The 5<sup>th</sup> percentile value of optical density value for the limit of quantitation was 0.071 (corresponding to a concentration of 17 mg/L). The lower 5<sup>th</sup> percentile value for the optical density value of the limit of quantitation does not overlap with the 95<sup>th</sup> percentile value for the limit of detection.

*e. Analytical specificity:*

Hemoglobin and bilirubin interference testing in a serum matrix was carried out by spiking 2 separate sera samples with the potential interferents and comparing the analyte (Albumin) level with a negative control sample. Processed sera diluted with instrument diluent were used as the base pools. Interference effects were defined according to CLSI guidelines; In this evaluation the maximum level of acceptable interference was 10% of the analyte concentration. For the 2 interfering substances tested, the following maximum levels of tested interferent and the maximum percentage interference found was the following:

|                        |         |                           |       |
|------------------------|---------|---------------------------|-------|
| Hemoglobin spike level | 5g/L    | % Hemoglobin interference | -4.1% |
| Bilirubin spike level  | 200mg/L | % Bilirubin interference  | 1.8%  |

Interference testing in CSF matrix was carried out at 1 albumin concentration close to the top of the reference range (~500 mg/L). Pools of CSF for the interference study were prepared by pooling CSF samples and adjusting the albumin level to achieve the target concentration. The CSF base pool was prepared by pooling 8 different CSF samples and then spiked with 0.5 mg/L of pure albumin to bring the albumin concentration to 373.5 mg/L.

Susceptibility to interference in albumin concentration in the CSF matrix by hemoglobin (2.5 g/L), bilirubin (100 mg/L), acetaminophen (200 mg/L) and aspirin (600 mg/L) was assessed. This was done by adding the interferent to the CSF base pool containing added albumin. The negative samples were prepared by spiking the CSF base pool with the same volume of commercially obtained blanks. For the acetaminophen and aspirin testing, the interferents were prepared by dissolving the drug in distilled water (100 µL) which was spiked into the CSF base pools (900 µL). The negative control samples were spiked with the same volume of distilled water. Interference effects were defined according to CLSI guideline EP7-A2. Percentage interference was calculated from comparison with the sample blank. Deviations less than or equal to  $\pm 10\%$  of the blank value, and  $D_{obs}$  lower than  $D_{max}$  were chosen to show that any interference was within acceptable levels. The  $D_{max}$  was set as 10% of the albumin concentration at the medical decision point, equivalent to 35 mg/L. The following table summarizes results.

| Interferent                     | Albumin concentration (Blank sample) | Albumin concentration plus interferent | % difference from Blank | Acceptance result |
|---------------------------------|--------------------------------------|--|-------------------------|-------------------|
| Hemoglobin (2.5 g/L spiked)     | 375.53 mg/L                          | 366.30 mg/L                            | -2.46%                  | Acceptable        |
| Bilirubin (100 mg/L spiked)     | 356.10 mg/L                          | 373.93 mg/L                            | 5.01%                   | Acceptable        |
| Acetaminophen (200 mg/L spiked) | 338.60 mg/L                          | 341.47 mg/L                            | 0.85%                   | Acceptable        |
| Aspirin (600 mg/L spiked)       | 345.73 mg/L                          | 331.27 mg/L                            | -4.18%                  | Acceptable        |

Any observed interference with albumin measurement using the Albumin CSF Kit on the SPA<sub>PLUS</sub> was within the acceptable limits (<35 mg/L, <10% difference compared to the negative control).

The package insert notes in the ‘Limitations’ and the ‘Interference’ section that bacterial interference has not been assessed.

*f. Assay cut-off:*

The cutoff for albumin in CSF is defined as the upper limit of the normal reference range. The range was obtained from literature. The limit is <350mg/L.

The cutoff for albumin in human serum can also be obtained from the literature (35-55 g/L).

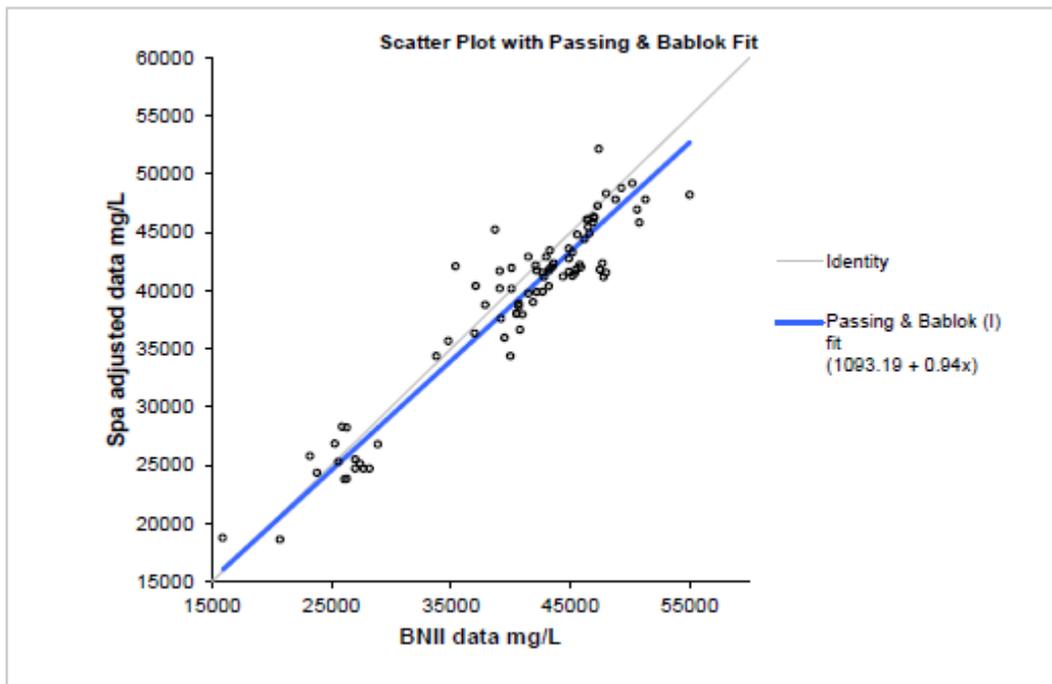
2. Comparison studies:

a. *Method comparison with predicate device:*

*Comparison of subjects with kidney or intestinal disease using serum samples*

85 serum samples (16 “clinical”, 59 normals and 10 diluted normals) which covered the measuring range of the assay as well as the medical decision point (35000 mg/L) were run on both the proposed and the predicate device. The listed diagnosis for some of the 16 “clinical” serum samples is related to kidney and intestinal disease. The samples run on the proposed albumin assay were initially diluted by 1/301 with sample diluent. The initial albumin concentration was adjusted by the dilution factor to give equivalent results as the predicate device. Ten samples were diluted a further 1/3 with sample diluent to give a wider spread of results. A graphical summary of results are as follows:

**Graph 1: Passing and Bablok analysis of comparison data**



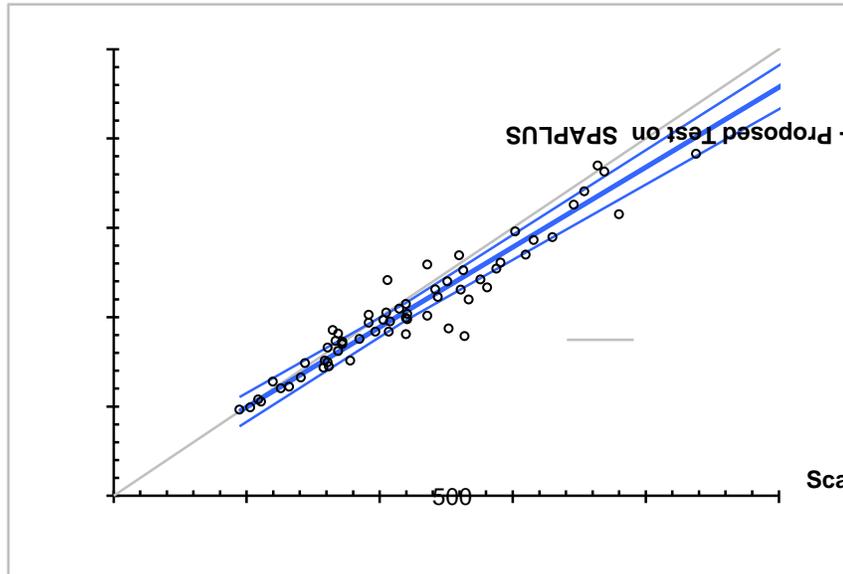
$$Y = 0.94x + 1093.19 \text{ mg/L}$$

The slope and intercept are 0.94 and 1093.19 respectively. The 95% confidence interval of the slope of the best fit line from Passing-Bablok regression analysis was 0.87 to 1.02, a value statistically equivalent with 1.0. The 95% confidence interval of the intercept of the best fit line was -2595.8 to 3916.9, a value statistically equivalent with 0. Regression analysis demonstrated that equivalent results were obtained on both assays. In only three cases a % difference between the methods of greater than 15% was seen.

*Comparison of CSF samples*

For CSF comparison, CSF samples from 67 subjects tested on the new device and the predicate. The following summarizes Passing-Bablok analysis of CSF samples.

|              | Bias  | 95% CI |        |
|--------------|-------|--------|--------|
| Constant     | 9.595 | -0.227 | 19.238 |
| Proportional | 0.895 | 0.843  | 0.948  |



For CSF samples from the 67 subjects the proportional bias is estimated at 10%. There is no apparent constant bias in CSF albumin results.

*b. Matrix comparison:*

The specimen matrix for this assay is human serum and cerebrospinal fluid. Human serum albumin concentrations are known to be considerably higher than CSF albumin concentrations. A direct comparison of albumin in serum and CSF is not necessary since concentrations are known to be different.

3. Clinical studies:

Studies utilizing samples from patients representing the target clinical diseases were not performed due to difficulty in obtaining CSF specimens in sufficient volume and from a sufficient number of subjects with any potentially diagnosable disease.

*a. Clinical Sensitivity:*

No information presented

*b. Clinical specificity:*

No information presented.

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

Not applicable.

4. Clinical cut-off:

No information presented.

5. Expected values/Reference range:

The normal reference range for albumin in CSF was obtained from literature. The limit is <350mg/L.

The cutoff for albumin in human serum was also from literature (35-55 g/L).

**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.