

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

k140664

B. Purpose for Submission:

New assay

C. Measurand:

Human alpha-2- Macroglobulin

D. Type of Test:

Quantitative immunoturbidometric

E. Applicant:

The Binding Site Group, Ltd.

F. Proprietary and Established Names:

Human alpha-2-Macroglobulin kit for use on SPAPLUS

G. Regulatory Information:

1. Regulation section:

21 CFR §866.5620, Alpha-2-Macroglobulin Immunological Test System

2. Classification:

Class II

3. Product code:

DEB, Alpha-2-Macroglobulin, Antigen, Antiserum, Control

4. Panel:

Immunology (82)

H. Intended Use:

1. Intended use(s):

This kit is designed for the quantitative *in vitro* measurement of alpha-2-Macroglobulin in human serum using the SPA_{PLUS} turbidimetric analyser. Measurement of alpha 2-

Macroglobulin may aid in the diagnosis of blood-clotting or clot lysis disorders. This test should be used in conjunction with other laboratory and clinical findings.

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}TM turbidimetric analyzer (k040958)

I. Device Description:

The kit contains the following reagents:

1. 1 x 100 Tests Human α 2-Macroglobulin Antiserum SPA_{PLUS} (monospecific in stabilized liquid form with sodium azide and preservatives)
2. 1 x Human α 2-Macroglobulin SPA_{PLUS} Calibrator set 1-6 (6 x 1.0mL)
3. 2 x 1.5 mL Human α 2-Macroglobulin SPA_{PLUS} High Control (pooled human serum in stabilized liquid form with sodium azide and preservatives)
4. 2 x 1.5mL Human α 2-Macroglobulin SPA_{PLUS} Low Control (pooled human serum in stabilized liquid form with sodium azide and preservatives)
5. 1 x 100 Test α 2-Macroglobulin Reaction Buffer SPA_{PLUS}

J. Substantial Equivalence Information:

1. Predicate device name(s):

N Antisera to Human α 2-Macroglobulin test system (Siemens)

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

k053073

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Measurement of α 2-Macroglobulin to aid in the diagnosis of blood clotting or blood lysis disorders.	Same
Measuring range	0.2-6.4 g/L	Same

Differences		
Item	Device	Predicate
Sample type	Serum	Serum & heparinized plasma
Method	Turbidimetry	Nephelometry
Analyzer	SPA _{PLUS}	BNII
Antibody	Goat anti-human α 2-Macroglobulin	Rabbit anti-human α 2-Macroglobulin
Open vial stability	Three months at 2 to 8°C	Four weeks at 2 to 8°C
On board stability	30 days	Minimum 5 days at 8 hours per day for 5mL vials. Minimum 3 days at 8 hours per day for 2mL vials.
Shelf life stability	12 months at 2-8°C	Not stated
Reference Interval	0.74–2.98 g/L	1.3–3.0 g/L

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

CLSI EP06-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach

1. CLSI EP05-A2 Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition
2. CLSI EP17-A Protocols for Determination of Limits of Detection and Limits of Quantitation, Approved Guideline
3. CLSI EP07-A2 Interference Testing in Clinical Chemistry, Approved Guideline
4. CLSI C28-A3: Defining, Establishing and Verifying Reference Intervals in a Clinical Laboratory

L. Test Principle:

This test measures levels of α 2-Macroglobulin by turbidimetric methods, which involves the reaction of α 2-Macroglobulin in serum with specific antiserum resulting in the formation of insoluble antigen/antibody complexes. When light is passed through the suspension, only a portion of the light is transmitted and focused onto a photodiode by an optical lens system; the remainder is scattered by the immunocomplexes. The amount of transmitted light is indirectly proportional to the specific antigen concentration in the test sample. α 2-Macroglobulin 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:*

This study was conducted according to the recommendations contained in CLSI EP-05-A2 Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition. Five samples with α 2-Macroglobulin concentrations covering the measuring range were tested on three SPA_{PLUS}

instruments and using three lots of assays over 21 days, with two runs per day in duplicate, for a total of 84 replicate measurements. The sponsor’s pre-defined acceptance criteria are total precision must be $\leq 10\%$ for all levels, within precision $\leq 5\%$, and between run and between day $\leq 8\%$ CV.

The between-day and between–lot results from the lowest concentration sample (0.34 g/L) exceeded the acceptance criteria of $<10\%$ CV; however, given that this concentration is at the bottom of the measuring range and is 10-fold above the medical decision point of 2.98 g/L, this imprecision is unlikely to have an effect on clinical decisions, and the overall precision is deemed acceptable.

Repeatability

Sample	Mean (g/L)	Within-Run		Between-Run		Between-Day		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	0.34	0.005	1.6	0.014	4.1	0.045	13.3	0.047	14.0
2	2.29	0.024	1.0	0.049	2.1	0.092	4.0	0.107	4.7
3	3.45	0.034	1.0	0.076	2.2	0.149	4.3	0.171	4.9
4	4.19	0.055	1.3	0.099	2.4	0.178	4.3	0.211	5.0
5	5.42	0.086	1.6	0.131	2.4	0.259	4.8	0.303	5.6

Instrument and Lot Reproducibility

Sample	Mean (g/L)	# Runs	# Days	Between-Instrument		Between-Lot	
				SD	CV%	SD	CV%
1	0.34	84	21	0.017	4.94	0.040	11.82
2	2.29	84	21	0.076	3.33	0.048	2.09
3	3.45	84	21	0.107	3.08	0.031	0.89
4	4.19	84	21	0.121	2.89	0.095	2.26
5	5.42	84	21	0.187	3.45	0.161	2.98

b. Linearity/assay reportable range:

- i. The analytical measuring range of the assay is 0.2-6.4 g/L
- ii. Linearity

A high and a low pool were prepared for Human $\alpha 2$ -Macroglobulin kit for use on SPAPLUS in order to evaluate linearity across the measuring range. The pools were prepared for each assay. Normal human serum was depleted of $\alpha 2$ -macroglobulin by affinity chromatography. For the high pool, a healthy donor serum sample was spiked with a processed serum sample, preserved with citrate

β -alanine, that consisted of pooled healthy donor sera and disease state sera. The pools were combined in a single proportional dilution series (e.g., 90+10, 80+20, etc.), containing from 100% high pool to 0% high pool.

Three replicates of each level of the dilution series were run and the mean value calculated. All of the concentrations within the analytical measuring range met the sponsor's acceptance criterion of %CV < 8% and all concentrations met the sponsor's regression analysis acceptance criteria:

Slope	r	%CV for each level	Allowable mean % recovery
0.90 – 1.10	≥ 0.975	<8%	<10%

% High pool	Mean Unweighted result (g/L)	Expected Unweighted Result (g/L)	% CV	Mean Recovery	Min	Max
100.0%	6.667	6.555	1.5%	103.45%	101.70%	104.60%
90.0%	5.897	5.897	2.0%	101.71%	99.42%	103.32%
80.0%	5.241	5.239	2.8%	101.74%	98.49%	103.54%
70.0%	4.567	4.581	0.9%	101.41%	100.42%	102.20%
60.0%	3.863	3.922	1.2%	100.16%	98.99%	101.37%
50.0%	3.142	3.264	1.8%	97.87%	95.85%	99.19%
40.0%	2.370	2.606	2.9%	92.48%	90.90%	95.58%
30.0%	1.823	1.948	1.1%	95.15%	94.19%	96.28%
20.0%	1.275	1.290	1.0%	100.42%	99.66%	101.55%
10.0%	0.658	0.631	1.1%	105.76%	104.43%	106.51%
5.0%	0.302	0.302	2.3%	101.12%	98.66%	103.35%
4.0%	0.246	0.237	1.1%	104.99%	104.14%	106.27%

Regression analysis

	Slope (95% CI)	Intercept (95% CI)	r
α 2-Macroglobulin	1.01 (0.98–1.04)	-0.06 (-0.16–0.04)	0.999

These data support linearity throughout the analytical measuring range of the assay 0.2–6.4 g/L.

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

a. Calibrators and Controls

Calibrator

The master calibrator is traceable to international reference standard DA470k.

Calibrators are prepared from pooled human sera collected from individual subjects and shown to contain suitable concentrations of α 2-Macroglobulin. The samples are pooled and processed to provide a stable fluid for use in turbidimetric assays. The assigned values of the calibrator set must be within $\pm 15\%$ of the target values to pass the sponsor's acceptance criteria.

b. Controls

Controls are prepared from pooled human sera collected from individual subjects and shown to contain suitable concentrations of α 2-Macroglobulin. The samples are pooled and processed to provide a stable fluid for use in turbidimetric assays. The final value assignment of the high and low controls is carried out by running five replicates of the control fluids on the previously generated five calibration curves. The mean result is multiplied by the same adjustment factor to give the final assigned value of the controls. A control assignment check is carried out by multiplying each of the control fluid results by the adjustment factor and calculating the percentage difference between the experimental and assigned value.

c. Stability

Shelf life: The sponsor provided data that supported the claim that the kits are stable for 12 months when stored unopened at 2-8°C.

d. On-board: The sponsor provided data that supported the claim that the kits are stable for up to 30 days when stored on-board the instrument provided the temperature is maintained at 8-12°C.

e. Open vial: The sponsor provided data that supported the claim that the kits are stable for 3 months when stored closed at 2-8°C.

ii. Antigen

The antiserum used in the Human α 2-Macroglobulin kit for use on SPA_{PLUS} is purchased from a commercial source. The anti- α 2-Macroglobulin immunoglobulins are chromatographically concentrated to increase the specific antibody concentration of the α 2-Macroglobulin antiserum. The antibodies are then characterized by immunoelectrophoresis (IEP) versus human serum.

d. *Detection limit:*

Analytical sensitivity was determined based on the recommendations contained in EP17-A Protocols for Determination of Limits of Detection and Limits of Quantitation, Approved Guideline.

i. Limit of Blank (LoB): The LoB sample consisted of an analyte-depleted sample pool. The LoB sample was tested 60 times and the mean and standard deviation (SD) were calculated.

LoB was calculated using non-parametric analysis. The LoB results were

ranked from lowest to highest concentration, and the LoB was estimated to be the result equivalent to the concentration at the 95th percentile, or between sample results 57 and 58. The LoB was determined to be 0.018 g/L.

- ii. **Limit of Detection (LoD):** The LoD was calculated from the LoB and the combined SDs of the LoQ samples: $LoD = LoB + [(1.645 \times SDs)]$. The LoD was determined to be 0.031 g/L.

Acceptance criterion	Result
LoD < LoQ	0.0313 g/L < 0.20 g/L
TE < 0.074 g/L	0.0212 g/L

- iii. **Limit of Quantitation (LoQ):** five US normal donor samples with known α 2-Macroglobulin concentrations were diluted with analyte-depleted serum so that the concentrations were close to the bottom of the standard measuring range (0.19-0.21 g/L). The five samples were run 12 times over five days using the Human α 2-Macroglobulin Kit for use on SPA_{PLUS} analyser. The samples were also tested in triplicate over the same time period on the predicate device (BN II analyser using N Antiserum to Human α 2-Macroglobulin reagents). LoQ was calculated according to the formula $TE = | [mean SPA_{PLUS} assay] - [mean predicate assay] | + 2 \times SDs$. The LoQ was determined to be 0.20 g/L, which is at the bottom of the measuring range.

e. Analytical specificity:

- i. **Interference**

This study was performed according to the recommendations contained in CLSI EP07-A2 Interference Testing in Clinical Chemistry, Approved Guideline, except that the cutoff for acceptable interference was set as $D_{obs} < D_{max}$ and <10% difference between the mean of the spiked sample and the unspiked sample. Twenty replicates of each of the serum base pools were run in order to calculate standard deviation. The D_{max} was set at 10% of the analyte concentration for each of the base pools. The standard deviation and the D_{max} were then used to calculate the number of replicates required in the interference study. Three replicates of each spiked and unspiked concentration were determined sufficient for this study.

Three (3) human serum base pools were prepared to target three analyte concentrations. These levels were chosen to evaluate the effects the interferents at critical clinical concentrations. The high concentration pool was diluted with analyte-free serum to achieve the desired target concentrations.

The serum pools were spiked separately with hemoglobin (5 g/L), bilirubin (200 mg/L), Intralipid (500 mg/dL) and triglyceride (1000 mg/dL). The spiked pools were analyzed, as were the unspiked pools, which were prepared by spiking the base pools separately with the same volume of commercially obtained blank for each potential interferent.

Hemoglobin 5 g/L

Interferent Hemoglobin	Mean blank result (g/L)	Mean interferent result (g/L)	% Difference	Dobs (g/L)	Dmax (g/L)	Pass/Fail
Level 1	1.524	1.414	-7.22%	-0.110	±0.174	Pass
Level 2	2.541	2.495	-1.81%	-0.046	±0.314	Pass
Level 3	3.794	3.900	2.79%	0.106	±0.426	Pass

Bilirubin 200 mg/L

Interferent Bilirubin	Mean blank result (g/L)	Mean interferent result (g/L)	% Difference	Dobs (g/L)	Dmax (g/L)	Pass/Fail
Level 1	1.711	1.689	-1.29%	-0.022	±0.174	Pass
Level 2	2.834	2.980	5.15%	0.145	±0.314	Pass
Level 3	4.161	4.105	-1.35%	-0.057	±0.426	Pass

Intralipid 500 mg/dL

Interferent Intralipid	Mean blank result (g/L)	Mean interferent result (g/L)	% Difference	Dobs (g/L)	Dmax (g/L)	Pass/Fail
Level 1	1.287	1.332	3.522%	0.045	±0.144	Pass
Level 2	2.591	2.587	-0.15%	-0.003	±0.292	Pass
Level 3	2.989	2.968	-0.70%	0.009	±0.364	Pass

Triglyceride 1000 mg/dL

Interferent Triglyceride	Mean blank result (g/L)	Mean interferent result (g/L)	% Difference	Dobs (g/L)	Dmax (g/L)	Pass/Fail
Level 1	1.414	1.451	2.62%	0.037	±0.154	Pass
Level 2	2.720	2.752	1.18%	0.033	±0.290	Pass
Level 3	3.834	3.755	-2.06%	-0.079	±0.394	Pass

These results demonstrate that no clinically significant interference was observed at the indicated concentrations.

ii. Hook effect

The sponsor provided data demonstrating that there is no hook effect at concentrations of antigen up to 12.8 g/L.

f. Assay cut-off:

Same as clinical cutoff; see below.

2. Comparison studies:

a. *Method comparison with predicate device:*

154 samples across the analytical measuring range of the assay (see below for demographics and sample distribution) were tested on the predicate method and on the α -2-Macroglobulin kit for use on SPAPLUS. Samples from subjects with diabetes, alcoholic liver disease, high levels of CRP and amyloid disease were included, as well as normal samples to ensure the measuring range was covered. Ten high concentration samples were constructed by spiking normal serum with purified α 2-macroglobulin. Linear, Passing-Bablok, and Deming regression analyses were performed and no outliers were removed.

Presenting Symptom or Admission Diagnosis	Number of Samples
UK Asian Diabetic	24
Amyloid	17
High CRP	19
Unknown	19
Alcoholic liver disease	23
Normal Human Serum	42
Constructed samples- normal human serum with purified α 2-Macroglobulin	10
Total	154

The sponsor's predetermined acceptance criterion was that substantial equivalence would be demonstrated if the results were within $\pm 10\%$ Passing-Bablok slope bias, and each individual result obtained on the test device should be within $\pm 20\%$ of the result obtained for that sample on the predicate device. Sample demographics: 154 samples, age range 19-93 years, 33% male, 38% female, and 29% unknown.

Sample Distribution	
N	154
Concentration Range	0.696–6.072 g/L
< 25% of cutoff	N =53
Cutoff \pm 25% (2.98 g/L)	N= 63
>25% of cutoff	N= 34

Regression Results:

Regression Analysis	Slope 95% CI	Intercept 95% CI
Passing- Bablok $y= 1.00x - 0.05$	0.97 to 1.04	-0.13 to 0.03
Weighted Deming $y= 1.01x - 0.07$	0.98 to 1.05	-0.13 to 0.00
Linear regression $y=0.98x + 0.03$ R= 0.977	0.94 to 1.01	-0.08 to 0.14

b. *Matrix comparison:*

Not applicable

3. Clinical studies:

a. *Clinical Sensitivity and specificity:*

Not applicable

b. Other clinical supportive data (when a. is not applicable):

Not applicable

4. Clinical cut-off:

The clinical decision point is set to 2.98 g/L, which is the top of the 95th percentile of the reference range for healthy individuals as determined in the reference range study, below.

5. Expected values/Reference range:

A reference range study was performed according to CLSI C28-A3: Defining, Establishing and Verifying Reference Intervals in a Clinical Laboratory. 166 normal healthy samples from US and German donors that were not used for the Method Comparison study were evaluated.

Results	
N	166
Range	0.390–3.739 g/L
Mean	1.580g/L
Male	108 (65%)
Median	1.500g/L
95 th percentile	0.74–2.98g/L
95% CI lower boundary	0.6765– 0.8134
95% CI upper boundary	2.7157–3.2655

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.