

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

- A. 510(k) Number:**
k100455
- B. Purpose for Submission:**
New Device
- C. Measurand:**
Human Complement C4
- D. Type of Test:**
Quantitative, turbidimetric.
- E. Applicant:**
The Binding Site Group, Ltd.
- F. Proprietary and Established Names:**
Human C4 Kit for use on SPA_{PLUS}TM
- G. Regulatory Information:**
1. Regulation section:
21 CFR § 866.5240 Complement components immunological test system
 2. Classification:
Class II
 3. Product code:
DBI, Complement C4, antigen, antiserum, control
 4. Panel:
Immunology (82)
- H. Intended Use:**
1. Intended use(s):
The Human C4 kit is intended for the quantitative in vitro determination of human C4 in serum using the Binding Site SPA_{PLUS}TM turbidimetric analyser. 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 (manufactured as Clinical Chemistry Analyzer under the names Prestige 24i, Sirrus, MGC240 by Tokyo Boeki, Japan, cleared under k040958). The SPA_{PLUS}TM is identical to the Prestige 24 clinical chemistry analyzer without the ISE module.

The software used by the SPA_{PLUS}TM is the same as that used by the Sirrus and Prestige 24i instruments. A minor modification has been made to the standard sample dilution for this assay. The standard sample dilution has been defaulted to 1/10 (rather than neat) for patient samples and control samples, with an auto rerun facility activated at 1/20 if the result obtained is over-range. The final result and test dilution appears on the instrument panel and printout. No changes have been made to the instruments functionality.

Following a review of the Guidance for Content of Premarket Submissions for Software Contained in Medical Devices the risk is assessed as 'minor level of concern'. These

modifications were previously covered in k062372 'Freelite for use on the SPA_{PLUS}[™]. No additional modifications have been made to the analyzer or software in order to run the C4 assay.

I. Device Description:

Human C4 Kit for Use on the SPA_{PLUS}[™] test includes:

1. Sheep anti-human C4 serum.
2. 6-level Calibrators and low and high controls (from control pooled human serum samples referenced to DA470k international reference material).
3. Reaction Buffer.

J. Substantial Equivalence Information:

1. Predicate device name(s):
Roche Diagnostics Corp Tina-Quant C4 Test system (for Roche/Hitachi 904/911/912/917/Modular analyzers)
2. Predicate 510(k) number(s):
k953239
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	Binding Site Human C4 kit	Tina-quant C4 (k953239)
Intended Use	For the quantitative in vitro determination of human C4 in serum.	Same.
Method	Turbidimetric Immunoassay	Same
Pediatric use	No pediatric range	Same
Traceability	DA 470k	Same

Differences		
Item	Device	Predicate
	Binding Site Human C4 kit	Tina-quant C4 (k953239)
Sample type	Human serum	Human serum and plasma
Instrument	SPA _{PLUS} [™] Analyser	Roche/Hitachi 904/911/912/91 and MODULAR P
Measuring range	0.064-0.9g/L	0.015 -1.0g/L
Healthy Adult reference interval	Adults 0.129-0.392g/L	Adults 0.1-0.4g/L
LOD, LOQ	LOD = 0.003g/L LOQ = 0.006g/L	LOD = 0.015g/L
Antibodies	Sheep	Goat
Calibrator and Controls	Low and High level Controls and 6 point calibrator, provided in kit	Normal and High level Controls and 6 point calibrator, sold separately
Reagent Stability	Unopened: 3 months at 2-8°C Opened (on-board) 30 days at 8-12°C.	Opened (on-board) 90 days

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

CLSI EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline.

CLSI EP06-A, Evaluation of the Linearity of the Quantitative measurement procedure: A statistical approach: Approved Guideline.

CLSI EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation.

L. Test Principle:

Human C4 Kit for Use on the SPA_{PLUS}TM test is a single-step immunoturbidimetric test. The determination of soluble antigen concentration by turbidimetric methods involves the reaction with specific antiserum to form insoluble complexes. When light is passed through the suspension formed, 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 (C4) in the test sample. 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:*

The precision studies were performed following CLSI Evaluation of Precision Performance of Clinical Quantitative Measurement Methods; Approved Guideline (CLSI Document EP5-A2).

The study was performed over 21 working days, with two runs per day. One user assessed three different samples, High level (towards the upper limit of the measuring range), Medium level (close to the bottom of the normal range) and Low level (between 140% and 180% of the lower limit of the measuring range), using three different reagent lots on three analyzers.

Results are summarized in the table below:

C4 Precision Summary									
	Mean	Within run		Between run		Between day		Total	
	(g/L)	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Serum 1	0.738	0.014	1.9	0.026	3.6	0.018	2.4	0.035	4.7
Serum 2	0.167	0.004	2.1	0.005	2.8	0.012	6.9	0.013	7.8
Serum 3	0.111	0.002	1.9	0.005	4.6	0.006	5.6	0.008	7.5

All within-run, between-run and between day precision coefficients of variation are below 7%. The total precision for Sample 2 has the highest coefficient of variation at 7.8%. The total precision %CV for the high level (Sample 1) is 4.7% and 7.5 % for Sample 3 at the bottom end of the calibration curve. The overall precision is within the acceptance criteria.

Results summarized in the table below show that the lot-to-lot reproducibility and Instrument-to-Instrument reproducibility were within acceptance criteria for the C4 SPAPLUS™ kit.

	Mean	Within Lots		Between Instrument	
	(g/L)	SD	%CV	SD	%CV
Serum 1	0.740	0.035	4.78	0.009	1.24
Serum 2	0.167	0.016	9.74	0.001	0.85
Serum 3	0.111	0.009	8.28	0.000	0.00

b. Linearity/assay reportable range:

Linearity was evaluated as per CLSI EP6-A (Evaluation of the Linearity of Quantitative measurement procedures; A statistical approach; Approved Guideline). Serum samples (see table) previously identified as containing high levels of C4 and stored at -20°C were pooled to form a high linearity fluid.

The high pool of samples for the linearity study was diluted in sample diluent (saline). Saline is the specified SPAPLUS™ instrument system diluent; low pool samples with undetectable levels of C4 were not available. The diluent does not correct for matrix effects; this is in accordance with the CLSI EP6-A section 4.3.2 (a patient-sample pool diluted in the recommended diluent constitutes the second most desirable situation if a low pool is not available). Dilutions were prepared to cover the kit measuring range and each dilution was measured three times on three different kit lots.

Control	Assigned Conc. (g/L)	Results (g/L)	% Difference (+/- 15% acceptance)
C4 Kit lot-1			
C4 High Control-1	0.550	0.559	1.6
C4 Low Control-2	0.310	0.305	-1.6
C4 internal reference	0.078	0.076	-2.6
C4 Kit lot-2			
C4 High Control-3	0.540	0.549	1.7
C4 Low Control-4	0.290	0.311	7.2
C4 internal reference	0.078	0.079	1.3
C4 Kit lot-3			
C4 High Control-5	0.480	0.494	2.9
C4 Low Control-6	0.290	0.302	4.1
C4 internal reference	0.078	0.075	-3.8

The results were plotted for each lot to inspect the linear relationship. This gave a regression plot of $y = 1.005x - 0.014\text{g/L}$ over the range of 0.06-1.14g/L. In addition percentage recovery was calculated for the mean of each dilution.

The data confirms the linearity of the assay over the measuring range of 0.06 - 1.14g/L at the standard sample dilution (1:10) and 0.008 - 0.12g/L the minimum sample dilution of 1:1 (neat).

The claimed reportable range for C4 is 0.064 – 0.9 g/L for initial 1:10 dilution of samples that are automatically processed by the instrument. Samples higher than the initial measuring range will automatically re-dilute using a 1:20 dilution, which allows samples up to 1.8g/L to be measured.

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

Controls and Calibrators

The calibrators and controls are manufactured from ‘pooled human serum’, and processed to stabilize C4. The control serum samples were traced to European Reference Material DA 470. The kit C4 calibrators are assigned to an internal reference (IR) which is directly calibrated to the external reference standard DA470. For the IR assignment both the IR fluid and DA470 are tested at different dilutions across the curve (33%, 16.5%, 8.3% and 4.2%). All dilutions are tested in triplicate using 2 or more different kit batches on the SPAPLUS™. A second set of dilutions are made and tested in the same way on a different SPAPLUS™ analyzer. From this information the IR value is calculated. The IR assignment is verified by testing it against DA470 in triplicate using all assays that measure C4.

The assayed controls have assigned values with a $\pm 10\%$ in-house tolerance and target values for the low control and for the high control. The controls are assigned on calibration curves validated with the internal reference standard which is directly assigned to the international reference standard DA470. The controls supplied with each lot are assayed on the kit and information on the values obtained, including the $\pm 15\%$ customer range, are found in the lot specific quality control certificate.

Stability

Three kit lots of C4, with each reagent originating from different batches, were tested after storage at 22°C for 1 week to simulate shipping conditions. The kits were tested at Time 0 and 3 months after storage at the 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.

Control	Assigned Conc. (g/L)	%	
		(+8.5%)	(+15%)
Lot-1:		0 months	3 months
IR	0.078	-3.8	5.1
		1.3	5.1
High	0.55	1.8	3.5
		-6.4	5.5
Low	0.31	-3.9	3.8
		-3.2	1.0

Control	Assigned Conc. (g/L)	% (+8.5%)	% (+15%)
Lot-2:		0 months	3 months
IR	0.078	-5.1	9.0
		-1.3	5.1
High	0.54	0.9	1.3
		3.9	0.7
Low	0.29	1.4	3.8
		1.7	3.1
Lot-3:		0 months	3 months
IR	0.078	-1.3	-6.4
		-5.1	1.3
High	0.48	2.9	3.3
		-1.9	-4.4
Low	0.29	0.0	-1.7
		-1.7	-4.1

The control and internal reference results obtained were well within the acceptable control result range of $\pm 15\%$ of the assigned value ($\pm 8.5\%$ at time 0), showing stability of the Kits reagents for at least 3 months from the date of manufacture when stored at the recommended storage temperature of 2-8°C.

On-Board stability tests were performed by storing vials 'on-board' the SPA_{PLUS}TM for a period of at least 30 days. Open vial Stability were tested by storing reagents in open vials for 3 months at 2 - 8°C. The results obtained from Low and High Controls were within $\pm 15\%$ of the assigned value.

d. *Detection limit:*

CLSI EPI7-A (Protocols for Determination of Limits of Detection and Limits of Quantitation, Approved Guideline) were employed for determining limit of detection. A blank, the lowest level calibrator and a sample with a known C4 concentration, diluted to give a value just greater than the blank, were tested 60 times on the SPA_{PLUS}TM analyzer. The standard deviation (SD) and the percentage coefficient of variation (%CV) were calculated in each case. The 60 blank absorbance readings gave a mean value of 0.0106 absorbance units with an SD of 0.0005 units.

The limit of quantitation (LoQ): The limit of quantitation for the assay was the lowest point of the calibration curve; LoQ was assigned a value of 0.006 g/L based upon a 1/1 sample dilution. Below LoQ point, samples are flagged as below the measurable range, and no concentration value is calculated

The limit of blank (LoB): The limit of blank was estimated as the mean of blank + 2 SD using the blank as a zero calibrator. The LoB was derived from the mean of the blank + 2 SD = 0.0106 + (0.0005 x 2) = 0.0116 abs units giving an estimated value of 0.0003g/L.

The limit of detection (LoD): The limit of detection was estimated as the lowest measurable analyte level that can be distinguished from zero. The very low level sample (diluted sample) gave a mean value of 0.018 absorbance units giving an estimated value of 0.003g/L which is considered to be the LoD.

e. *Analytical specificity:*

Cross-reactivity with other complement components:

The IEP and ouchterlony tests were performed against deep frozen normal human serum to demonstrate absence of any significant cross reaction with other complement components including major split products and other subunits (C1q etc.). Western blots analysis was used to demonstrate the absence of cross-reactivity to C3 and C3-components like C3a, iC3b, C3c and C3dg. Results showed the sheep anti-human C4 serum specifically binds to human C4.

Interference – endogenous substances

Susceptibility to interference was assessed by adding a high concentration of a potential interferent to a test sample containing known concentrations of C4. The method used to check for chyle, a mixture of biological lymph and chylomicrons (lipids and proteins), and hemoglobin was based on the Interference Check A plus™ (Sysmex, Japan). In the same way bilirubin interference was assessed by adding a high concentration of bilirubin to a test serum sample and comparing it to a blank. Percentage interference was calculated from comparison with the sample blank. Deviations less than or equal to $\pm 10\%$ of the blank value were considered to show 'no significant interference'.

	Bilirubin (200 mg/mL)	Hb (5 g/L)	Chyle (1,500 FTU)
Mean C4 (g/L)	0.048	0.051	0.049
% interference	-5.3%	4.8%	-2.0%

No significant interference was seen when high concentrations of hemoglobin (5 g/L), bilirubin (200 mg/L) and chyle (1500 FTU) were added to serum samples.

f. *Assay cut-off:*
Not applicable

2. **Comparison studies:**

a. *Method comparison with predicate device:*

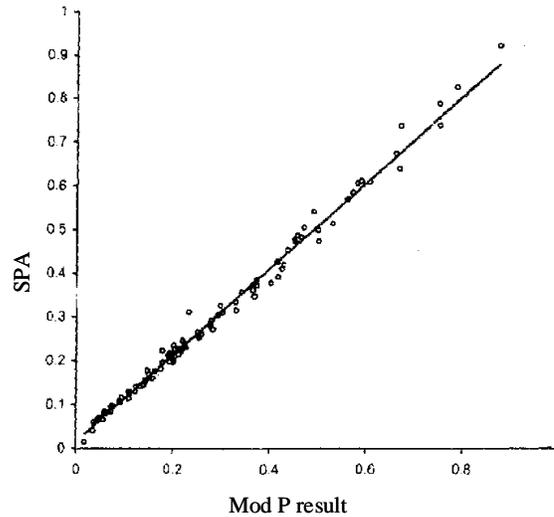
Method comparison between Binding Site's C4 SPA_{PLUS}™ assays to the already 510k-cleared Tina-Quant complement C4 Test system (k953239 Roche Diagnostics Corp.) was done by using 101 retrospective human serum samples. The clinical samples were collected and stored at -80°C in order to preserve the complement levels as much as possible. No validation data is available to support these storage conditions. Thirty one normal samples and 70 clinical samples were tested on SPA_{PLUS}™ and Roche/Hitachi Modular P analyzer C4 kit. Kit controls were included with each assay run. The samples were fairly distributed over the measuring range.

The following results were obtained for 101 samples over the range of 0.01720 to 0.8756 g/L.

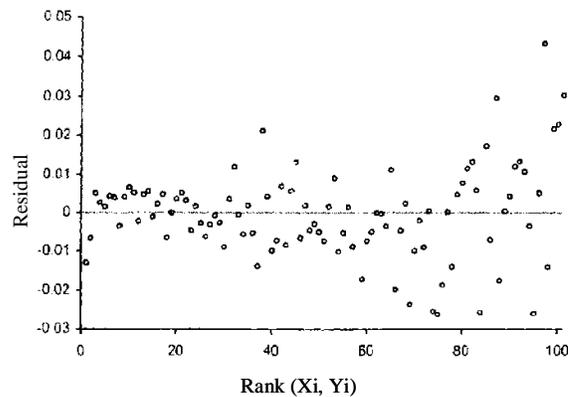
Passing & Bablok fit $y = 0.99x + 0.02$; $r = 0.996$

Linear fit $y = 1.00x + 0.01$

Scatter Plot with Passing & Bablok Fit:



Residual Plot:



b. Matrix comparison:
Not applicable

3. Clinical studies:

a. Clinical Sensitivity:
Not applicable

b. Clinical specificity:
Not applicable

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:

Following ranges were obtained using this kit, by measuring the C4 concentration of the human serum. The reference interval was calculated using non-parametric statistics and represents the central 95% of the population.

	Number (n)	Mean (g/L)	Median (g/L)	95 Percentile Range (g/L)
C4	120	0.241	0.234	0.129-0.392

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.