

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

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

k100179

**B. Purpose for Submission:**

New Device

**C. Measurand:**

Human complement C3c

**D. Type of Test:**

Quantitative, turbidimetric.

**E. Applicant:**

The Binding Site, Ltd

**F. Proprietary and Established Names:**

Human C3c Kit for use on SPA<sub>PLUS</sub><sup>TM</sup>

**G. Regulatory Information:**

1. Regulation section:  
21 CFR § 866.5240 Complement components immunological test system
2. Classification:  
Class-II
3. Product code:  
CZW, Complement C3, antigen, antiserum, control
4. Panel:  
Immunology (82)

**H. Intended Use:**

1. Intended use(s):  
The Human C3c Kit is intended for the quantitative in vitro determination of human C3c in serum using the Binding Site SPA<sub>PLUS</sub><sup>TM</sup> turbidimetric analyser. The 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<sub>PLUS</sub><sup>TM</sup> Turbidimetric Analyzer (manufactured as Clinical Chemistry Analyzer under the names Prestige 24i, SIRRUS, MGC240 by Tokyo Boeki, Japan, cleared under k040958). The SPA<sub>PLUS</sub> is identical to the Prestige 24 clinical chemistry analyzer without the ISE module.

The software used by the SPA<sub>PLUS</sub><sup>TM</sup> 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 all auto rerun facility activated at 1/20 if the results 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 Submission 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<sub>PLUS</sub>'. No additional modifications have been made to the analyzer or software in order to run the C3c assay.

**I. Device Description:**

Human C3c Kit for Use on the SPA<sub>PLUS</sub><sup>TM</sup> test includes:

1. Sheep anti-human C3c serum.
2. Calibrators and controls (from control pooled human serum samples referenced to DA470k international reference material).
3. Sample diluent.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):  
Roche Diagnostics Corp, Tina Quant Complement C3c Test System.
2. Predicate 510(k) number(s):  
k012361
3. Comparison with predicate:

Similarities		
	Device	Predicate
Item	Binding Site C3c SPA <sub>PLUS</sub> Kit	k012361 Tina-Quant C3c
Intended Use	For the quantitative in vitro determination of human C3c	Same
Method	Turbidometric immunoassay	Same
Pediatric use	No pediatric range	Same
Traceability	DA-470k	Same
Control	Normal and High level liquid ready-to-use controls	Same

Differences		
	Device	Predicate
Item	Binding Site C3c SPA <sub>PLUS</sub> Kit	k012361 Tina-Quant C3c
Sample type	Human Serum	Human Serum and Plasma
Instruments	SPA <sub>PLUS</sub> analyser	COBAS Integra analyzer
Measuring range	0.25-3.0 g/L at 1/10 dilution	0.11-6.0 g/L
Healthy Adult Reference Interval	Adults 0.81-1.57g/L	Adults 0.9-1.8g/L
LOD and LOQ	LOD = 0.012 g/L LOQ = 0.24 g/L	LOD = 0.11 g/L
Healthy Adult Reference Interval	Adults 0.81-1.57 g/L	Adults 0.9-1.8 g/L
Antibodies	Sheep (latexed)	Goat

Differences		
	Device	Predicate
Item	Binding Site C3c SPA <sub>PLUS</sub> Kit	k012361 Tina-Quant C3c
Calibrator and Controls	Provided in kit	Sold separately
Reagent Stability	Unopened: 3 months at 2-8°C Opened (on-board) 28 days.	Opened (on-board) 42 days

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

CLSI EP5-A2: Evaluation of Precision Performance of Quantitation Measurement Methods; Approved Guideline-Second Addition

CLSI EP6-A: Evaluation of the Linearity of Quantitative measurement procedures; A statistical approach; Approved Guideline

CLSI EPI7-A: Protocols for Determination of Limits of Detection and Limits of Quantitation, Approved Guideline.

Protocols for Determination of Limits of Detection and limits of Quantitation; Approved Guideline

**L. Test Principle:**

Human C3c Kit for Use on the SPA<sub>PLUS</sub><sup>TM</sup> test is a single-step immunoturbidimetric test.

The assay determines concentration of C3c through photometric measurement of suspension formed (insoluble antigen-antibody complex agglutination) between antibodies to human C3c and C3c present in the sample. Concentrations are automatically calculated by reference to a calibration curve stored with in 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 carried out over 21 working days, with two runs per day. One user assessed three different samples (High, Medium and Low) using three different reagent lots on three different analyzers.

Results are summarized in the table below:

Level	Mean	Within run		Between run		Between day		Total precision	
	(g/L)	SD	%CV	SD	%CV	SD	%CV	SD	%CV
High	3.114 g/L	0.053	1.7%	0.065	2.1%	0.122	3.9%	0.148	4.7%
Medium	0.899 g/L	0.017	2.0%	0.023	2.6%	0.028	3.2%	0.039	4.6%
Low	0.421 g/L	0.009	2.5%	0.015	4.0%	0.017	4.7%	0.025	6.6%

All within-run, between-run and between day precision coefficients of variation are below 7%. The Total Precision for Sample 3 has the highest coefficient of variation at 6.6%. The Total Precision %CV for the medium level (Sample 2) is 4.6% and

4.7% for Sample 1 at the top end of the calibration curve. The study shows overall precision for the C3c SPA<sub>PLUS</sub> kit to be good.

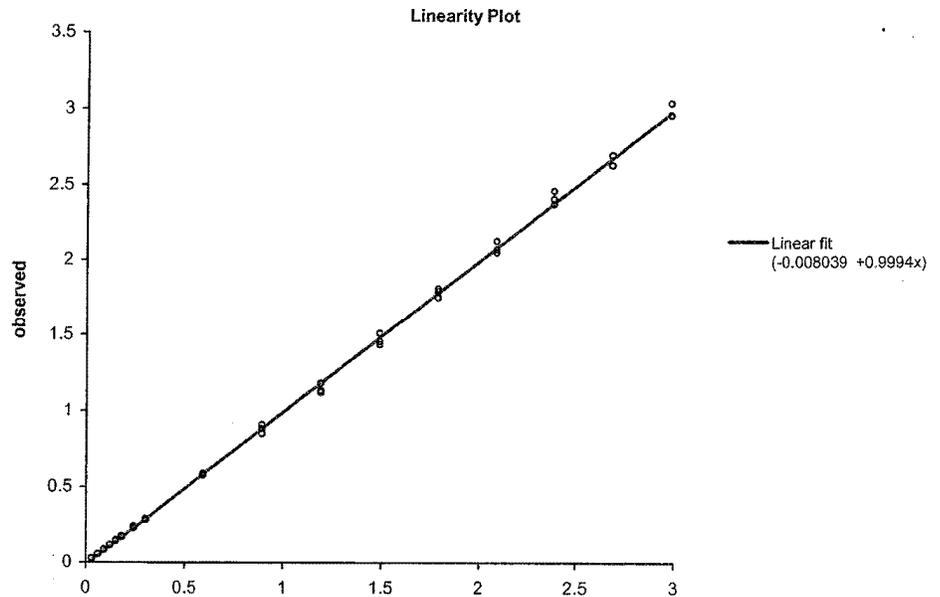
*b. Linearity/assay reportable range:*

Linearity was evaluated as per CLSI EP6-A (Evaluation of the Linearity of Quantitative measurement procedures; A statistical approach; Approved Guideline). Seven undiluted human serum samples (see table) previously identified as containing high levels of C3c and stored at -20°C were pooled to form a high linearity fluid.

This high level fluid was diluted with sample diluent (not reaction buffer) as it was not possible to obtain sufficient samples with adequate (low) levels to produce a low level linearity pool. Dilutions were prepared to cover the kit measuring range and each dilution was measured three times on three different kit lots at neat and a 1/10 sample dilution.

Control	Assigned Conc. (g/L)	Results (g/L)	% Difference (+/-15% acceptance)
<b>C3c Kit Lot-1:</b>			
C3c High Control	2.720	2.710	-0.5
C3c Low Control	0.840	0.850	1.0
		0.830	-1.0
Internal reference	0.941	0.953	1.3
		0.954	1.4
<b>C3 Kit Lot-2:</b>			
C3c High Control	2.730	2.660	-2.6
		2.660	-1.3
C3c Low Control	0.790	0.770	-2.4
		0.750	-4.8
Internal reference	0.941	0.901	-4.3
		0.918	-4.8
<b>C3 Kit Lot-3:</b>			
C3c High Control	2.850	2.870	0.8
		2.910	2.0
C3c Low Control	0.870	0.910	5.4
		0.920	5.1
Internal reference	0.941	0.977	3.8
		1.006	6.9

The results were plotted for each lot to inspect the linear relationship (batch-1 shown in figure below). In addition percentage recovery was calculated for the mean of each dilution.



Linear - Term	Coefficient	SE	p
Intercept	-0.0080	0.0053	0.1349
X	0.9994	0.0037	<0.0001

Source of variation	SSq	DF	MSq	F	p
Linear Fit	50.3503	1	50.3503	72081.27	<0.0001
Residual	0.0342	49	0.0007	-	-
Lack of Fit	0.0115	15	0.0008	1.15	0.3555
Pure error	0.0227	34	0.0007		
Total	50.3845	50			

This data confirms the linearity of the assay over the range of 0.03-3g/L across 3 different kit lots.

Measuring range (reportable range) for C3s is 0.03 - 3 g/L for initial 1:10 dilution of samples that are automatically processed by the instrument. Samples with results in excess of 3g/L are repeated on a higher dilution (1:20).

#### Auto-dilution vs. manual dilution

Studies were done to support the accuracy and the precision of the SPA<sup>PLUS</sup><sup>TM</sup> turbidimetric analyser automated sample dilution protocol, by comparing auto-dilution vs. manual-dilution. Three samples of different levels were tested on the SPA<sup>PLUS</sup> using off-line (manual) and on-line (auto) dilution method. The samples were all diluted in SPA<sup>PLUS</sup> instrument system diluent (SN080.S) which is the recommended diluent for this analyzer. Each sample dilution was tested 20 times and the mean, standard deviation and % coefficient of variation were calculated for each data set. In all three cases the manual (off-line) 1/20 dilution was compared to the 1/20 on-line dilution.

The test results are summarized in the table below:

sample ID	77		97		85	
SPA sample dilution	1/1	1/20	1/1	1/20	1/1	1/20
offline dilution	1/20	none	1/20	none	1/20	none
1	3.160	3.208	2.400	2.351	1.260	1.301
2	3.180	3.178	2.420	2.414	1.300	1.320
3	3.060	3.137	2.360	2.381	1.240	1.288
4	3.160	3.197	2.380	2.412	1.320	1.317
5	3.100	3.149	2.320	2.417	1.260	1.294
6	3.160	3.228	2.420	2.393	1.320	1.315
7	3.100	3.159	2.400	2.800	1.280	1.307
8	3.200	3.128	2.380	2.371	1.320	1.267
9	3.060	3.088	2.400	2.329	1.260	1.316
10	3.200	3.069	2.440	2.369	1.320	1.279
11	3.120	3.121	2.360	2.395	1.260	1.313
12	3.160	3.190	2.440	2.353	1.300	1.302
13	3.120	3.188	2.380	2.400	1.260	1.200
14	3.160	3.188	2.440	2.348	1.300	1.280
15	3.100	3.231	2.380	2.395	1.240	1.300
16	3.140	3.184	2.420	2.399	1.300	1.280
17	3.100	3.199	2.400	2.417	1.220	1.274
18	3.160	3.140	2.460	2.343	1.280	1.254
19	3.160	3.151	2.420	2.387	1.240	1.282
20	3.100	3.207	2.420	2.435	1.280	1.281
Mean	3.135	3.167	2.402	2.405	1.278	1.289
SD	0.041	0.044	0.034	0.097	0.031	0.028
CV	1.32%	1.39%	1.40%	4.04%	2.43%	2.16%
% diff from offline 1/20		1.02%		0.14%		0.82%

The within run precision show coefficient of variation below 5% and the difference between the mean off-line and mean on-line dilution are 1% or less. This study shows good agreement between the manual 1/20 and auto 1/20 sample dilutions.

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

The calibrators and controls are manufactured from 'pooled human serum'. The control serum samples were traceable to European Reference Material DA 470. The kit C3c calibrators are assigned to an internal reference (IR) which is directly calibrated to the external reference standard DA-470. For the IR assignment both the IR fluid and DA-470 are tested at different dilutions across the curve (50%, 25%, 12.5% and 6.25%). 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 DA-470 in triplicate using all assays that measure C3c.

The assayed controls have assigned values with a +/-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 DA-470. The controls supplied with each lot are assayed on the kit and information on the values obtained, including the +/-15% customer range.

The comparison study provided show good agreement with no bias seen across the measuring range and a mean percentage difference between the two assays of -1.1%

### Stability

Three kit lots of C3c, 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, 3, 7 and 10 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 was also run at time 0.

Control	Assigned Conc. (g/L)	% (+8.5%)	% (+15%)	% (+15%)	% (+15%)
<b>Lot-1:</b>		0 months	3 months	7 months	10 months
IR	0.941	3.9		0.7	5.2
		2.9		2.6	4.4
High	2.72	-3.9	-7.0	-2.2	1.7
		-5.2	-10.5	-1.0	3.5
Low	0.84	-2.0	-7.9	3.8	10.2
		-2.9	-10.7	2.0	11.5
<b>Lot-2:</b>		0 months	3 months	7 months	10 months
IR	0.941	-3.3		0.1	3.1
		-3.3		-3.7	1.7
High	2.73	-4.7	-3.8	2.4	1.8
		-5.9	-1.4	2.5	4.9
Low	0.79	-3.9	-6.6	7.1	-2.9
		-2.4	-6.2	9.2	0.3
<b>Lot-3:</b>		0 months	3 months	7 months	10 months
IR	0.941	-2.7		-4.7	1.1
		-4.3		-3.5	2.0
High	2.85	-3.9	-9.9	-0.8	0.1
		-3.9	-9.6	-2.4	0.2
Low	0.87	-6.3	-0.6	2.8	0.8
		-3.3	-0.5	-3.3	2.9

The results obtained from Low and High Controls were within  $\pm 15\%$  of the assigned value ( $\pm 8.5\%$  at time 0), showing stability of the Kit 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<sub>PLUS</sub> for a period of at least 28 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 bottom calibrator and a sample known to contain C3c, diluted to give a value just greater than the blank, were tested 60 times on the SPA<sub>PLUS</sub> 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 0.0027 absorbance units with an SD of 0.0008 units.

The limit of quantitation (LoQ): The bottom calibrator point was the lowest point of the measuring range and therefore was considered as the limit of quantitation (LoQ). The LoQ was assigned a value of 0.24g/L for kit lot N0054.

The limit of blank (LoB): Below LoQ point, samples were flagged as below the measurable range, and no concentration value was calculated. LoB was estimated as the mean of blank +2 SD using the blank (sample diluent) as a zero calibrator. The results derived from  $0.0027 + (0.0008 \times 2) = 0.0040$  abs units gave an estimated value of 0.005g/L (LoB).

The limit of detection (LoD): The limit of detection (LoD) was estimated as the mean value obtained for the very low level sample using the blank as a zero calibrator. The very low level sample (diluted sample) gave a mean value of 0.0088 absorbance units giving an estimated value of 0.012g/L which was considered to be the limit of detection (LoD).

e. *Analytical specificity:*

Cross-reactivity with other complement components:

The IEP and ouchterlony tests were performed to demonstrate the absence of cross reaction with other complement components including major split products (C4a etc.) and other subunits (C1q etc.). Western blots analysis was used to demonstrate the absence of cross-reactivity to C3a and C3dg. Results showed the sheep anti-human C3c serum specifically binds to human C3c.

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 C3c. The method used to check for chyle, a mixture of biological lymph and chylomicrons (lipids and proteins), and haemoglobin was based on the Interference Check A plus<sup>TM</sup> (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 (200mg/mL)	Haemoglobin (5g/L)	Chyle (1500FTU)
Mean C3c (g/L)	0.313	0.294	0.293
% interference	4.6%	0.1%	1%

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

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

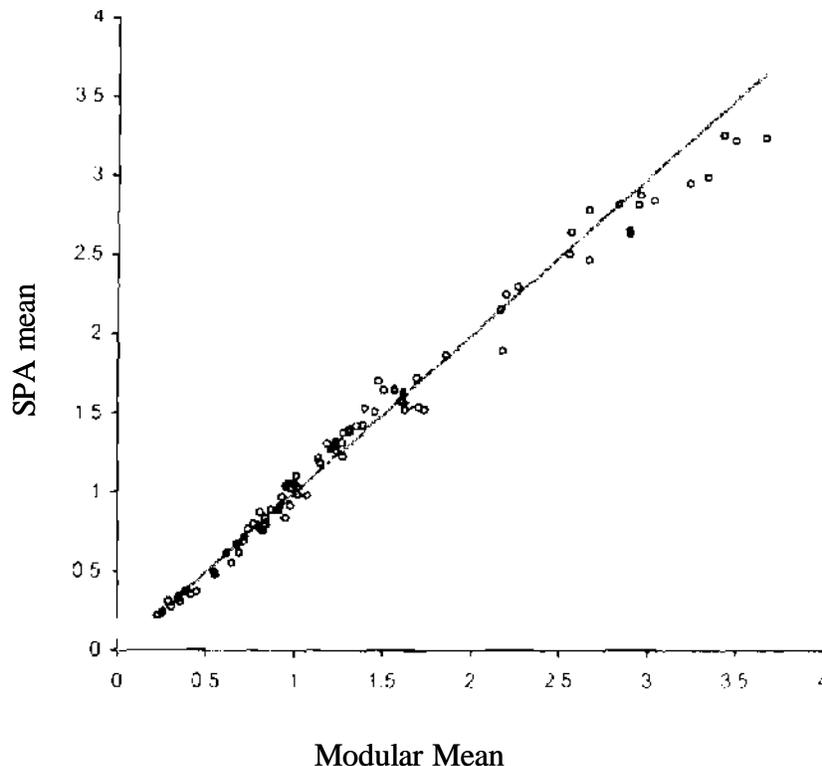
## 2. Comparison studies:

### a. Method comparison with predicate device:

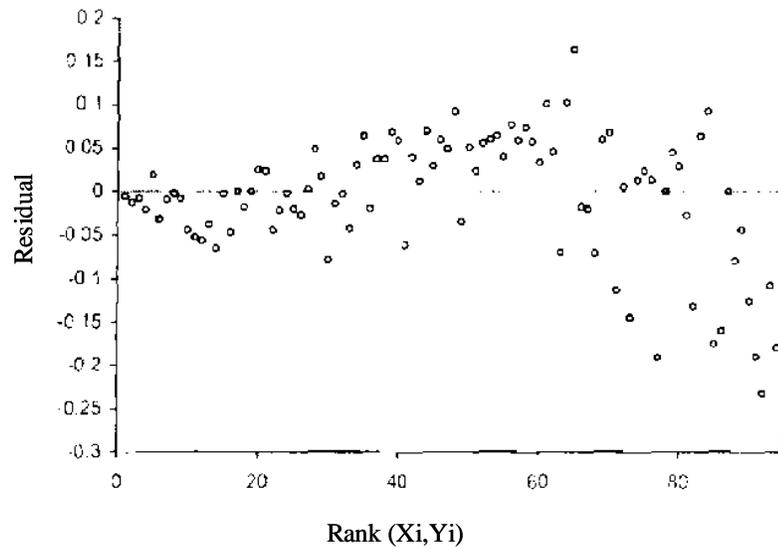
Method comparison between Binding Site's C3c SPAPLUS™ assays to the already 510k approved Tina-Quant C3c Test system (k012361 Roche Diagnostics Corp.) was done by using 92 human serum retrospective samples. The clinical samples were collected and stored at -80°C in order to preserve the complement levels as much as possible. No validation data is available to support these storage conditions. Thirty six normal samples and 56 clinical samples were tested on SPAPLUS™ and Tina-Quant C3c kit. Three of the clinical samples were diluted in SPAPLUS instrument system diluent to give adequate sample numbers over the measuring range. The samples were fairly distributed over the measuring range. The following results were obtained for 95 samples over the range of 0.230 to 3.670g/L:

Passing and Bablok fit  $y=0.99x + 0.00$   
 Linear regression  $y=0.9284 + 0.075$   $R^2=0.99$   $r=0.993$

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:**

These ranges were obtained using this kit, by measuring the C3c concentration of human serum. The reference interval was calculated using nonparametric statistics and represents the central 95% of the population.

	Number (n)	Mean (g/L)	Median (g/L)	95 Percentile Range (g/L)
C3c	120	1.168	1.151	0.811 - 1.570

**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.