

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

A. 510(k) Number:

k122304

B. Purpose for Submission:

New device

C. Measurand:

Complement C1 inactivator (inhibitor)

D. Type of Test:

Quantitative immunonephelometry

E. Applicant:

The Binding Site

F. Proprietary and Established Names:

Human C1 Inactivator Kit for use on SPAPLUS

Complement C1 inhibitor

G. Regulatory Information:

1. Regulation section:

21 CFR 866.5250 Complement C1 inhibitor (inactivator) immunological test system

2. Classification:

Class II – Device and Calibrator

3. Product code:

DBA, Complement C1 inhibitor (inactivator) antigen, antiserum, control

1. Panel:

Immunology (82)

H. Intended Use:

1. Intended use(s):

This Human C1 Inactivator kit for use on SPAPLUS is intended for the *in vitro* measurement of human C1 inactivator in human serum using the SPAPLUS Analyzer. Measurement of C1 inactivator levels in serum is an aid in the diagnosis of hereditary angioedema (HAE) 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:

SPAPLUS Analyzer (k040958)

I. Device Description:

The Human C1 Inactivator Kit for use on SPAPLUS is comprised of the following reagents:

Human C1 inactivator antiserum: This antiserum is monospecific for C1 inactivator and is supplied in stabilized liquid form. It contains 0.099% sodium azide, 0.1% EACA, 0.1% EDTA and 0.01% benzamidine as preservatives.

Calibrator set 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.

Reaction Buffer: Containing 0.099% sodium azide as a preservative

J. Substantial Equivalence Information:

1. Predicate device name(s) and 510(k) number(s):

Siemens N antiserum to C1 inhibitor k960257

2. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended use	Quantification of C1 inactivator in human serum. It is intended to aid in the diagnosis of hereditary angioedema in conjunction with other laboratory and clinical findings	Same
Assay type	Quantitative	Same

Differences		
Item	Device	Predicate
Test method	Turbidometry	Nephelometry
Specimen type	Serum	Serum and plasma (citrated and EDTA)
Detection antibody	Sheep anti-human C1 inactivator	Rabbit anti-human C1 inactivator
Sample dilution	1:10 and 1:20	1:5
Measuring range	0.06 – 0.40 g/L (1/10 dilution) 0.12 – 0.80 g/L (1/20 dilution)	0.03 to 0.40 g/L
Instrument	SPAPLUS Analyzer	Behring Nephelometer II System
Calibration	Six calibrators (provided): 0.006, 0.01, 0.015, 0.02, 0.03, 0.04 g/L	One N Protein Standard PY (not provided) serially diluted by the instrument
Control	Two controls (provided): high (0.3 g/L) and low (0.15g/L)	One N/T Protein Control PY (not provided) serially diluted by the instrument
Open vial stability	3 months	4 weeks
On-board stability	30 days	5 days at 8 hours per day

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

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

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

CLSI EP7-A2: Interference Testing in Clinical Chemistry, Approved Guideline – Second Edition

CSLI EP17-A: Protocols for Determination of Limits of Detection and Limits of

Quantitation; Approved Guideline

CLSI C28-A3: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory

L. Test Principle:

Human C1 inactivator protein contained in human plasma and serum samples reacts with sheep anti-human C1 inactivator to form immune complexes. These complexes scatter a beam of light passed through the sample and the intensity of the scattered light is proportional to the concentration of the protein in the sample. Protein concentration is automatically calculated by reference to a calibration curve stored within the SPAPLUS Analyzer.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The studies followed CLSI EP5-A2, where three pooled sera samples with high (0.359 g/L), mid (0.162 g/L) and low (0.108 g/L) analyte level were each tested in 2 runs per day (each of the 2 runs in duplicate) for over 21 days using 3 reagent lots on 3 analyzers. The same high sample was tested at 1/20 dilution. Results met the Acceptance criteria for total precision (%CV<10%), within-run precision (%CV <7%), between-run precision (%CV <8%), and between-day precision (%CV <8%). The precision of the 1/20 dilution is equivalent to that for the 1/10 standard dilution.

Sample	Low	Mid	High at 1/10 dilution	High at 1/20 dilution
Mean concentration	0.108 g/L	0.162 g/L	0.359 g/L	0.370 g/L
Total precision SD/ %CV	0.0058 5.4%	0.0070 4.3%	0.0170 4.7%	0.0171 4.6%
Within-run precision SD/ %CV	0.0029 2.6%	0.0023 1.4%	0.0057 1.6%	0.0055 1.5%
Between-run precision SD/%CV	0.0011 1.0%	0.0029 1.8%	0.0054 1.5%	0.0057 1.5%
Between-day precision SD/%CV	0.0050 4.6%	0.0059 3.6%	0.0151 4.2%	0.0151 4.1%
Between-batch precision SD/%CV	0.001 0.62%	0.002 1.51%	0.007 1.94%	0.005 1.33%
Between-instrument precision SD/%CV	0.005 4.31%	0.004 2.37%	0.014 3.89%	0.011 2.98%

b. *Linearity/assay reportable range:*

The studies followed CLSI EP6-A, where eleven serial dilutions were prepared by diluting a high (0.458 g/L) in a low pool (0.044 g/L) of unprocessed serum. The resulting series of 13 samples (100, 90, 80, 70, 60, 50, 40, 30, 20, 10, 5, 2.5, 0 % of the high pool) was tested in 3 replicates (Rep). Results met the Acceptance criteria for CV<8%. The linear regression equation is $y = 0.962x + 0.0122$ and R^2 value is 0.9982. The assay is linear between 0.044 - 0.458 g/L and the claimed measuring range is 0.06 - 0.4 g/L. The labeling states that for samples giving results outside of the measuring range at standard 1/10 sample dilution, auto dilution to 1/20 will be performed. Results which are still outside of the measuring range following dilution are reported as < 0.06 g/L (from 1/10 dilution) or > 0.8 g/L (from 1/20 dilution).

Sample	% High pool	Rep 1 (g/L)	Rep 2 (g/L)	Rep 3 (g/L)	Mean (g/L)	% Mean Recovery	SD	% CV
1	100	0.465	0.456	0.454	0.458	100	0.0023	5.3
2	90	0.411	0.416	0.412	0.413	116.6	0.0020	3.2
3	80	0.380	0.370	0.361	0.370	112.3	0.00313	4.2
4	70	0.325	0.326	0.324	0.325	117.5	0.00363	3.6
5	60	0.290	0.292	0.289	0.290	109.8	0.0026	1.9
6	50	0.250	0.258	0.249	0.252	109.7	0.0032	1.7
7	40	0.212	0.212	0.226	0.217	103.4	0.0081	3.7
8	30	0.188	0.183	0.182	0.184	100.5	0.0049	2.0
9	20	0.137	0.142	0.138	0.139	99.3	0.0015	0.5
10	10	0.097	0.104	0.099	0.100	97.3	0.0010	0.3
11	5	0.075	0.073	0.069	0.072	98.7	0.0095	2.6
12	2.5	0.065	0.063	0.061	0.063	99.1	0.0026	0.6
13	0	0.045	0.045	0.041	0.044	100	0.0059	1.3

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

i) *Traceability:*

The calibration of the assay is traceable to the international reference standard ERM-DA470k.

ii) *Kit Stability:*

Real-time stability – A study to establish shelf-life stability (from the date of manufacture when stored at recommended temperature 2-8°C) of the Human C1 Inactivator Kit is on-going. Currently available data supported a 3-month stability claim.

Open-vial stability - The Human C1 Inactivator Kit reagents can be stored, opened at 2 – 8°C for up to 3 months.

On-board stability – The Human C1 Inactivator Kit reagents can be stored onboard the SPAPLUS Analyzer at 8 – 12°C for at least 30 days.

d. *Detection limit:*

The analytical sensitivity was determined in accordance with CLSI EP17-A. The Limit of Blank (LoB) was based on 60 determinations of a blank sample and was estimated as the 95% percentile of the distribution. The Limit of Detection (LoD) was calculated according to the equation: the LoB + c_{β} x SD where SD, the standard deviation, was based on 40 determinations of a sample with analyte level near the lower limit of the reportable range. The LoQ was calculated and the bias (-0.001 g/L) was within the maximum allowable bias.

LoB	LoD	LoQ
0.0015	0.003	0.06

e. *Analytical specificity:*

Interferences were assessed according to CLSI EP7-A2 by testing 3 serum samples with low (0.094 g/L), mid (0.222 g/L) and high (0.341 g/L) C1 inactivator concentrations. Each sample was spiked with interfering substances and tested in replicates of 7 (for high sample) or 3 (for mid and low samples). For non-interference to be claimed, the mean results from the spiked samples must be within 10% of the mean of the neat samples. The data demonstrated that the assay was not affected by high levels of the following substances: hemoglobin (5 g/L), bilirubin (200 mg/L), and chyle (1500 formazine turbidity units or FTU).

Hook effect

The possibility of a hook effect due to antigen excess was assessed as follows: 3 batches of a C1 Inactivator Internal Reference preparation with a value of 2.31 g/L which is 5.7 times the highest calibrator were assayed. The resulting OD values were higher than the normal curve top point for all three batches. The data demonstrated the assay is not susceptible to antigen excess up to a concentration of 0.231 g/L which is equivalent to 2.31 g/L at standard 1/10 dilution.

f. *Assay cut-off:*

Not determined

2. Comparison studies:

a. *Method comparison with predicate device:*

A total of 94 sera samples spanning the dynamic range were assayed in singleton by both the Human C1 Inactivator Kit on the SPAPLUS Analyzer and the N antiserum to C1 inhibitor test on the BN II System. The serum samples included 53 normal donors and 29 clinical samples (HAE (7), unknown diagnosis (18), recurrent periorbital edema (1), urticaria (1), IgG kappa paraprotein (1), SLE (1)). In addition, 4 contrived normal serum samples and 8 serum samples from patients with Kappa Myeloma (5) and Lambda Myeloma (3) with depressed levels of C1 inactivator were included to cover the lower end of the claimed measuring range. Regression statistics are based on the balance of the paired results and the inclusion or exclusion of 4 between-

method outliers outside 95% limits, and the data are as follows:

Regression fit	Inclusion of outliers	Regression Equation	Slope (95% CI)	Intercept (95% CI)
Ordinary Linear	Yes	$y = 0.92x + 0.02$	0.86 to 0.98	0.00 to 0.03
	No	$y = 0.96x + 0.01$	0.91 to 1.01	-0.01 to 0.02
Passing-Bablok	Yes	$y = 0.98x$	0.93 to 1.04	-0.01 to 0.02
	No	$y = 0.99x$	0.94 to 1.05	-0.02 to 0.01
Deming	Yes	$y = 0.96x$	0.90 to 1.02	-0.01 to 0.02
	No	$y = 0.98x$	0.93 to 1.03	-0.01 to 0.01

When considering the lower limit of the reference range as the medical decision point, the percentage positive and negative agreement in the serum samples (n=94) analyzed including all 4 outliers is as follows:

Human C1 Inactivator Kit on the SPAPLUS Analyzer	Predicate		
	Positive	Negative	Total
Positive	17	4	21
Negative	2	73	75
Total	19	77	96

Positive percentage agreement: 89.47 % (95% CI: 83.33 - 95.61)
 Negative percentage agreement: 94.81 % (95% CI: 90.37 - 99.25)
 Overall agreement: 93.75 % (95% CI: 88.91 – 98.59)

When considering the lower limit of the reference range as the medical decision point, the percentage positive and negative agreement in the serum samples (n=90) analyzed excluding all 4 outliers is as follows:

Human C1 Inactivator Kit on the SPAPLUS Analyzer	Predicate		
	Positive	Negative	Total
Positive	17	4	21
Negative	1	70	71
Total	18	74	92

Positive percentage agreement: 94.44 % (95% CI: 89.95 – 98.93)
 Negative percentage agreement: 94.59 % (95% CI: 90.16 – 99.02)
 Overall agreement: 94.57% (95% CI: 90.13 - 99.01)

b. Matrix comparison:

None

3. Clinical studies:

a. Clinical Sensitivity:

None determined

b. Clinical specificity:

None determined

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

Not applicable

4. Clinical cut-off:

Based on lower limit of reference range which is 0.21 g/L.

5. Expected values/Reference range:

The reference range (0.21 -0.38 g/L) selected is comparable to that of the predicate assay (0.21 -0.39 g/L) and those used by several US Reference Centers: Mayo Clinic (0.19-0.37 g/L); ARUP Laboratories (0.21-0.39 g/L); Duke University Hospital (0.21-0.39 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.