Human C1 Inactivator Kit for use on SPAPLUS

For *in-vitro* diagnostic use

Product Code: NK019.S.A

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FDA (USA) Information
Analyte name: Complement C1 Inhibitor
Complexity Cat.: Moderate

1 INTENDED USE

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 analyser. 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 SUMMARY AND EXPLANATION

C1 inactivator (also known as C1 inhibitor) is a 105 kDa heavily glycosylated protein which belongs to the serpin family. C1 inactivator is an important regulator of the classical complement pathway. It inhibits the activation of the first complement component in the cascade therefore limiting any potentially harmful effects of over-activation. It acts by inhibiting the activity of C1s and C1r serine proteases. C1 inactivator is a major regulator of the inflammatory response. Measurement of C1 inactivator aids in the diagnosis of HAE which are the most common complement deficiencies (refs 1-3).

3 PRINCIPLE

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 in the test sample. Concentrations are automatically calculated by reference to a calibration curve stored within the instrument.

4 REAGENTS

4.1 Human C1 inactivator Antiserum
This antiserum is monospecific for C1 inactivator and is supplied in stabilised liquid form. It contains 0,099% sodium azide, 0.1% EACA, 0.1% EDTA and 0.01% benzamidine as preservatives.

4.2 Calibrator and Controls: These consist of pooled human serum and are supplied in stabilised liquid form. They contain 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives. The concentration of C1 Inactivator given in the quality control certificate has been obtained by comparison with European Reference Material ERM – DA470k.

4.3 Reaction Buffer: Containing 0.099% sodium azide as a preservative.

5 CAUTION

All donors of human serum supplied in this kit have been serum tested and found negative for hepatitis B surface antigen (HBsAg) and antibodies to human immunodeficiency virus (HIV1 and HIV2) and hepatitis C virus. The assays used were either cleared by the FDA (USA) or cleared for *in vitro* diagnostic use in the EU (Directive 98/79/EC, Annex II); however, these tests cannot guarantee the absence of infective agents. Proper handling and disposal methods should be established as for all potentially infective material, including (but not limited to) users wearing suitable protective equipment and clothing at all times. Only personnel fully trained in such methods should be permitted to perform these procedures.

WARNING: This product contains sodium azide and must be handled with caution; suitable gloves and other protective clothing should be worn at all times when handling this product. Do not ingest or allow contact with the skin (particularly broken skin or open wounds) or mucous membranes. If contact does occur wash with a large volume of water and seek urgent medical advice. Explosive metal azides may be formed on prolonged contact of sodium azide with lead and copper plumbing; on disposal of reagent, flush with a large volume of water to prevent azide build up.

This product should only be used by suitably trained personnel for the purposes stated in the Intended Use. Strict adherence to these instructions is essential at all times. Results are likely to be invalid if parameters other than those stated in these instructions are used.

Reagents from different batch numbers of kits are **NOT** interchangeable. If large numbers of tests are performed care should be taken to ensure that all the reagents are from the same batch.

6 STORAGE AND STABILITY

The unopened kit should be stored at 2-8°C and can be used until the expiry date shown on the kit box label. DO NOT FREEZE. The Human C1 inactivator Antiserum, Reaction Buffer, calibrators and controls may be stored for up to three months after opening providing that they are capped to avoid evaporation and kept at 2-8°C in a refrigerator. The Human C1 inactivator Antiserum and Reaction Buffer may be stored, uncapped, on the SPAPLUS analyser for up to 30 days, provided that the main power switch (located at the rear of the left hand panel) is left switched on.

7 SPECIMEN COLLECTION AND PREPARATION

Use fresh or deep frozen serum samples. Samples should be obtained by venepuncture and separated as soon as possible. Blood should be allowed to clot and the serum separated as soon as possible to prevent haemolysis. Samples may be stored at 2-8°C for no more than 8 days. Samples can be stored below -20°C for up to 1 year if they are frozen within 24 hours after collection (ref 4). **Frozen samples must be clarified by centrifugation prior to testing.** Repeated freeze/thaw cycles should be avoided. Microbially contaminated serum samples, samples containing particulate matter and lipaemic or haemolysed serum samples should not be used.

8 METHODOLOGY

8.1 Materials provided

8.1.1 1 x 50 Tests *Human C1 Inactivator Antiserum SPAPLUS*
8.1.2 1 x *Human C1 Inactivator SPAPLUS Calibrator set 1-6* (6 x 1.0mL)
8.1.3 2 x 1.5mL *Human C1 Inactivator SPAPLUS High Control*
8.1.4 2 x 1.5mL *Human C1 Inactivator SPAPLUS Low Control*
8.1.5 1 x 50 Tests *C1 Inactivator Reaction Buffer SPAPLUS*

8.2 Materials required but not provided

8.2.1 Equipment for collection and preparation of test samples e.g. sample tubes, centrifuge etc.
8.2.2 A fully operational and equipped SPAPLUS analyser.
8.2.3 Current analyser operating instructions: SPAPLUS Reference Guide, Insert Code FIN012.
8.2.4 Sample Diluent (99: Dil 1) Product Code: SN080.S

8.3 Reagent preparation

Before loading, gently mix by inversion ensuring no foam or bubbles are generated or remain on the surface as these may interfere with reagent aspiration.

8.4 Test procedure

The user should be familiar with the operation of the SPAPLUS analyser before attempting to carry out the test procedures. The analyser should be prepared for use according to the manufacturer's instructions and the assay protocol entered as described below.
For full details of analyser operation refer to the SPAPLUS Reference Guide (FIN012) supplied with the analyser.

8.4.1 Test parameters

Assay parameters are entered into item number 18.

Item Name 18 C1i
DATA INFORMATION
Units g/L
Decimals 3

ANALYSIS
Type End ▼

Main W.Length 1 340 ▼
Sub W.Length ▼
Method

CORR.
SLOPE INTER
Y = 1 X + 0

CALIBRATION
Type Logit 2 ▼
Standard
1 # 4 #
2 # 5 #
3 # 6 #

NORMAL RANGE

	MALE LOW	MALE HIGH	FEMALE LOW	FEMALE HIGH
Serum	[ ]	[ ]	[ ]	[ ]
Urine	[ ]	[ ]	[ ]	[ ]
Plasma	[ ]	[ ]	[ ]	[ ]
CSF	[ ]	[ ]	[ ]	[ ]
Dialysis	[ ]	[ ]	[ ]	[ ]
Other	[ ]	[ ]	[ ]	[ ]

Page : 1 Print Hard Copy Next Page Save Return

Item Name 18 C1i

ASPIRATION
KIND ○ Single ● Double
VOLUME
SAMPLE 18
REAGENT1 VOL 160 µL
REAGENT2 VOL 25

Third mix ● OFF ○ ON
R1 Blank ● Water – Blank

MONITOR
0 LEVEL SPAN 1
SPAN 3.0

DATA PROCESS
READ

	START	END
MAIN	53	54
SUB	30	31

ABSORBANCE LIMIT
LOW -3.0
HIGH 3.0

FACTOR
Blank correction *
ENDPOINT LIMIT 2.0
LINEAR CHECK (%) 0

Reaction Check
○ ON ● OFF
CHECK POINT
LOW -3
HIGH 3

DILUTION
Diluent ● 99: Dil 1 ○ 100: Dil 2
Pre Dilution Rate 10 ▼
Auto Rerun Dilution Rate High 20 ▼
Auto Rerun Dilution Rate Low ▼

PROZONE CHECK

	START	END	LIMIT (%)	
FIRST	[ ]	[ ]		
SECOND	[ ]	[ ]	[ ]	○ Low ● High
THIRD	[ ]	[ ]	[ ]	○ Low ● High

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*automatically calculated

Item Name 18 C1i

Auto Rerun SW
● On ○ Off

Auto Rerun Range (Result)
○ On ● Off ● On ○ Off

	Lower	Higher
Serum	Cal 1 #	Cal 6 #
Urine		
Plasma		
CSF		
Dialysis		
Other		

Bottle Size (ml)

24 Items		36 Items	
Reagent1	60	Reagent1	0
Reagent2 R1	8.50	Reagent2 R1	0
Reagent2 R2	1.75	Reagent2 R2	0

Auto Rerun Condition (Absorbance)

Absorbance Range
Lower ○ On ● Off
Higher ● On ○ Off

Prozone Range ○ On ● Off

Page : 3 Print Prev Page Save Return

NB. The calibrator (Standard #) values to be entered on **Page: 1** are found on the quality control certificate (SIN294.QC) and should be entered in ascending order, i.e. lowest value first. **IMPORTANT:** the analyser will automatically calculate and enter the correct measuring ranges on Item pages 3 and 4 providing **the ENTER button is pressed after typing the value for calibrator 6 on Page: 1**. View Item parameter page 4 to ensure correct value entry.

8.4.2 Control volumes

Transfer 150µL of control fluid into a sample cup and place onto the rack.

8.5 Measuring range

The approximate measuring range of the C1 inactivator assay is shown in the table below.

SPAPLUS Analyser Dilution	Approximate range (g/L)
1/10	**0.06-0.4**
1/20	0.12-0.8

Results outside the measuring range should be reported as a 'less than' or 'greater than' result.

9 QUALITY CONTROL

We recommend that the controls provided should be included in all assays performed. The C1 inactivator concentration is stated on the accompanying QC certificate (SIN294.QC). Sample results obtained should only be accepted if the control results are within ±15% of the concentration(s) stated.

10 LIMITATIONS

10.1 Turbidimetric assays are not suitable for measurement of highly lipaemic or haemolysed samples or samples containing high levels of circulating immune complexes (CICs) due to the unpredictable degree of non-specific scatter these sample types may generate. Unexpected results should be confirmed using an alternative assay method.

10.2 This assay has not been validated using paediatric samples.

10.3 Should a control measurement be out of range when assayed with a stored curve the assay must be recalibrated. If on recalibration the control values measured with the new curve are still out of range, the instrument and the assay parameters should be checked before repeating the assay. If problems persist, refer to supplier.

10.4 Diagnosis cannot be made and treatment must not be given on the basis of C1 inactivator measurements alone. Clinical history and other laboratory findings must be taken into account.

10.5 Variation in reagent temperature may affect results. Ensure that reagents are transferred directly from the refrigerator to the refrigerated reagent compartment of the analyser – do not allow to warm to room temperature.

10.6 Samples stored frozen must be clarified by centrifugation prior to testing. Failure to do so may result in suppression of the result.

10.7 In patients with hereditary angioedema (HAE), it has been reported that C1 inactivator can form stable covalent complexes with its target proteases[5]. The potential effect of this on patient results obtained with this assay has not been assessed.

10.8 In patients with acquired angioedema (AAE), it has been reported that antibodies to C1 inactivator are produced [6,7,8]. The potential interference from such antibodies on patient results obtained with this assay has not been assessed.

11 EXPECTED VALUES

The reference range for this kit was transferred from an alternative commercially available assay in accordance with CLSI document *EP C28-A3 "Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory"* and was validated by measuring the C1 Inactivator concentration of sera taken from 20 healthy UK adults. Wherever possible it is strongly recommended that local ranges are generated.

	95 Percentile Range (g/L)
Serum	0.21-0.38

12 PERFORMANCE CHARACTERISTICS

12.1 Precision

A study was 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 four different samples using three different reagent lots on three analysers.

C1 inactivator Precision summary									
	Mean (g/L)	Within run		Between run		Between day		Total	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %
Serum 1	0.359	0.006	1.6	0.005	1.5	0.015	4.2	0.017	4.7
Serum 2	0.162	0.002	1.4	0.003	1.8	0.006	3.6	0.007	4.3
Serum 3	0.108	0.003	2.6	0.001	1.0	0.005	4.6	0.006	5.4
Serum 4*	0.370	0.006	1.5	0.006	1.5	0.015	4.1	0.017	4.6

*Repeat of Serum 1, performed at a 1/20 sample dilution

12.2 Comparison

A correlation study was performed on 94 samples (using a variety of normal and clinical serum samples) using this kit on a SPAPLUS and an alternative commercially available C1 inactivator assay. The study demonstrated excellent agreement with the following Deming regression plot:

$y = 0.98 + 0.00$ (g/L) (y = SPAPLUS C1 inactivator; x = alternative assay)

correlation coefficient $R^2 = 0.905$ (calculated by linear regression)

12.3 Limit of Quantitation

Based on *CLSI document EP17-A - Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline* the limit of quantitation for this assay is defined as the lowest point of the calibration curve i.e. 0.06g/L based upon the minimum sample dilution.

12.4 Linearity

A linearity study was performed following CLSI (formally NCCLS) *Evaluation of the Linearity of Quantitative Measurement Procedures* document EP6-A. One user assessed the linearity of a pool of high samples using one lot of reagent on one analyser. This gave a regression plot of $y = 0.96x + 0.012$ (y = measured C1 inactivator concentration, x = theoretical concentration) over the range of 0.044 – 0.458g/L using the 1/10 sample dilution.

12.5 Interference

No significant assay interference by 1500 formazine turbidity units (FTU) of chyle, 200mg/L bilirubin, 5g/L haemoglobin (Hb) has been demonstrated for a sample with a concentration of 0.161g/L at the minimum sample dilution (1/10).

	Bilirubin	Hb	Chyle
Mean C1 inactivator (g/L)	0.147	0.141	0.157
% interference	-2.78%	0%	4.26%

12.6 Antigen excess

No antigen excess was observed to a level of 5.7 times the top point of the assay, approximately 2.31g/L (at the standard sample dilution).

13 BIBLIOGRAPHY

1. Zuraw B.L. (2003), Diagnosis and management of hereditary angioedema: an American approach *Transfusion and Apheresis Science,* 29. pp 239–245
2. Rosen F.S. and Davis A.E. (2005) Deficiencies of C1 inhibitor. *Best Practice & Research Clinical Gastroenterology* Vol. 19, No. 2, pp. 251–261
3. Gompels M. M., *et al* (2005) C1inhibitor deficiency: consensus document British Society for Immunology, *Clinical and Experimental Immunology*139:pp379–394
4. Use of Anticoagulants in Diagnostic Laboratory Investigations WHO/DIL/LAB/99.1 Rev.2 2002.
5. Cugno M. *et al* (1990), Plasma levels of C1 inhibitor complexes and cleaved C1 inhibitor in patients with hereditary angioneurotic edema. *J Clin Invest 85: 1215-1220.*
6. Alsenz J, Bork K, Loos M, (1987), Autoantibody-mediated acquired deficiency of C1 inhibitor. *N Engl J Med **316**: 1360-1366.*
7. Chevailler A. *et al* (1996). C1-inhibitor binding monoclonal immunoglobulins in three patients with acquired angioneurotic edema. *J Clin Immunol 97 (4): 998-1008.*
8. Fremeau-Bacchi V. *et al* (2002). Prevalence of monoclonal gammopathy in patients presenting with acquired angioedema type 2. *Am J Med 113 (3) 194-199.*