Human C3c Kit for use on SPA*PLUS*®

For *in vitro* diagnostic use only

Product Code: NK023.S

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FDA (USA) Information
Analyte name Complement C3
Complexity Cat.: Moderate

CE

1 INTENDED USE

This kit is intended for the quantitative *in vitro* determination of human C3c in serum using the Binding Site SPA*PLUS* turbidimetric analyser. This test should be used in conjunction with other laboratory and clinical findings.

2 SUMMARY AND EXPLANATION

C3c is the major protein component of the complement system, and has a fundamental role in the inflammatory response and immune system functionality. C3c is produced in the liver, and accordingly reduced complement activity is associated with severe liver failure (Ref 1). Deficiencies in C3c, either acquired or otherwise, manifest in severe recurrent infections such as pneumococcal and meningococcal infections, and an increased risk of systemic lupus erythematosus (Refs 1, 2). C3c deficiency is also associated with glomerulonephritis and vasculitis, as well as other conditions. Elevated complement levels and serum C3c concentrations are indicative of acute inflammatory reactions and untreated inflammatory conditions such as rheumatoid arthritis (Ref 2, 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 C3c Antiserum:** This is supplied in stabilised liquid form. Preservatives: 0.099% sodium azide, 0.1% E-amino-n-caproic acid (EACA) and 0.01% benzamidine.

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 C3c given on the quality control certificate has been obtained by comparison with the DA470k international reference material.

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 C3c Antiserum, Reaction Buffer, Calibrators and Controls may be stored for up to three months after opening provided that they are capped to avoid evaporation and kept at 2-8°C in a refrigerator. The Human C3c Antiserum and Reaction Buffer may be stored at 8-12°C, uncapped, on the SPA*PLUS* 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 frozen serum samples. Blood samples should be collected by venepuncture, allowed to clot naturally and the serum separated as soon as possible to prevent haemolysis. **Note:** Upon storage, C3 breaks down to C3c. Depending on the storage conditions, the C3c level in very fresh samples is lower than aged sample (ref. 4). Sera may be stored at 2-8°C for up to three days, otherwise aliquot and freeze at -20°C or below and store for up to 8 days; do not freeze and thaw sera more than once. Sample dilutions should be freshly prepared on the day of assay.

8 METHODOLOGY

8.1 Materials provided

8.1.1 1 x 100 Tests *Human C3c Antiserum SPA*PLUS
8.1.2 1 x *Human C3c SPA*PLUS *Calibrator set 1-6* (6 x 1.0mL)
8.1.3 2 x 1.3mL *Human C3c SPA*PLUS *High Control*
8.1.4 2 x 1.3mL *Human C3c SPA*PLUS *Low Control*
8.1.5 1 x 100 Tests *C3c Reaction Buffer SPA*PLUS

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 SPA*PLUS* analyser.
8.2.3 Current analyser operating instructions: SPA*PLUS* 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 SPA*PLUS* 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 SPA*PLUS* Reference Guide (FIN012) supplied with the analyser.

8.4.1 Test parameters

Assay parameters are entered into item number 19.

Item Name 19 C3c
DATA INFORMATION
Units g/L
Decimals 3

ANALYSIS
Type End ▼

Main W.Length 1 380 ▼
Sub W.Length ▼

Method

CORR.
SLOPE INTER
Y = 1 X + 0

CALIBRATION
Type Spline 1 ▼
Auto Fill
Standard
1 # 4 #
2 # 5 #
3 # 6 #

NORMAL RANGE

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

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Item Name 19 C3c

ASPIRATION
KIND ○ Single ● Double
VOLUME
SAMPLE 17
REAGENT1 VOL 195 µL
REAGENT2 VOL 40

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 (%) Min dOD	
FIRST	[ ]	[ ]		0
SECOND	[ ]	[ ]	[ ] ○ Low	● High
THIRD	[ ]	[ ]	[ ] ○ Low	● High

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*Automatically calculated

Item Name 19 C3c

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

Auto Rerun Condition (Absorbance)

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

Prozone Range ○ On ● Off

Bottle Size (ml)

24 Items		36 Items
Reagent1	60	Reagent1
Reagent2 R1	20.5	Reagent2 R1
Reagent2 R2	5	Reagent2 R2

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N.B. The calibrator (Standard #) values are found in the Quality Control Certificate (SIN167.QC). Calibrator values on **Page 1** should be entered in ascending order, i.e. the lowest value first. The analyser will automatically calculate and enter the correct measuring ranges on item pages 3 and 4 providing the Autofill button is pressed after typing the value for calibrator 6 on page 1. View Item parameter pages 3 and 4 to ensure correct value entry.
* The Blank correction factor is automatically calculated by the instrument.

8.5 Measuring range

The approximate measuring range of the C3c assay when using the standard 1/10 sample dilution is 0.25-3.0g/L.

9 QUALITY CONTROL

9.1 At least two levels of appropriate control material should be tested a minimum of once a day. In addition, controls should be tested after calibration, with each new lot of reagent and after specific maintenance or troubleshooting steps described in the SPAPLUS Operation Manual.

9.2 Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure. 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 the local technical support organisation.

9.3 The concentrations of the controls provided are stated on the accompanying QC certificate (SIN167.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 e.g. radial immunodiffusion.

10.2 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.

10.3 Diagnosis cannot be made and treatment must not be given on the basis of C3c measurements alone. Clinical history and other laboratory findings must be taken into account.

11 EXPECTED VALUES

The ranges provided have been obtained from a limited number of adult samples and are intended for guidance purposes only. Wherever possible it is strongly recommended that local ranges are generated. Paediatric data is not available.

Adult serum ranges

These ranges were obtained using this kit, by measuring the C3c concentration of sera taken from healthy adult UK blood donors. 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)
C3c	120	1.168	1.151	0.811-1.570

12 PERFORMANCE CHARACTERISTICS

12.1 Precision

A study was performed following CLSI (formerly NCCLS) *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 using three different reagent lots on three analysers.

C3c Precision Summary									
	Mean (g/L)	Within run		Between run		Between day		Total	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %
Serum 1	3.114	0.053	1.7	0.065	2.1	0.122	3.9	0.148	4.7
Serum 2	0.899	0.017	2.0	0.023	2.6	0.028	3.2	0.04	4.6
Serum 3	0.421	0.009	2.5	0.015	4.0	0.0174	4.7	0.025	6.6

12.2 Comparison

A correlation study was performed on 95 samples (36 normal serum and 59 clinical serum) using this kit on a SPAPLUS and an alternative commercially available C3c assay. The study demonstrated agreement with the following Passing Bablok plot:

y = 0.99x + 0.00 (g/L) (y = SPAPLUS C3c; x = alternative assay)

correlation coefficient r = 0.993 (calculated by linear regression)

12.3 Limit of Blank and Limit of Detection

Based on *CLSI document EP17-A - Protocols for Determination of Limits of Detection and Limits Quantitation; Approved Guideline* the limit of detection represents the lowest measurable analyte level that can be distinguished from zero. This has been estimated at 0.012 g/L (n = 60). The limit of quantitation for this assay is defined as the lowest point of the calibration curve i.e. 0.24g/L based upon a 1/10 sample dilution.

12.4 Linearity

A linearity study was performed based on CLSI *Evaluation of the Linearity of Quantitative Measurement Procedures* (CLSI 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 = 0.9994x - 0.008 (y = measured C3 concentration, x = theoretical concentration) over the range of 0.03 to 2.98g/L using the neat and 1/10 sample dilutions.

12.5 Interference

No significant assay interference by 1500 formazine turbidity units (FTU) of chyle, 200mg/L bilirubin, 5.0g/L haemoglobin has been demonstrated at the minimum sample dilution (1/1).

	Bilirubin	Hb	Chyle
Mean C3c (g/L)	0.31	0.29	0.29
% interference	5.0%	0.1%	1.0%

12.6 Antigen excess

No antigen excess was observed to a level of eight times the top point of the assay; approximately 27.2g/L.

13 BIBLIOGRAPHY

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