Human CH50 reagent pack for use on the SPAPLUS®

For *in vitro* diagnostic use only

Product Codes: NK095.S

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

CE

1 INTENDED USE

These reagents are intended for the quantification of total classical complement activity (CH50) in human serum on the Binding Site SPAPLUS analyser. Measurement of complement activity aids in the diagnosis of immunological disorders, especially those associated with deficiencies of complement components. The test results are to be used in conjunction with clinical findings and other laboratory tests.

2 SUMMARY AND EXPLANATION

The complement cascade comprises over 20 serum proteins that form part of the innate immune system. Complement acts in a number of ways to help clear invading organisms, with a major function being the lysis of bacteria through formation of the membrane attack complex (MAC). The complex interactions of the complement cascade mean that functionality of the MAC cannot necessarily be inferred by apparently normal levels of any single complement component. Therefore the traditional method for measuring total complement activity in serum is the CH50 test, based on complement mediated haemolysis of antibody sensitised erythrocytes[1]. However this method can be complicated, time consuming and reagent stability limited. The SPAPLUS CH50 assay alleviates these issues by directly testing the function of the MAC, thereby quantifying total complement activity (CH50). Complement activity has been correlated with the active stage of systemic lupus erythematosus, rheumatoid arthritis, some forms of nephritis, and inherited deficiencies of the complement system.[2,3,4]

3 PRINCIPLE

Liposomes, encapsulating glucose-6-phosphate dehydrogenase (G6PDH) are used to mimic an invading microorganism. On addition of sample, antibodies in the reagent combine with dinitrophenyl groups on the surface of the liposomes. The resultant complex activates complement in the sample, which lyses the liposome, releasing G6PDH to react with glucose-6-phosphate and NAD in the reagent. The change in absorbance can be measured and is proportional to the complement activity in the sample. Comparison to a calibration curve gives a value for the unknown patient sample.[5]

4 REAGENTS

4.1 CH50 Liposome reagent R1: Liquid reagent containing liposome G6PDH

4.2 CH50 Substrate R2: Lyophilised, containing anti-DNP antibody (goat), 24 mmol/L G6P and 9 mmol/L NAD

4.3 CH50 Substrate diluent R2a: Liquid reagent containing 10 mmol/L maleate buffer (pH 5.0)

5 CAUTION

The R2a Substrate diluent (NA095.R2a) is irritating to eyes and skin.
Potential health effects:
Eye contact: may cause irritation, redness and swelling of the eyes
Skin contact: May cause irritation and skin rash (dermatitis).
Inhalation: High vapour concentrations are irritating to the eyes, nose, throat and lungs.
Ingestion: May cause abdominal pain.
Avoid skin contact and wear suitable gloves when handling the substrate.

This product should only be used by suitably trained personnel for the purposes stated. Adherence to the given advice is recommended. **The validity of results obtained using parameters other than those stated cannot be guaranteed.**

6 STORAGE AND STABILITY

The unopened reagent pack 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 CH50 R1 Liposome reagent and reconstituted R2 Substrate 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. It is recommended to measure the complement activity in the specimen immediately after separation of serum. For prolonged storage, specimens should be stored at -70^{0}C or lower. Repeated freezing and thawing should be avoided. Samples containing high levels of lipid, haemoglobin, bilirubin or ascorbic acid may cause interference and should be avoided.

8 METHODOLOGY

8.1 Materials provided

8.1.1 2 x 20mL *CH50 Liposome reagent R1* (supplied as a clear liquid)
8.1.2 1 x 20mL *CH50 Substrate R2* (supplied lyophilised)
8.1.3 1 x 20mL *CH50 Substrate diluent R2a* (supplied as a clear liquid)
8.1.4 1x *Liposome reagent bottle* (*R1*) (supplied empty)
8.1.5 1x *Substrate bottle* (*R2*) (supplied empty)

8.2 Materials required but not provided

8.2.1 NC095.S Human CH50 calibrator set for use on the SPAPLUS
8.2.2 NQ095.S Human CH50 controls for use on the SPAPLUS
8.2.3 Equipment for collection and preparation of test samples e.g. sample tubes, centrifuge etc.
8.2.4 A fully operational and equipped SPAPLUS analyser.
8.2.5 Current analyser operating instructions: SPAPLUS Reference guide, Insert Code FIN012.
8.2.6 Sample Diluent (99: Dil 1) Binding Site Product Code: SN080.S

8.3 Reagent preparation

8.3.1 **R1 Liposome reagent:** pour 40mL of R1 Liposome reagent into the liposome reagent bottle (R1)
8.3.2 **R2 substrate:** reconstitute 20mL of R2 Substrate with 20mL of R2a Substrate diluent to prepare the substrate solution and mix by gentle inversion. Ensure that the substrate is completely reconstituted before pouring into the Substrate bottle (R2)

Ensure that both R1 and R2 bottles are free from bubbles before loading onto the analyser.

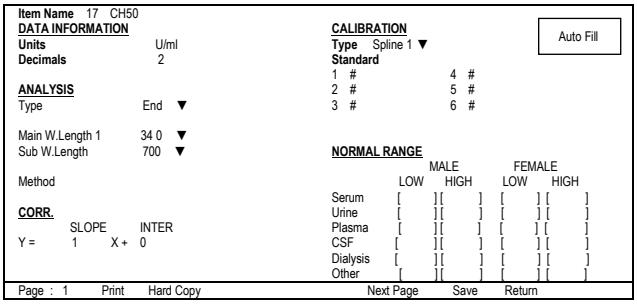
8.4 Test procedure

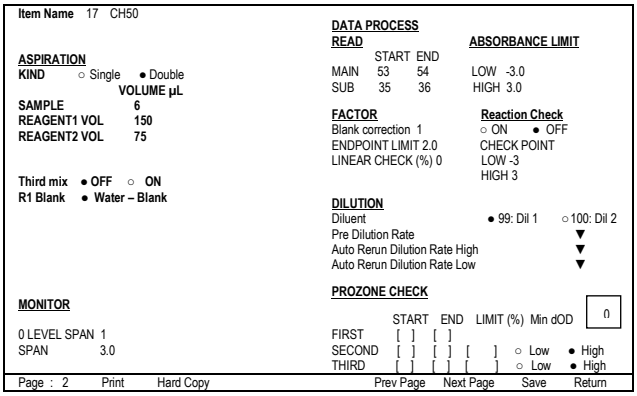
The user should be familiar with the operation of the SPAPLUS analyser before attempting to carry out the test procedures. The analyser should be prepared for use according to the manufacturer's instructions and the assay protocol entered as described below.

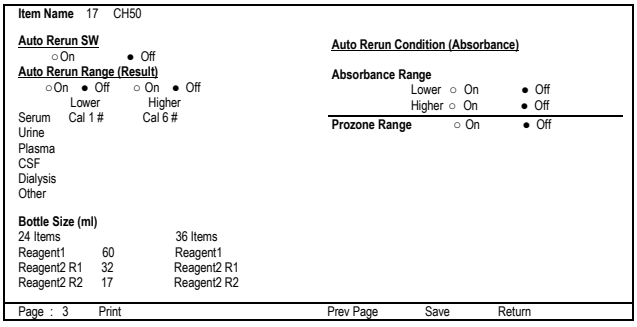
For full details of analyser operation refer to the SPAPLUS Reference Guide (FIN012) supplied with the analyser.

8.4.1 Test parameters

Assay parameters are entered into item number 17.







The calibrator (Standard #) values are found in the Quality Control Certificate (SIN164.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 page 3 and 4 providing the Autofill button is pressed after typing the value for calibrator 6 on page 1. View Item parameter page 3 and 4 to ensure correct value entry.

8.5 Measuring range

The approximate measuring range of the CH50 assay when using the standard 1/1 sample dilution is 12-95 U/mL. Samples measuring above this range should be manually diluted offline (1/2 in sample diluent SN080.S). Present the 1/2 diluted sample for analysis. Multiply the result by 2.

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 vial 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 (SIN194.QC). Sample results obtained should only be accepted if the control results are within ±20% 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 Diagnosis cannot be made and treatment must not be given on the basis of CH50 measurements alone. Clinical history and other laboratory findings must be taken into account.

10.3 This assay has not been validated for the paediatric population.

11 EXPECTED VALUES

These ranges were obtained on the SPAPLUS analyser using normal UK blood donor sera. They are intended for guidance purposes only. The sera reference interval was calculated using parametric statistics and represents the central 95% of the population. It is strongly recommended that each user generates their own CH50 reference ranges.

	Number (n)	Mean (U/mL)	Median (U/mL)	95 Percentile Range (U/mL)
CH50	120	68.37	68.70	41.68 - 95.06

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.

		CH50 Precision Summary							
	Mean (U/mL)	Within run		Between run		Between day		Total	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %
Serum 1	78.73	1.67	2.1	3.04	3.9	3.26	4.1	4.76	6.0
Serum 2	55.22	0.66	1.2	0.65	1.2	1.86	3.4	2.08	3.8
Serum 3	46.05	0.84	1.8	1.03	2.2	1.00	2.2	1.66	3.6
Serum 4	25.34	0.34	1.4	0.39	1.5	0.99	3.9	1.12	4.4

12.2 Comparison

A correlation study was performed on 110 serum samples using this kit on a SPAPLUS and an alternative commercially available CH50 assay. The study demonstrated a good agreement giving the following Passing & Bablok plot. (Please note that 62 samples were excluded from the plot as they gave '<' results).

y = 1.15x - 4.29 (U/mL) (y = SPAPLUS CH50; x = alternative assay)

correlation coefficient r = 0.961 (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. 11.98 U/mL based upon the minimum sample dilution.

12.4 Linearity

A linearity study was performed following CLSI *Evaluation of the Linearity of Quantitative Measurement Procedures* (NCCLS Document EP6-A) using a diluted high level sample. This gave a regression plot of *y = 0.996x – 2.709 (U/mL), r = 0.994* (y = measured CH50, x = theoretical concentration) over the range of 12.68 - 95.47 U/mL.

12.5 Interference

No significant assay interference by 1500 formazine turbidity units (FTU) of chyle, 200mg/L bilirubin, 5g/L haemoglobin and 0.5g/L ascorbic acid has been demonstrated at the standard sample dilution (1/1).

	Bilirubin	Hb	Chyle (FTU)	Ascorbic acid
Mean CH50 (U/mL)	36.26	35.40	35.51	15.50
% interference	-0.21	3.39	-3.90	6.23

13 BIBLIOGRAPHY

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