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INSTRUCTIONS FOR USE

Reagents for professional use,
for *In Vitro* use only in clinical laboratory (IVD)

N C5 Kit

C5 Complement
for *BN™ Series and Atellica® NEPH 630*
REF TD-42570

INTENDED USE

Quantitative determination of C5 Complement (C5), in human serum, by nephelometric method, in *BN™ Series and Atellica® NEPH 630* nephelometers of *Siemens Healthcare* (*BN™*, *ProSpec®* and *Atellica®* are registered trademarks of *Siemens Healthcare Diagnostics Products GmbH*, Marburg, Deutschland).

SUMMARY AND EXPLANATION

C5 component of the complement system is a protein with an approximate molecular weight of 190 kDa, composed of two polypeptide chains (alpha and beta) linked by disulphide bridges.
Cleavage of C5 by the C5 convertases, of the different complement pathways, determines its separation into C5a and C5b.
C5a is a powerful anaphylatoxin and also a chemotactic factor that produces an inflammatory response.
C5b binds sequentially to C6, C7, C8 and C9 to form the complement membrane attack complex (MAC), responsible for the lysis of invader cells by forming pores in their membrane.
Deficiency of C5 is associated with increased susceptibility to recurrent severe bacterial infections and has also been linked to susceptibility to autoimmune diseases (e.g. Systemic Lupus (SLE), Rheumatoid Arthritis, Liver Fibrosis ...).
Low levels of C5 and normal levels of C3 and C4 are consistent with C5 deficiency, while if reduced levels of C3 and C4 are also found, complement consumption is indicated.

PRINCIPLE OF THE METHOD

The specific antibodies (Ab) of the reagent, when combined with the antigens (Ag) of the patient sample, form insoluble compounds causing a change in the absorbance and dispersion of the light, proportional to the antigen concentration, which can be quantified by turbidimetric (TIA) or nephelometric (NIA) method, by comparison with calibrators of known concentration.

CONTENTS - COMPOSITION - PREPARATION

- Antiserum Reagent: REAG | Ab | C5
  REF TD-42565-RA 100 test - 4 ml
  Solution of anti-human C5 antibodies.
- Enhancer Reagent: REAG | Enh | C5
  REF TD-42565-B 100 test - 2 ml
  Solution of Ab/Ag reaction accelerators.
- Low Calibrator: CAL | L | C5
  REF TD-42578 CONT 1 ml
  Human serum solution, delipidated, filtered by 0.2 µm.
- High Control: CONTROL | H | C5
  REF TD-42579-H CONT 1 ml
  Human serum solution, delipidated, filtered by 0.2 µm.
- Low Control: CONTROL | L | C5
  REF TD-42579-L CONT 1 ml
  Human serum solution, delipidated, filtered by 0.2 µm.

As preservatives, all the components contain <0.1% (1 g/l) Sodium Azide (NaN_3), and calibrator and controls also contain <0.02% (0.2 g/l) Methylisothiazolone and <0.02% (0.2 g/l) Bromonitrodioxane.
The components are ready for use and require no preparation.
Before each use it is convenient that the components are homogenized, shaking them gently avoiding the formation of foam or bubbles (capped with their original caps, never with the anti-evaporation stoppers on).
Before use, it is always advisable to bring the components to their use temperature, waiting a while before using them.
The values of calibrator and controls are lot dependent and are indicated in their table of assigned values.

WARNINGS - PRECAUTIONS

- Sodium Azide is toxic. Even if at the concentrations present neither Sodium Azide nor the other preservatives are harmful, take the necessary precautions to avoid accidental ingestion or contact with the eyes.
- Sodium Azide can react with lead or copper to give explosive compounds. For disposal it is recommended to rinse with plenty of running water to avoid accumulation in drains.
- Materials of human origin have been tested and found negative for the presence of HBsAg, HCV, and anti-HIV 1 and 2 antibodies.
- Since the absence of infectious agents can not be proven with absolute certainty, components containing materials of human or animal origin must be handled with caution, as potentially infectious, following the recommended safety standards for biological risk.
- Do not mix components belonging to different lot kits.
- Clinical diagnosis should never be based on the results of a single test, but should always integrate all relevant clinical and laboratory data.

STORAGE - SHELF LIFE

- Store refrigerated at +2...+8ºC. Do not freeze, as the functionality of the components may be altered.
- Properly stored and unopened, the components are stable until the expiration date indicated on the label.
- Once opened, provided they are handled with adequate precautions to avoid contamination, the shelf life of the components is at least 4 weeks, provided that after each use they are stored immediately in the original containers, tightly capped with their original caps, and refrigerated at +2...+8ºC.
  During use, it is advisable to use the following optional *Siemens Healtcare*'s anti-evaporation caps:
  - REF OVLE 21, for reagent vials on *BN II*,
  - REF OVLC 31, for reagent vials on *BN ProSpec®* or *Atellica® NEPH 630*, and
  - REF OVLC 21, for control vials on *BN ProSpec®* or *Atellica® NEPH 630*.

  The indicated shelf life must be taken as a guideline given that, obviously, it depends on the particular environmental and use conditions, which may differ from those of the stability studies carried out.

MATERIALS NEEDED, NOT SUPPLIED

- *BN™ Series or Atellica® NEPH 630* nephelometers of *Siemens Healthcare*, and accessories: racks, cuvettes, etc..
- *N Reaction Buffer*, from *Siemens H.* REF OUMS 65
- *N Diluent*, from *Siemens Healthcare* REF OUMT 65
- *Cleaner SCS*, from *Siemens Healthcare* REF OQUB 19

SAMPLES

Fresh Serum.

Samples with presence of fibrin should be centrifuged.

Do not use hemolyzed, lipemic or contaminated samples.

In bibliography[1] it is reported the following stability in serum:

- Refrigerated: 7 days
- Freezed: 60 days

Specific guidelines[2] establish that it is the responsibility of each laboratory to consult all available references or to carry out its own studies to determine its specific stability criteria.

PROCEDURE

To program and calibrate assays, follow the instructions for use of the analyzer used. Detailed information on the parameters to program the assay is available in the documentation section (select folders *C5* and *Applications*) of the website (www.3diag.com) or on request to the Customer Support Service (✉ support@3diag.com - ☎ +34 93 244 86 79).

Warning - Calibration

The calibration curves have a limited validity, which depends on the particular conditions of use.

The assay should be recalibrated when:

- a new lot of reagents, buffer, or diluent is used,
- established internal quality control procedures do not deliver the expected results, or
- after performing maintenance operations on the analyzer.

The stability of the calibrator dilutions is limited to its immediate use. If the calibration must be repeated, it is recommended to request that the analyzer carry out a new set of dilutions.

PERFORMANCES OF THE METHOD

Detailed information on the characteristics and performances of the assay is given in the Technical Report, available in the documentation section (select folders *C5* and *Technical Reports*) of the website (www.3diag.com) or upon request to the Customer Support Service (✉ support@3diag.com - ☎ +34 93 244 86 79).

QUALITY CONTROL

To monitor performances, it is recommended to use the controls included in the Kit.

The insertion of internal controls is recommended:

- in each analytical series,
- when using a new reagent vial from the same lot, and
- after performing a calibration.

Each laboratory should establish its own quality scheme and corrective actions if controls do not meet the assigned tolerances.

The reagents have been subjected to quality control checks and should react as described in these instructions. Therefore, as a general recommendation, in the event that controls do not give the expected results, the following should be done:

- repeat controls,
- if the deviation persists, repeat with new controls,
- if the deviation persists, calibrate again, and
- if the deviation persists, contact the Customer Support Service (✉ support@3diag.com - ☎ +34 93 244 86 79), or please contact your local Siemens Healthineers Remote Services Center.

As a precaution, until the causes of the deviation have been identified and corrected:

- all reagents should be considered unreliable, and
- sample results should not be validated.

Warning

Like samples, controls are run diluted. The stability of the dilutions is limited to their immediate use, therefore, if it is wanted to repeat the measurement, it is recommended to request the analyzer to carry out new dilutions.

TRACEABILITY

Values in IU/ml are referred to the *International Ref. Preparation for human serum complement Factors* (*NIBSC* code: W1032) of the *WHO* (*World Health Organization*)[3][4].

For the values in mg/dl, given that certified reference materials in these units are not available, the values are referred to internal standards of highly purified proteins. Traceability is ensured by measuring the C5 in the *WHO* standard.

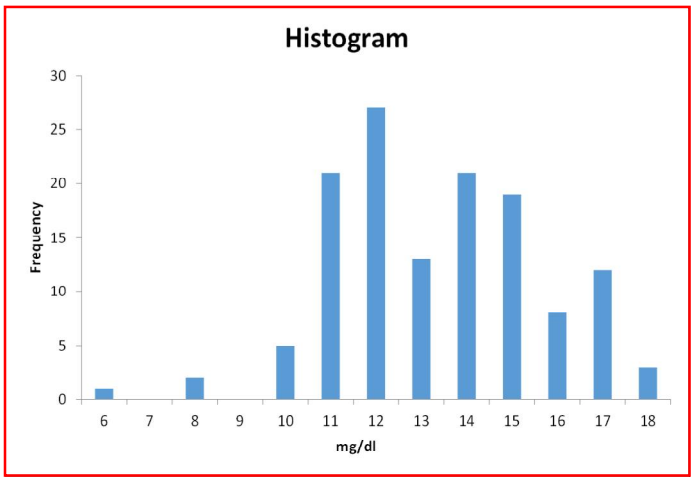
REFERENCE INTERVALS

It is always advisable for each laboratory to establish its own reference values.

The bibliography reports variable reference values between publications, depending on the method used and the population analyzed: between 10.6 - 26.3 mg/dl[1], 5.1 - 7.7 mg/dl[5], and 9.7 - 20.3 mg/dl[6].

Analyzing, by nephelometric method in a *BN™ II System*, serum samples of 132 presumably healthy adults from the Barcelona area, the following results have been obtained (see table and histogram):

units	mean	SD	range	95 percentile	90 percentile
IU/ml	102	17.6	45.4 - 140	71.3 - 132	77.8 - 130
mg/dl	12.9	2.22	5.75 - 17.7	9.02 - 16.7	9.85 - 16.5



In view of the results, a concentration lower than about 75 IU/ml, equivalent to about 9.5 mg/dl, can be taken as a significant value, indicative of a deficiency or consumption.

LIMITATIONS OF THE PROCEDURE

- Hemolyzed, lipemic or turbid samples, which cannot be clarified by centrifugation, should not be used in turbidimetric or nephelometric assays as turbidity and particles can interfere with the determination.
- Samples containing circulating immune complexes (CICs) / heterophilic antibodies can lead to erroneously increased or decreased results in immunoassays. Unexpected or inconsistent results should be confirmed using alternative methods.
- The product must be used as described in these instructions by suitably trained personnel. Any modification made to the assay and its necessary validation is the sole responsibility of the user, as well as its possible use in other analyzers.
- Samples from internal quality controls other than the recommended one, or from external quality controls, may give different results than those obtained by other methods, due to matrix effects. To evaluate the results it may be necessary to establish specific target values for the method.

SYMBOLS

In addition to the harmonized symbols provided on the European Standard EN 980:2008, in the labels and instructions of use has been used the complementary symbology proposed[7] by the *EDMA* (*European Diagnostic Manufacturers Association*), whose meaning is detailed below.

REAG	Reagent
Ab	Antibody / Antiserum
Enh	Enhancer
C5	C5 Complement
CAL	Calibrator
CONTROL	Control
H	High
L	Low
CONT	Contents

BIBLIOGRAPHY

(1) *Mayo Medical Laboratories* website (www.mayomedicalcliniclabs.com), date of consultation: 7th June 2017.

(2) Clinical and Laboratory Standards Institute (CLSI), Doc. GP44-A4, May 2010: "*Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Test; Approved Guideline - Fourth Edition*"

(3) National institute for Biological Standards and Control: "*International Ref. Preparation - Human serum complement factors - NIBSC code W1032 - Instructions for Use (Version 1.0, Dated 31/01/2011)*".

(4) International Union of Immunological Societies, Standardisation Committee: "*WHO International Reference Preparation of Four Human Serum Complement Proteins: C1q, C4, C5 and Factor B, and Functional Whole Complement - Recommendations for use*".

(5) *"AEFA/AEBM Nomenclator de Laboratorio Clínico"* (*ISBN*: 84-486-0117-3).

(6) M.C. Sánchez Pozo et al.: "*Estudio de Valores de Referencia del Complemento*" - Poster, XXII Congreso Nacional del Laboratorio Clínico, Bilbao, Oct-2018.

(7) EDMA Labelling Task Force: "*EDMA Symbols for IVD Reagents and Components - Revision, October 2009*".

TEXT REVISION DATE

26th May 2021.