SIEMENS Healthineers

N Antiserum to Human C3c

N AS C3

CE 0197

Revision bar indicates update to previous version.

Atellica® NEPH 630 System / BN II System / BN ProSpec® System

Intended Use

N AS C3 is an in vitro diagnostic reagent for the quantitative, IFCC-standardized determination of complement factor C3/C3c as aid to diagnosis and monitoring of complement-associated disorders in patients at risk or with signs of complement-associated disorders in human serum, heparin and EDTA plasma by means of automated Siemens Healthineers immuno-nephelometry systems.

Summary and Explanation

The complement system is an integral part of the antigen-nonspecific immune defense. It can be activated via three reaction pathways, the classical pathway which is triggered primarily by cell-bound immune complexes, the alternative pathway which is activated primarily by foreign bodies such as microorganisms, and the lectin pathway triggered via the mannose binding protein - lectin complex. The complement component C3 is a key protein in all the reaction pathways whereas C4 belongs to the classical pathway of complement activation. Complement activation is associated with consumption of components C3 or C4 so that a reduction in their concentrations can allow diagnostic conclusions to be reached. Diminished serum concentrations of C3 and C4 are observed primarily in active systemic lupus erythematosus (SLE), in forms of membrane proliferative glomerulonephritis and in immune complex diseases (serum sickness). In the case of SLE the serum concentrations of the complement factors reflect the activity of the disease. Diminished C3 values occur in acute glomerulonephritis and in membrane proliferative glomerulonephritis whereas isolated diminished levels of C4 can occur in hereditary angioedema (HAE) and in cases of autoimmune hemolytic anemia. Both complement components react as acute-phase proteins and may therefore show elevated serum concentrations in patients with inflammatory diseases. Hereditary deficiency states of both complement factors have been reported[1,2,3,4].

Principles of the Procedure

Proteins contained in human body fluids form immune complexes in an immunochemical reaction with specific antibodies. These complexes scatter a beam of light passed through the sample. The intensity of the scattered light is proportional to the concentration of the relevant protein in the sample. The result is evaluated by comparison with a standard of known concentration.

Reagents

Reagent	Description	Storage	Stability
N Antiserum to Human C3c N AS C3	Ready to use liquid containing: • animal serum produced by immunization of rabbits with highly purified human complement factor C3c (concentration of active antibodies: < 6.4 g/L) • Preservative: • Sodium azide (< 1 g/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened. Do not freeze!	2–8 °C: once opened, 4 weeks[a,b]

[a] if securely capped immediately after use
[b] if contamination (e.g. by microorganisms) is precluded

During storage, N AS C3 can develop precipitates or turbidity which are not caused by microbial contamination and which do not affect their activity. In such cases, the antiserum should be filtered prior to use. Disposable filters with a pore size of 0.45 µm are suitable for this purpose.

On-board stability
A minimum of 5 days at 8 hours each for 5 mL vials, and 3 days at 8 hours each for 2 mL vials or a comparable period of time.

Note: On-board stability may vary, depending on the system used and laboratory conditions. For further details, refer to the respective Assay Protocols document.

Warnings and Precautions

For *in-vitro* diagnostic use only.

For laboratory professional use.

According to EU regulation 2017/746, any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or patient is established.

Safety data sheets (MSDS/SDS) available on siemens-healthineers.com/sds.

Caution
This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

Contains sodium azide as a preservative. Sodium azide can react with copper or lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides. Disposal into drain systems must be in compliance with prevailing regulatory requirements.

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with all government requirements.

Preparing Reagents

N AS C3 is ready-for-use as supplied and requires no additional preparation.

Specimen Collection and Handling

Suitable samples are human serum or heparinized or EDTA plasma. Serum samples must be completely coagulated and, after centrifugation, must not contain any particles or traces of fibrin. Lipemic samples or frozen samples which became turbid after thawing must be clarified by centrifugation (10 minutes at approximately 15 000 × g) prior to testing.

In the assay of C3, one should note that the antiserum is directed against the C3c fragment of the C3 molecule. The extent of fragmentation of C3 to the C3c fragment varies depending on the age of the sample and storage conditions. For fresh samples the C3 values obtained in the immunonephelometric assay have been observed to be up to 30 % lower than those obtained for stored samples, depending

on the extent to which fragmentation has advanced[5]. During storage for eight days at 2 to 8 °C or for three months at below –20 °C, serum or heparinized or EDTA plasma specimens may increase in C3c concentration by up to 17 %. Therefore, complement protein results for stored samples need to be assessed against reference intervals determined under similar conditions.

Procedure

Materials Provided

REF	Contents		
OSAP15	N Antiserum to Human C3c N AS C3	1 ×	5 mL
OSAP09	N Antiserum to Human C3c N AS C3	1 ×	2 mL

Materials Required but not Provided

Item	Description
REF OQIM13	N PROT STANDARD SL, N Protein Standard SL (human)
REF OQIN13	N/T PROT CONTROL SL/L, N/T Protein Control SL/L (human)
REF OQIO13	N/T PROT CONTROL SL/M, N/T Protein Control SL/M (human)
REF OQIP13	N/T PROT CONTROL SL/H, N/T Protein Control SL/H (human)
REF OUMS65	N BUFFER, N Reaction Buffer
REF OUMT65	N DILUENT, N Diluent
REF OVLE21	BN II Evaporation Stoppers (optional)
Instruments[c], such as:	• Atellica® NEPH 630 System • BN II System • BN ProSpec® System

Additional materials and supplies as described in the respective System's Instruction Manual.

[c] Availability of analyzers may vary by country.

Notes

Consult your respective System's Instruction Manual for details regarding operation of the instrument.

For the Atellica® NEPH 630 System, BN II System or BN ProSpec® System, reagents and samples stored at 2 to 8 °C can be used immediately.

Assay Protocols on Atellica® NEPH 630 System and BN Systems

The assay protocols for serum and plasma are given in the respective Assay Protocols document and software of the instrument. All steps are performed automatically by the system.

Performing Calibration

Reference curves are generated by multi-point calibration. Serial dilutions of N PROT STANDARD SL are automatically prepared by the instrument using N DILUENT. The standard dilutions are to be used within four hours.

The reference curves can be used for as long as controls with corresponding method-dependent target values, e.g. N/T PROT CONTROL SL/L, N/T PROT CONTROL SL/M and N/T PROT CONTROL SL/H, are reproduced within their respective range. If a different lot of antiserum is used, a new reference curve must be generated.

The exact measuring range depends upon the concentration of the protein in each lot of N PROT STANDARD SL. Typical measuring ranges are given in the respective Assay Protocols document.

Assay of Specimens

Serum and plasma samples are automatically diluted 1:20 with N DILUENT. The diluted samples must be measured within four hours. If the results obtained are outside the measuring range, the assay can be

repeated using a higher or lower dilution of the sample. Refer to the respective System's Instruction Manual for information on repeat measurements using other dilutions.

Internal Quality Control

Assay N/T PROT CONTROL SL/L, N/T PROT CONTROL SL/M and N/T PROT CONTROL SL/H after each establishment of a reference curve, the first use of an antiserum vial as well as with each run of samples. The controls are assayed and evaluated as for patient samples. Assigned value and range are listed in the Table of Assigned Values of the respective control.

Follow government regulations or accreditation requirements for quality control frequency.

If the result of a control is outside the range, the determination must be repeated. If the repeated determination confirms the deviation, a new reference curve must be established. Do not release patient results until the cause of deviation has been identified and corrected.

Results

Evaluation is performed automatically in g/L or in a derived unit selected by the user.

Limitations

No interference was detected for concentrations of triglycerides up to 5.7 g/L, of bilirubin up to 600 mg/L, and of free hemoglobin up to 10 g/L. No interference from commonly used drugs is known. Turbidity and particles in the samples may interfere with the determination. Therefore, samples containing particles must be centrifuged prior to testing. Lipemic or turbid samples which cannot be clarified by centrifugation (10 minutes at approximately 15 000 × g) must not be used.

Siemens Healthineers has validated use of these reagents on various analyzers to optimize product performance and meet product specifications. Please note that the applications on other analyzers can be validated by the instrument manufacturer in accordance with the requirements of the REGULATION (EU) 2017/746 under their responsibility as long as the intended purpose and performance are not modified. User defined modifications are not supported by Siemens Healthineers as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these instructions or use of the reagents on analyzers other than those included in Siemens Healthineers Application Sheets or these Instructions for Use.

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

Due to matrix effects, interlaboratory survey samples and control samples may yield results that differ from those obtained with other methods. It may therefore be necessary to assess these results in relation to method-specific target values.

Expected Values

The following reference intervals apply for serum and plasma samples from healthy adults[6]:

C3/C3c	0.9 to 1.8 g/L

Fresh samples can be expected to have lower C3 concentrations. The reference intervals for C3 will depend on sample age and storage (refer to "Specimen Collection and Handling", page 2).

Therefore, each laboratory should determine its own reference intervals since values may vary depending on the individual population studied and age and storage of the sample.

Performance Characteristics

Note: The values cited for specific performance characteristics of the assay represent typical results and are not to be regarded as specifications for N AS C3.

Measuring Range

The measuring range of the assays is established by the lower limit of the reference curve and depends therefore upon the concentrations of the proteins in the N PROT STANDARD SL. Typical measuring ranges are given in the respective Assay Protocols document.

Specificity

There are no known cross-reactivities of the antiserum used.

Sensitivity

The analytical sensitivity of the assay is determined by the lower limit of the reference curve and depends therefore upon the concentration of the protein in the N PROT STANDARD SL. A typical Limit of Detection (LoD) for C3 is < 0.041 g/L.

Precision

The following coefficients of variation (CV) were obtained with N AS C3 on a BN System (n = 40):

C3/C3c	n	Mean [g/L]	Repeatability CV [%]	Within-Device/Lab Precision CV [%]
N/T PROT CONTROL SL/L	40	0.84	2.4	2.9
N/T PROT CONTROL SL/M	40	1.17	4.2	4.5
N/T PROT CONTROL SL/H	40	1.65	2.8	3.8
Serum pool (low)	40	1.21	3.2	3.5
Serum pool (high)	40	1.71	2.0	2.7

The results were generated by analysis of variance.

Equivalency for the Atellica® NEPH 630 System has been confirmed.

The reproducibility was assessed by Siemens Healthineers for N AS C3 based on publicly available proficiency testing information in 2018/2019. The overall reproducibility median CV% was found to be < 10 % including lot, instrument, laboratory and operator variability factors.

Method Comparison

Serum samples were assayed with N AS C3 on a BN ProSpec® System (y) and a BN II System (x). Correlation of the results yielded the following data:

Protein	n		Correlation Coefficient
C3/C3c	79	y (BN ProSpec®) = 0.900 x + 0.053 g/L	0.998

Equivalency for the Atellica® NEPH 630 System to a BN System has been confirmed.

Technical Assistance

For customer support, contact your local technical support provider or distributor.

siemens-healthineers.com

Current Version of Assay Protocols

N AS C3 can be used in combination with various automated analyzers. Siemens Healthineers provides Assay protocols for instruments listed in section "Materials Required but not Provided", page 3 under the dedicated link below:

siemens-healthineers.com/ap

As Siemens Healthineers continuously monitors the product performance and safety, the users are required to ensure that they work with the correct revision of the instructions for the product lots in use. Please periodically review the availability of new electronic labeling revisions to ensure safe use of the product.

The IFU version number is visible on each product box label. Siemens Healthineers ensures that all products lots bearing the same IFU version number are compatible with the electronic labeling provided via siemens-healthineers.com/eIFU.

References

1. Grumach AS, Kirschfink M. Are complement deficiencies really rare? Overview on prevalence, clinical importance and modern diagnostic approach. Mol Immunol 2014;61(2):110-7.
2. Hebert LA, Cosio FG, Neff JC. Diagnostic significance of hypocomplementemia. Kidney Int 1991; 39: 811-21.

3. Botto M, Kirschfink M, Macor P, et al. Complement in human diseases: Lessons from complement deficiencies. Mol Immunol 2009;46(14):2774-83.
4. West CD. The complement profile in clinical medicine. Inherited and acquired conditions lowering the serum concentrations of complement component and control proteins. Complement Inflamm 1989; 6: 49-64.
5. Okumura N, Nomura M, Tada T, et al. Effects of sample storage on serum C3 assay by immunonephelometry. Clin Lab Sci 1990; 3: 54-57.
6. Dati F, Schumann G, Thomas L, et al. Consensus of a group of professional societies and diagnostic companies on guidelines for interim reference ranges for 14 proteins in serum based on the standardization against the IFCC/BCR/CAP Reference Material (CRM 470). International Federation of Clinical Chemistry. Community Bureau of Reference of the Commission of the European Communities. College of American Pathologists. Eur J Clin Chem Clin Biochem 1996; 34: 517-20.

Definition of Symbols

The following symbols may appear on the product labeling:

	Do not reuse		Use By
LOT	Batch Code	REF	Catalogue Number
	Caution		Manufacturer
EC REP	Authorized representative in the European Community		Contains sufficient for <n> tests
	Biological Risks	IVD	*In Vitro* Diagnostic Medical Device
	Temperature Limitation		Consult instruction for Use
	Non-sterile	CE	CE marking of conformity
CE 0197	CE marking of conformity with notified body ID number. Notified body ID number can vary.	CONTENTS	Contents
	Reconstitution volume	LEVEL	Level
	Keep away from sunlight and heat	WARNING	Warning
DANGER	Danger	RxOnly	Prescription device (US only)
UDI	Device Identification (UDI) barcode	REACH xx/xx/xx	REACH Authorization Number

Legal Information

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