SIEMENS Healthineers

N Antiserum to Human IgG

NAS IGG

CE 0197

Revision bar indicates update to previous version.

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

Intended Use

NAS IGG is an in vitro diagnostic reagent for the quantitative, IFCC-standardized determination of immunoglobulin G (IgG) as aid to diagnosis and monitoring of immune deficiencies and gammopathies in human serum, heparin and EDTA plasma by means of automated Siemens Healthineers immuno-nephelometry systems.

Additionally NAS IGG can be used in human urine as aid to differential diagnosis of glomerular proteinuria, and in cerebrospinal fluid (CSF) as aid to diagnosis and characterization of intra-thecal immunoglobulin production.

Summary and Explanation

Immunoglobulins are formed by plasma cells as a humoral immune response to contact of the immune system with antigens. The primary reaction after the initial contact is the formation of antibodies of the IgM class followed later by IgG and also IgA antibodies. Quantitative determination of the immunoglobulins can provide important information on the humoral immune status[1]. Decreased serum immunoglobulin concentrations occur in primary immunodeficiency conditions as well as in secondary immune insufficiencies, e.g. in advanced malignant tumours, lymphatic leukemia, multiple myeloma and Waldenstrom's disease. Increased serum immunoglobulin concentrations occur due to polyclonal or oligoclonal Ig proliferation, e.g. in hepatic diseases (hepatitis, liver cirrhosis), acute and chronic infections, autoimmune diseases as well as in the cord blood of neonates with intra-uterine and perinatal infections[2]. Monoclonal immunoglobulin proliferations are observed e.g. in multiple myeloma, Waldenstrom's disease and heavy-chain disease[2]. Monoclonal immunoglobulinemia requires detailed differential diagnostic investigations in addition to the quantitative immunoglobulin determination.

Determination of IgG, IgA and IgM in serum or plasma is indicated in the evaluation of immunodeficiency states and can be used as aid to diagnosis and monitoring of

- immune deficiencies, which can be due to reduced synthesis, increased loss or hypercatabolism
- polyclonal and monoclonal gammopathies with increase of one or several immunoglobulin classes.

IgG determination in urine aids in the differentiation of glomerular proteinuria; elevated IgG levels in urine indicate non-selective glomerular proteinuria[3].

Local immune reactions within the central nervous system result in elevated immunoglobulin levels, particularly IgG, in the cerebrospinal fluid[4,5].

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 IgG NAS IGG	Ready to use liquid containing: • animal serum produced by immunization of rabbits with highly purified human immunoglobulin[a] • 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[b,c]

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

During storage, NAS IGG can develop precipitates or turbidity which are not caused by microbial contamination and which do not affect their activity. In such cases, NAS IGG 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 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.

Summary of Safety and Performance (SSP) is available in the European database on medical devices (see Eudamed public website: https://ec.europa.eu/tools/eudamed). In case Eudamed is not available, SSP can be delivered by Siemens Healthineers on request.

Preparing Reagents

NAS IGG is ready-for-use as supplied and requires no additional preparation.

Specimen Collection and Handling

Suitable samples are human serum, heparinized plasma or EDTA plasma, either as fresh as possible (stored no more than eight days at 2 to 8 °C) or stored frozen. Fresh human urine and CSF samples are also suitable for the IgG determination. If paired serum and CSF samples are to be analyzed, they

should be drawn simultaneously. Serum and plasma samples can be stored at below –20 °C for up to three months if they are frozen within 24 hours after collection and if repeated freezethaw cycles are avoided. 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. Random and timed urine collections are suitable specimens for testing IgG in urine. Urine and CSF samples which have been stored frozen must not be used. Each urine and CSF sample must be centrifuged prior to testing.

Procedure

Materials Provided

REF	Contents		
OSAS15	N Antiserum to Human IgG NAS IGG	1 ×	5 mL
OSAS09	N Antiserum to Human IgG NAS IGG	1 ×	2 mL

Note: Consult your respective System's Instruction Manual for details regarding operation of the instrument.
For the Atellica® NEPH 630 System or the BN Systems, reagents and samples stored at 2 to 8 °C can be used immediately.

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 OQLW13	N/T PROT CONTROL LC, N/T Protein Control LC (human)
REF OPFT03	N CON LC1, N Protein Control LC1
REF OPFU03	N CON LC2, N Protein Control LC2
REF OUMS65	N BUFFER, N Reaction Buffer
REF OUMT65	N DILUENT, N Diluent
REF OVLE21	BN II Evaporation Stoppers (optional)
Instruments[d], 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.

[d] Availability of analyzers may vary by country.

Assay Protocols on the Atellica® NEPH 630 System and the BN Systems

The assay protocols, for serum and plasma, as well as for IgG in urine and CSF, respectively, 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 4 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 for the serum/

plasma assay and N/T PROT CONTROL LC, N CON LC1 and N CON LC2 for the IgG in urine and CSF assays, are reproduced within their respective confidence interval. 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 1:400 (IgG) with N DILUENT. The diluted samples must be measured within 4 hours. IgG in urine or CSF is measured from undiluted samples using separate assay protocols. IgG in serum and CSF can also be analyzed using a single reference curve, provided that the appropriate sample dilution is selected manually.

If the results obtained are outside the measuring range, the assay can be repeated using a higher or lower dilution of the sample (IgG in the serum/plasma assay protocol). 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 serum or plasma samples. For the determination of IgG in urine or CSF samples the N/T PROT CONTROL LC, N CON LC1 and N CON LC2 should be used accordingly. The controls are assayed and evaluated as for patient samples. The assigned value and confidence interval are listed in the Table of Assigned Values of the respective control.

The values can be entered via data storage device on the Atellica® NEPH 630 System and on the BN ProSpec® System.

Follow government regulations or accreditation requirements for quality control frequency.

If the result of a control is outside the confidence interval, the determination must be repeated. If the repeated determination confirms the deviation, a new reference curve should 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 on the BN System/ Atellica® NEPH 630 System.

Limitations

No interference with the determinations in serum was detected for concentrations of triglycerides up to 19 g/L, bilirubin at 600 mg/L, and free hemoglobin at 10 g/L.

No interference from commonly used drugs is known.

Turbidity and particles in the sample 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.

The immunoglobulin assays have been designed to minimize antigen excess in the initial sample dilutions. However, it cannot be completely eliminated and in rare cases very high immunoglobulin concentrations may produce falsely-low results. Especially monoclonal immunoglobulins may show reactivity different from the polyclonal standard, which in isolated cases may lead to artificially decreased or non-linear results. In case of serum or plasma determinations, the constellation of IgG, IgA and IgM should be assessed. A check of IgG results in CSF should be performed by means of ratio diagrams[6,7].

In case of questionable results, the determinations should be repeated using the next higher sample dilution. For patient monitoring, consecutive immunoglobulin determinations should be performed from the same sample dilution, as far as possible.

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, inter-laboratory 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 interval applies for serum and plasma samples from healthy adults[8]:

IgG 7.0 - 16.0 g/L

Pediatric reference ranges for IgG are dependent on age and can vary over a wide range[9].

Reference interval for IgG in urine samples:

IgG in urine: in 24-hour-urine collections of healthy adults IgG is below 1.15 mg/dL [11.5 mg/L] (97.5th percentile)[7].

Reference interval for IgG in CSF samples:

IgG in the CSF: below 34 mg/L[6].

Note: This value is for guidance only. Reference intervals in the strict sense exist only for CSF/serum ratios[6,7]

Nevertheless, each facility should determine its own reference intervals since values may vary depending on the individual population studied.

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 NAS IGG.

Measuring Range

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

Specificity

NAS IGG is specific for the immunoglobulin µ-chain. There are no known cross-reactivities of the antiserum used.

Sensitivity

The analytical sensitivity of the assays is determined by the lower limit of the reference curve and depends therefore upon the concentration of IgG in the N PROT STANDARD SL. A typical Limit of Quantitation (LoQ) is

- IgG: 0.07 g/L
- IgGU: 3.6 mg/L
- IgGC: 3.6 mg/L

Precision

The following coefficients of variation (CV) were obtained with NAS IGG (n = 40) in the different assays on a BN System:

IgG

Sample	n	Mean [g/L]	Repeatability CV [%]	Within-Device/Lab Precision CV [%]
N/T PROT CONTROL SL/L	40	5.0	3.0	3.4
N/T PROT CONTROL SL/M	40	8.4	1.8	2.1
N/T PROT CONTROL SL/H	40	12.1	2.6	2.7

Sample	n	Mean [g/L]	Repeatability CV [%]	Within-Device/Lab Precision CV [%]
Serum pool (low)	40	8.8	1.8	2.3
Serum pool (high)	40	12.7	2.2	2.4

IgGU

Sample	n	Mean [mg/L]	Repeatability CV [%]	Within-Device/Lab Precision CV [%]
N/T PROT CONTROL LC	40	18.7	1.1	1.3
Urine pool (low)	40	5.5	2.9	4.1
Urine pool (high)	40	19.9	1.2	2.8

IgGC

Sample	n	Mean [mg/L]	Repeatability CV [%]	Within-Device/Lab Precision CV [%]
N/T PROT CONTROL LC	40	18.7	1.2	1.3
CSF pool (low)	40	8.1	1.3	1.4
CSF pool (high)	40	42.1	1.8	1.9

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 NAS IGG 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

One hundred (100) serum samples were assayed with NAS IGG on a BN System (y) and radial immunodiffusion (x) (NOR Partigen® IgG).

Correlation of the results yielded the following data:

Protein	n		Correlation Coefficient
IgG	100	y (BN) = 1.09 × (RID) – 0.12 g/L	0.97

A comparison of urine samples (n = 53) assayed using NAS IGG in the IgGU assay on a BN ProSpec® System (y) versus a BN II System (x) yielded the following correlation result:

y = 1.03 × - 0.102 mg/L; correlation coefficient r = 0.999.

A comparison of CSF samples (n = 53) assayed using NAS IGG in the IgGC assay on a BN ProSpec® System (y) versus a BN II System (x) yielded the following correlation result:

y = 0.998 × + 0.882 mg/L; correlation coefficient r = 0.996.

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

Antigen Excess

The NAS IGG reagent shows no high-dose hook effect in the assay for

- IgG up to 83.8 g/L
- IgGU up 648 mg/L
- IgGC up to 2 290 mg/L

Technical Assistance

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

siemens-healthineers.com

Current Version of Assay Protocols

NAS IGG 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

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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 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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