SIEMENS Healthineers

N Latex IgG4

N IGG4

CE 0197

Revision bar indicates update to previous version.

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

Intended Use

N IGG4 is an in vitro diagnostic reagent for the quantitative determination of IgG subclass 4 as aid to diagnosis and monitoring of immune deficiencies in individuals with suspected immune deficiency in human serum, heparin and EDTA plasma by means of automated Siemens Healthineers immuno-nephelometry systems.

N IGG4 assay can be used as aid in diagnosis of IgG4-related diseases in individuals with suspicion of IgG4-related disorders or autoimmune disease.

For IgG4 testing no international reference preparation or method is available.

Summary and Explanation

Human IgG antibodies are composed of the four subclasses IgG1, IgG2, IgG3 and IgG4. The structural differences between the IgG subclasses are reflected in different biologically important functions such as antigen recognition, complement activation and cell surface receptor binding[1,2].

While absolute concentrations of the IgG subclasses are subject to a large biological variance, their relative proportions with regard to total IgG content is regulated within relatively narrow limits, with about 60 to 75 % for IgG1, 15 to 25 % for IgG2 and < 10 % each for IgG3 and IgG4. Diminished serum IgG subclass concentrations can be linked to different disease states[3–6].

A deficiency of IgG subclasses is often observed in patients with recurring infections of the upper and lower respiratory passages.

Polyclonal increases of IgG subclass concentrations may occur with chronic antigen stimulation, but have only a secondary diagnostic role. Monoclonal IgG increases are due to the increased production of one IgG subclass.

The determination of IgG subclasses is indicated for diagnostic clarification in patients with increased susceptibility to infection. A determined IgG subclass deficiency is an indication of a malfunctioning immune defense and requires additional diagnostic investigation.

Elevated IgG4 levels are observed in patients with sclerosing pancreatitis and other IgG4-related, autoimmune disorders[7,8].

Principles of the Procedure

Polystyrene particles coated with specific antibodies to human IgG4 form aggregates that cause irradiated light to be scattered when mixed with samples containing IgG4. The intensity of the scattered light is dependent on the concentration of the respective protein in the sample. The evaluation is performed by comparison with a standard of known concentration.

Reagents

Reagent	Description	Storage	Stability

N Latex IgG4
N IGG4 REAGENT
	Ready to use liquid containing:
	• suspension of polystyrene particles coated with specific antibodies (sheep) against IgG4 (< 1 g/L)
	• Preservative:
	  • Amphotericin B (0.625 mg/L)
	  • Gentamicin sulfate (6.25 mg/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, 2 weeks[a]

[a] if re-closed tightly immediately following use and stored at 2 to 8 °C

On-board stability
At least 5 days at 8 hours each or a comparable period of time (maximum 40 hours).

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.

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 IGG4 is ready-for-use as supplied and requires no additional preparation.

Specimen Collection and Handling

For testing, use the freshest possible (stored for a maximum of 8 days at 2 to 8 °C) or frozen human serum samples as well as heparinized and EDTA plasma samples. If samples are frozen within 24 hours after collection, they may be stored below –20 °C for up to one month, if repeated thawing and freezing are avoided. Serum samples must be fully coagulated and must not contain any particles or traces of fibrin after centrifuging. Lipemic samples or frozen samples that are cloudy after thawing must be clarified prior to the assay by centrifuging (10 minutes at approx. 15 000 × g).

Procedure

Materials Provided

REF	Contents		
OPAU03	N Latex IgG4 N IGG4	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 OUMT65	N DILUENT, N Diluent
REF OUMU15	N SUPPLEMENT P, N Supplementary Reagent/Precipitation
REF OQUB19	Cleaner SCS (for BN II System)
REF OVLE21	BN II Evaporation Stoppers (optional)
Instruments[b], such as:	• Atellica® NEPH 630 System • BN II System • BN ProSpec® System

Disposable supplies and equipment as described in the respective System's Instruction Manual.

[b] Availability of analyzers may vary by country.

Notes

Consult the respective System's Instruction Manual for detailed instructions.

Reagents and samples stored at 2 to 8 °C may be used immediately for testing in the Atellica® NEPH 630 System, BN II System and BN ProSpec® System.

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

The assay protocols for N IGG4 are included in the Assay Protocols document as well as the software for the respective device. All steps are performed automatically by the system.

Performing Calibration

Reference curves are plotted by multiple point calibration. The calculation is automatically performed by producing dilution series of the N PROT STANDARD SL with N DILUENT. The dilutions must be used within 4 hours. The reference curves may be used as long as accuracy controls, e.g. N/T PROT CONTROL SL/L, N/T PROT CONTROL SL/M and N/T PROT CONTROL SL/H, remain within the respective range. A new reference curve must be generated for each change in reagent lot.

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.

Measuring patient samples

Samples are automatically diluted 1:2 000 with N DILUENT. The dilutions must be measured within 4 hours. For results that lie outside the measuring range, the measurement may be repeated with a higher or lower sample dilution.

Repeat measurements from further sample dilutions are described in the respective System's Instruction Manual.

Internal Quality Control

The N/T PROT CONTROL SL/L, N/T PROT CONTROL SL/M and N/T PROT CONTROL SL/H should be analyzed after each calculation of a reference curve, after the first time a reagent vial is used, as well as with each series of samples.

The controls are assayed and evaluated like patient samples. The target value and range for the controls are given in the corresponding Table of Assigned Values.

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 a result of the control measurements lies outside of the range, the control determination must be repeated. If the deviation is confirmed by the repeat measurement, a new reference curve should be

calculated. Patient results should not be released until the cause of the deviation is identified and corrected.

Results

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

Limitations

Samples containing particles must be centrifuged prior to testing. Lipemic samples that cannot be clarified by centrifuging (10 minutes at approx. 15 000 × g) must be excluded from testing. In individual cases, monoclonal immunoglobulins may exhibit reactions that deviate from that of the polyclonal standard, with the possibility of lower or nonlinear results. In case of suspicious results, the measurement should be repeated with a higher sample dilution.

As an additional control, the determination of all four IgG subclasses (N AS IgG1, REF OQXI09, N AS IgG2, REF OQXK09 and N Latex IgG3, REF OPAV03) and the comparison of their sum to the total IgG (determined with N Antiserum to Human IgG, REF OSAS15) is recommended. The sum of subclasses 1 - 4 should fall within 80 to 120 % of the total IgG; otherwise, the presence of a monoclonal component or another disturbance should be tested.

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 ranges (2.5th to 97.5th percentile in g/L) were established by testing samples from 405 apparently healthy individuals from North America and Central Europe as well as 279 apparently healthy adults from Central Europe with N IGG4 on a BN System:

Age Group	[g/L]
≤ 1 year	0.004 – 0.464
> 1–≤ 3 years	0.009 – 0.742
> 3–≤ 6 years	0.013 – 1.446
> 6–≤ 12 years	0.012 – 1.699
> 12–≤ 18 years	0.049 – 1.985
> 18 years	0.03 – 2.01

In addition, each laboratory should determine its own reference ranges, since these are subject to many variables that may be different for each population tested.

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

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 IGG4.

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 assays is determined by the lower limit of the reference curve and depends therefore upon the concentration of IgG4 in the N/T PROT CONTROL SL/L. A typical Limit of Detection (LoD) for IgG4n is < 0.000484 g/L.

Precision

The precision was calculated by measuring samples with different analyte concentrations and calculating the coefficients of variation (CV) of the results by means of variance analysis (n = 40):

IgG4	Mean [g/L]	Repeatability CV [%]	Within-Device/Lab Precision CV [%]
N/T PROT CONTROL SL/L	0.195	3.7	4.0
N/T PROT CONTROL SL/M	0.507	2.4	2.6
N/T PROT CONTROL SL/H	0.729	3.5	3.2
Serum pool 1	0.079	4.4	5.2
Serum pool 2	1.62	4.5	6.2

The reproducibility was assessed by Siemens Healthineers for N IGG4 based on publicly available proficiency testing information in 2019/2020. The overall reproducibility median CV% was found to be < 10 % including lot, instrument, laboratory and operator variability factors.

Method Comparison

69 serum samples were tested with N IGG4 in the BN System (y). For comparison, these samples were also tested by radial immunodiffusion methods (RID, x) found on the market. Correlation of the results provided the following data:

Protein	n	Regression line comparison	Correlation Coefficient
IgG4	69	y (BNS) = 1.12 × (RID) - 0.09 g/L	r = 0.99

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

Antigen Excess

The N IGG4 reagent shows no high-dose hook effect in the IgG4n assay, up to: 35.1 g/L.

Technical Assistance

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

siemens-healthineers.com

Current Version of Assay Protocols

N IGG4 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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2. Vlug A, et al. The structure and function of human IgG subclasses. Eur Clin Lab 1989; 8: 26.
3. Morell A. Clinical relevance of IgG subclass deficiencies. Ann Biol Clin (Paris) 1994; 52: 49-52.
4. Stiehm ER. The four most common pediatric immunodeficiencies. J Immunotoxicol. 2008; 5: 227-34.
5. Herrod HG. Clinical significance of IgG subclasses. Curr Opin Pediatr. 1993; 5: 696-9.
6. Hamilton RG. Human IgG subclass measurements in the clinical laboratory. Clin Chem 1987; 33: 1707-25.
7. Kubo S, Nakayamada S, Tanaka Y. Immunophenotype involved in IgG4-related disease. Mod Rheumatol. 2018;18:1-13.
8. Dorn L, Finkenstedt A, Schranz M, et al. Immunoglobulin subclass 4 for the diagnosis of immunoglobulin subclass 4-associated diseases in an unselected liver and pancreas clinic population. HPB (Oxford). 2012; 14:122-5.

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

Siemens Healthineers Headquarters
Siemens Healthcare GmbH
Henkestraße 127
91052 Erlangen
Germany
Phone: +49 9131 84-0
siemens-healthineers.com

Siemens Healthcare Diagnostics Products GmbH
Emil-von-Behring-Str. 76
35041 Marburg
Germany
siemens-healthineers.com