SIEMENS Healthineers

N Latex RF Kit

N RF

CE 0197

Revision bar indicates update to previous version.

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

Intended Use

N RF is an in vitro diagnostic reagent for the quantitative, WHO-standardized determination of rheumatoid factors (RF) as aid to diagnosis of rheumatoid arthritis in patients at risk or with signs of rheumatoid arthritis in human serum, heparin and EDTA plasma by means of automated Siemens Healthineers immuno-nephelometry systems.

Summary and Explanation

RF are autoantibodies against the Fc region of human IgG which has been altered in its tertiary structure. These autoantibodies also react with animal IgG. RF belong predominantly to the IgM class, but they also occur in all the other immunoglobulin classes[1].

The detection of RF is one of the criteria of the European and American Rheumatism Association (ARA) for the diagnosis of rheumatoid arthritis (RA)[2,3], since 70 to 90 % of patients with RA exhibit rheumatoid factors. RF play an important role in the differential diagnosis between RA and other rheumatic diseases[1]. Moreover, they permit prognostic statements with regard to RA[3].

High RF concentrations are often associated with a more severe course of disease. There are, however, also seronegative types of RA without detectable RF, and RF can occur in connection with other rheumatic and non-rheumatic diseases such as hepatitis, endocarditis and parasitic or viral infections and other autoimmune diseases[4]. With increasing age there is also an increase in the ratio of RF positive findings without corresponding signs of disease[1]. Therefore, the detection of RF alone cannot serve as diagnosis, but must be interpreted in conjunction with further clinical findings.

Principles of the Procedure

Polystyrene particles coated with an immunocomplex consisting of human immunoglobulin and anti-human IgG from sheep are aggregated when mixed with samples containing RF. These aggregates scatter a beam of light passed through the sample. The intensity of the scattered light is proportional to the concentration of the respective protein in the sample. The result is evaluated by comparison with a standard of known concentration.

Reagents

Reagent	Description	Storage	Stability
N Latex RF Kit N RF			

N RF REAGENT	Ready to use liquid containing:
	• polystyrene particles coated with an immunocomplex of human-γ-globulin (0.2 mL/L) / anti-human-γ-globulin from sheep (2.7 mL/L)[a]
	• Preservative:
	  • Gentamicin sulfate (6.96 mg/L)
	  • Amphotericin B (0.696 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,
			4 weeks[b,c]

N RF SUPPLEMENT	Ready to use liquid containing: • polyethylene glycol (≤ 70 g/L) • Detergent • 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] i. e. a combination of human and animal γ-globulin
[b] if securely capped immediately after use
[c] if contamination (e.g. by microorganisms) is precluded

During storage at 2 to 8 °C crystals may form in N RF Supplement which go into solution again at room temperature (15 to 25 °C) and have no effect on the usability of the product.

On-board stability
On-board stability may vary, depending on the system used and laboratory conditions. The reagents should not remain open on the BN II System, but instead be stored again securely capped immediately after use at 2 to 8 °C. 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! POTENTIAL BIOHAZARD
N RF REAGENT
Each donor or donor unit was tested and found to be negative for human immunodeficiency virus (HIV) 1 and 2, hepatitis B virus (HBV) and hepatitis C virus (HCV) using either tests that are CE marked or FDA approved for this purpose. Because no known test can offer complete assurance of the absence of infectious agents, all human derived products should be handled with appropriate caution.

Caution
N RF REAGENT
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

The N RF REAGENT is liquid and can be used without additional preparation. It must be mixed carefully before use, avoid vigorous shaking and foam formation.

N RF SUPPLEMENT: The solution is ready-for-use.

Specimen Collection and Handling

Collecting the Specimen

Suitable samples are human serum, lithium heparin or EDTA plasma, either as fresh as possible (stored for no more than seven days at 2 to 8 °C) or stored frozen. Serum and plasma samples can be stored below –20 °C up to three months if frozen within 24 hours after collection and if repeated freeze-thaw cycles are avoided. Serum samples must be completely coagulated and, after centrifugation, must not contain any particles or traces of fibrin. Lipemic samples should be avoided.

Turbid samples must be clarified by centrifugation (10 minutes at approximately 15 000 × g) prior to testing.

Procedure

Materials Provided

REF	Contents		
OPCE03	N Latex RF Kit N RF		
	N RF Reagent N RF REAGENT	3 ×	2 mL
	N RF Supplement N RF SUPPLEMENT	3 ×	2.4 mL
OPCE05	N Latex RF Kit N RF		
	N RF Reagent N RF REAGENT	4 ×	4 mL
	N RF Supplement N RF SUPPLEMENT	4 ×	4.8 mL

Materials Required but not Provided

Item	Description
REF OQKZ13	N RHEUMA STANDARD SL, N Rheumatology Standard SL
REF OQDB13	N/T RHEUMA CONTROL SL/1, N/T Rheumatology Control SL/1
REF OQDC13	N/T RHEUMA CONTROL SL/2, N/T Rheumatology Control SL/2
REF OUMT65	N DILUENT, N Diluent

Item	Description
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.

Notes

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.

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

Only components of test kits with the same batch number may be combined with one another.

N RF REAGENT and N RF SUPPLEMENT must be replaced on the systems at the same time.

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

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

Performing Calibration

Reference curves are generated by multi-point calibration. Serial dilutions of N RHEUMA STANDARD SL are automatically prepared by the instrument using N DILUENT.

The reference curve is valid for 4 weeks and can be used beyond this period of time as long as controls with corresponding method-dependent target values, e.g. N/T RHEUMA CONTROL SL/1 and N/T RHEUMA CONTROL SL/2, are reproduced within their respective range.

If a different lot of reagent 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 RHEUMA 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.

Internal Quality Control

Assay N/T RHEUMA CONTROL SL/1 and N/T RHEUMA CONTROL SL/2 after each establishment of a reference curve, the first use of a reagent vial as well as with each run of samples. The controls are to be assayed and evaluated as for patient samples. The assigned value and range 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 a control value is outside the range, 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 IU/mL or in a unit selected by the userrespective system.

If the results obtained are above the measuring range, the assay can be repeated using a higher dilution of the sample. Refer to the respective System's Instruction Manual for information on repeat measurements using other dilutions.

Limitations

No interference in serum was detected for concentrations of bilirubin up to 0.6 g/L and of free hemoglobin up to 10 g/L.

Turbidity and particles in the samples may interfere with the determination. Therefore, samples containing particles must be centrifuged prior to testing. Turbid samples which cannot be clarified by centrifugation (10 minutes at approximately 15 000 × g) as well as heat inactivated samples 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.

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

Testing of serum from 253 European blood donors with N RF resulted in a 97.5th percentile of 15.9 IU/mL.

In a normal population, RF is below the measuring range of this method. However, apparently healthy, asymptomatic individuals may have RF of low titer. The incidence of a false positive result increases with age and is similar in males and females. Each facility should determine its own reference intervals, since values may vary depending on the population studied.

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

Performance Characteristics

Note: The values cited for specific performance characteristics were obtained on a BN II System and represent typical values; they are not to be regarded as specifications for the N RF.
Equivalency for the Atellica® NEPH 630 System has been confirmed.

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 RHEUMA STANDARD SL. Typical measuring ranges are given in the respective Assay Protocols document.

Specificity

Particle-enhanced agglutination reactions predominantly detect RF of the IgM class[5].

Sensitivity

The analytical sensitivity of the assay is determined by the lower limit of the reference curve and therefore depends upon the concentration of RF in the N RHEUMA STANDARD SL. A typical Limit of Detection (LoD) for RFn is < 0.441 IU/mL for measurements performed using a sample dilution of 1:20.

Precision

Precision testing was conducted in accordance with CLSI guideline EP5-A2[6] with two control samples as well as four serum pools with different RF concentrations. The analysis of variance (n = 80) of the results produced the following coefficients of variation (CV):

Sample	Mean [IU/mL]	Repeatability CV [%]	Within-Device/Lab Precision CV [%]
N/T RHEUMA CONTROL SL/1	69.3	2.2	5.7
N/T RHEUMA CONTROL SL/2	170.2	2.2	3.8
Serum pool (low) 1	81.3	2.2	5.3
Serum pool (low) 2	27.9	2.7	7.9

Sample	Mean [IU/mL]	Repeatability CV [%]	Within-Device/Lab Precision CV [%]
Serum pool (high) 1	600.8	5.3	8.1
Serum pool (high) 2	582.7	5.1	7.7

The reproducibility was assessed by Siemens Healthineers for N RF 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

90 serum samples (RF concentrations from 15.8 to 589.8 IU/mL) were assayed with the N RF (y) and N Latex RF (x). Correlation of the results yielded the following data:

y = 1.098 × – 12.94 IU/mL, r = 0.98.

Antigen Excess

The N RF reagent shows no high-dose hook effect in the RFn assay up to, 4 159 IU/mL.

Technical Assistance

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

siemens-healthineers.com

Current Version of Assay Protocols

N RF 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. Nakken B, Papp G, Bosnes V, et al. Biomarkers for rheumatoid arthritis: From molecular processes to diagnostic applications-current concepts and future perspectives. Immunol Lett 2017;189:13-18.
2. Aletaha D, Neogi T, Silman AJ, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum 2010;62(9):2569-81.
3. Hua C, Daien CI, Combe B, et al. Diagnosis, prognosis and classification of early arthritis: results of a systematic review informing the 2016 update of the EULAR recommendations for the management of early arthritis. RMD Open 2017;3(1):e000406.
4. Ali Y. Rheumatologic Tests: A Primer for Family Physicians. Am Fam Physician 2018;98(3):164-170.
5. Mierau R, Genth E. Autoantibodies in rheumatoid arthritis. In: Thomas L ed. Clinical Laboratory Diagnostics, TH-Books, Frankfurt/Main 1998;810-3.
6. NCCLS. Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition. NCCLS document **EP5-A2** [ISBN 1-56238-542-9]. NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2004

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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