SIEMENS Healthineers

N Latex Lp(a) Reagent

N LP(A) **REAGENT**

CE 0197

Revision bar indicates update to previous version.

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

Intended Use

N LP(A) **REAGENT** is an in vitro diagnostic reagent for the quantitative determination of lipoprotein(a) [Lp(a)] as aid in risk assessment for cardiac and vascular diseases, and for diagnosis of elevated Lp(a) in patients at increased risk for cardiovascular disease in human serum and heparin plasma by means of automated Siemens Healthineers immuno-nephelometry systems.

N LP(A) **REAGENT** uses a calibration against an internal protein reference preparation.

Summary and Explanation

Lipoprotein(a) [Lp(a)] particles are formed by the covalent binding of apolipoprotein(a) [apo(a)] to apolipoprotein B of an low-density lipoprotein (LDL) particle. Apo(a) is highly polymorphic in size because of a wide variation in number of kringle IV repeats resulting in > 40 iso-forms and thus > 40 different sizes of Lp(a) particles. Apo(a) has high-sequence homology with the coagulation factor plasminogen and, like LDL, Lp(a) contains apolipoprotein B100 (ApoB). Thus, Lp(a) is both proatherogenic and prothrombotic[1].

Lp(a) is an established causal, independent and genetically determined risk factor for coronary heart disease (CHD), ischemic stroke, and aortic valve stenosis[2–5].

The European Atherosclerosis Society recommends that Lp(a) be tested for all individuals with moderately high or high CHD risk (≥ 10 % 10-year risk) who present with[3,4]:

- Premature CVD
- A family history of premature CVD and/or elevated Lp(a)
- Familial hypercholesterolemia
- Recurrent CVD despite statin treatment

The mechanism of increased risk is unclear but most likely involves progression of atherosclerotic stenosis via intimal deposition of cholesterol and promotion of thrombosis via homology to plasminogen.

Lp(a) serum levels are > 90 % genetically determined, and are largely unaffected by diet, exercise and lipid-lowering pharmaceuticals. However, in a patient with additional modifiable CHD risk factors, more aggressive therapy to normalize these factors may be indicated if the Lp(a) value is also increased.

Lp(a) concentrations > 30 mg/dL are associated with 2- to 3-fold increased risk of cardiovascular events independent of conventional risk markers[1,5].

Principles of the Procedure

Polystyrene particles coated with specific antibodies to human Lp(a) are aggregated when mixed with samples containing Lp(a). 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 Lp(a) Reagent N LP(A) REAGENT	Lyophilized reagent containing: • polystyrene particles coated with anti-human Lp(a)-γ-globulin fraction, rabbit (reconstituted: 62.5 mg/L) • Preservative: • Sodium azide (reconstituted: < 1 g/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	2–8 °C: reconstituted, 4 weeks[a,b] Do not freeze!

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

On-board stability
A minimum of five days, at eight hours per day, 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.

Danger! N LP(A) REAGENT
Hazardous ingredient: Sodium azide (1.59 % [w/w]), Imidazole (4.57 % [w/w]).
H302 + H312: Harmful if swallowed or in contact with skin. **H318**: Causes serious eye damage. **H315**: Causes skin irritation. **H360D**: May damage the unborn child. **H412**: Harmful to aquatic life with long lasting effects.
P201: Obtain special instructions before use. **P264**: Wash hands thoroughly after handling. **P280**: Wear protective gloves/protective clothing/eye protection/face protection. **P273**: Avoid release to the environment. **P308 + P313**: IF exposed or concerned: Get medical advice/attention. **P305 + P351 + P338**: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. **P310**: Immediately call a POISON CENTER or doctor/physician. **P501**: Dispose of contents and container in accordance with all local, regional, and national regulations.

CAUTION! POTENTIAL BIOHAZARD
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
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 LP(A) REAGENT: Resuspend the lyophilized contents of a vial in 2.0 mL distilled water and allow to sit for 30 minutes before use. Shake carefully to mix before first use.

Specimen Collection and Handling

Suitable samples are human serum and heparinized plasma, either as fresh as possible (stored for no more than 7 days at 2 to 8 °C) or stored frozen. Samples can be stored below –20 °C for up to 1 month if they are frozen within 24 hours after collection and if repeated freeze-thaw cycles are avoided. Serum samples must have completely coagulated and, after centrifugation, must not contain any particles or traces of fibrin.

Procedure

Materials Provided

REF	Contents		
OQHL11	N Latex Lp(a) Reagent N LP(A) REAGENT	3 × →	2 mL

Materials Required but not Provided

Item	Description
REF OQCV05	N LP(A) STANDARD SY, N Lp(a) Standard SY
REF OQCW05	N LP(A) CONTROL SY, N Lp(a) Control SY
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

For the Atellica® NEPH 630 System, BN II System or BN ProSpec®, 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. The lyophilized reagent must not be used until properly reconstituted (at least 30 minutes after the addition of distilled water).

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

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

Performing Calibration

Reference curves are generated by multi-point calibration. Serial dilutions of N LP(A) STANDARD SY are automatically prepared by the instrument using N DILUENT. The standard dilutions are to be used within 4 hours. The reference curve is valid for 2 weeks and can be used beyond this period, as long as controls with corresponding method-depending target values, e.g., N LP(A) CONTROL SY is reproduced

within its 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 LP(A) STANDARD SY. Typical measuring ranges are given in the respective Assay Protocols document.

Assay of Specimens

Samples are automatically diluted 1:400 with N DILUENT. The diluted samples must be measured within 4 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 your respective System's Instruction Manual for information on repeat measurements using other dilutions.

Internal Quality Control

Assay N LP(A) CONTROL SY after each establishment of a reference curve, the first use of a reagent vial as well as with each run of samples. The control is to be assayed and evaluated as for patient samples. The assigned value and range of the N LP(A) CONTROL SY are listed in the respective table.

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 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 the deviation has been identified and corrected.

Results

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

Limitations

No interference was detected for concentrations of triglycerides up to 11.6 g/L, free hemoglobin up to 10 g/L, bilirubin up to 0.6 g/L. No interference was observed with the drugs ASPIRIN, β-estradiol (estrogen), niacin, pravastatin or simvastatin.

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.

N Latex Lp(a) assay was not tested with age-matched pairs in a disease population. When testing Lp(a) as a risk factor, patients should be free from acute inflammatory states, as an acute-phase response will lead to an increase of the Lp(a) concentration[7].

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

Lp(a) serum concentrations in healthy persons exhibit an asymmetrical distribution and may exceed 1 g/L[6], and are known to vary by ethnicity. Reference intervals for Lp(a) assay were determined using the N LP(A) REAGENT assay and sera from ostensibly healthy blood donors. The results obtained are shown in the following table:

Lp(a) Concentration g/L									
Population	Total			Males			Females		
	n	25th–75th	5th–95th	n	25th–75th	5th–95th	n	25th–75th	5th–95th
Caucasian	628	0.04–0.30	< 0.02–0.72	331	0.05–0.32	< 0.02–0.74	297	0.04–0.25	< 0.02–0.72

These values are for reference only. Each facility should determine its own reference intervals, since values may vary depending on the population studied. The Lp(a) concentration of 0.3 g/L, which corresponds to the 75th percentile in a male Caucasian reference population[8], is frequently used as a cardiac risk differentiation level.

Performance Characteristics

The N LP(A) REAGENT assay performance characteristics were determined using BN Systems.

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

Note: The values cited for specific performance characteristics of the assay represent typical results and are not to be regarded as specifications for N LP(A) REAGENT.

Measuring Range

The measuring range of the N LP(A) REAGENT assay is established by the lower limit of the reference curve and depends therefore upon the concentrations of the protein in the N LP(A) STANDARD SY. Typical measuring ranges are given in the respective Assay Protocols document.

Specificity

The N LP(A) REAGENT assay shows no cross-reactivity by apolipoprotein B (< 1 %). Cross-reactivity by plasminogen is < 5 %. Apo(a) size heterogeneity only has a moderate effect on Lp(a) recovery with the N LP(A) REAGENT assay[9].

Sensitivity

The analytical sensitivity of the assay is determined by the lower limit of the reference curve and therefore depends upon the concentration of the protein in the N LP(A) STANDARD SY. A typical limit of detection for Lp(a) is < 0.0241 g/L for measurements performed using a sample dilution of 1:100.

The exact assay ranges depend upon the concentration of the protein in each lot of N LP(A) STANDARD SY. For typical ranges refer to your respective Assay Protocols documents.

Precision

The following coefficients of variation (CV) were obtained with N LP(A) REAGENT on a BN System:

Sample	n	Mean [g/L]	Repeatability CV [%]	Within-Device/Lab Precision CV [%]
N LP(A) CONTROL SY	40	0.328	2.8	2.7
Pool 1	40	0.288	4.5	6.0
Pool 2	40	0.698	3.4	4.7

The results were evaluated by analysis of variance.

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

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

Method Comparison

The N LP(A) REAGENT assay on a BN ProSpec® System (y) was compared to a BN II System (x) by evaluating 5 plasma samples and 55 serum samples, with Lp(a) concentrations ranging from 0.026 to 1.02 g/L. Regression analysis of the results yielded the following equation: $y = 0.96 \times + 0.0025$ g/L ($r = 0.997$).

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

Antigen Excess

The N LP(A) REAGENT shows no high-dose hook effect in the assay up to Lp(a) concentrations of: 1.79 g/L.

Technical Assistance

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

siemens-healthineers.com

Current Version of Assay Protocols

N LP(A) REAGENT 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. Enas EA, Varkey B, Dharmarajan TS, et al. Lipoprotein(a): An independent, genetic, and causal factor for cardiovascular disease and acute myocardial infarction. Indian Heart J 2019;71(2):99-112.
2. Scipione CA, Koschinsky ML, Boffa MB. Lipoprotein(a) in clinical practice: New perspectives from basic and translational science. Crit Rev Clin Lab Sci 2018;55(1):33-54.
3. Tsimikas S, Fazio S, Ferdinand KC, et al. NHLBI Working Group Recommendations to Reduce Lipoprotein(a)-Mediated Risk of Cardiovascular Disease and Aortic Stenosis. J Am Coll Cardiol 2018;71(2):177-192.
4. Rawther T, Tabet F. Biology, pathophysiology and current therapies that affect lipoprotein (a) levels. J Mol Cell Cardiol 2019;131:1-11.
5. Shai I1, Rimm EB, Hankinson SE, et al. Lipoprotein (a) and coronary heart disease among women: beyond a cholesterol carrier? Eur Heart J 2005;26(16):1633-9.
6. Koschinsky ML, Marcovina SM. Lipoprotein(a): structural implications for pathophysiology. Int J Clin Lab Res. 1997; 27: 14-23.
7. Danesh J, Collins R, Peto R. Lipoprotein(a) and coronary heart disease. Meta-analysis of prospective studies. Circulation 2000; 102: 1082-5.
8. Kostner GM, Avogaro P, Cazzolato G, et al. Lipoprotein Lp(a) and the risk for myocardial infarction. Atherosclerosis 1981; 38: 51-61.
9. Marcovina SM, Albers JJ, Scanu AM, et al. Use of a reference material proposed by the International Federation of Clinical Chemistry and Laboratory Medicine to evaluate analytical methods for the determination of plasma lipoprotein (a). Clin Chem 2000; 46: 1956-67.

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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