

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

k122722

B. Purpose for Submission:

New device

C. Measurand:

Lipoprotein (a) [Lp(a)]

D. Type of Test:

Quantitative immunoturbidimetric assay

E. Applicant:

Roche Diagnostics

F. Proprietary and Established Names:

cobas c Tina-quant Lipoprotein (a) Gen.2 Test System
Preciset Lp(a) Gen.2 calibrator set
PeciControl Lp(a) Gen.2 control set

G. Regulatory Information:

<u>Product</u>	<u>Classification</u>	<u>Regulation</u>	<u>Panel</u>
<u>DFC</u>	<u>Class II</u>	<u>21 CFR 866.5600</u> <u>Low Density Lipoprotein</u> <u>Immunological Test System</u>	<u>Clinical Chemistry(75)</u>
<u>JIT</u>	<u>Class II</u>	<u>21 CFR 862.1150</u> <u>Calibrator, Secondary</u>	<u>Clinical Chemistry (75)</u>
<u>JJX</u>	<u>Class I, reserved</u>	<u>21 CFR 862.1660</u> <u>Quality control material</u>	<u>Clinical Chemistry(75)</u>

H. Intended Use:

1. Intended use(s):

The cobas c Tina-quant Lipoprotein (a) Gen.2 assay is an in vitro test intended for

the quantitative determination of lipoprotein(a) [Lp(a)] in human serum and plasma on the Roche/Hitachi cobas c systems. The measurement of Lp(a) is useful in evaluation of lipid metabolism disorders and assessing atherosclerotic cardiovascular disease in specific populations, when used in conjunction with clinical evaluation and other lipoprotein tests.

The Preciset Lp(a) calibrator set is intended for use in the calibration of quantitative Roche methods on Roche clinical chemistry analyzers as specified in the value sheets.

The PreciControl Lp(a) Gen.2 control set is intended for use in quality control by monitoring accuracy and precision for the quantitative methods as specified in the value sheets.

2. Indication(s) for use:

See intended use.

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

For use on the cobas c 501 Analyzer

I. Device Description:

The Tina-quant Lp(a) Gen.2 test principle is a particle-enhanced immunoturbidimetric assay. Reagents are packaged in two bottles labeled with their instrument positioning, R1 and R3. R1 is glycine buffer solution that contains BSA and 0.1% rabbit serum. R3 is a suspension of latex particles coated with polyclonal anti-human Lp(a) antibodies (rabbit) in glycine buffer.

The Preciset Lp(a) Gen.2 calibrator set consists of five lyophilized calibrators based on a stabilized and lyophilized pool of human plasma. Vials are reconstituted with water to yield approximate concentration values of 6.5, 12.5, 29.0, 62.5, and 90 mg/dL.

The PreciControl Lp(a) Gen.2 control set contains two lyophilized controls based on a human plasma matrix. Vials are reconstituted with water to yield approximate concentrations of 20 and 48 mg/dL.

All human material should be considered potentially infectious. All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV using

testing methods approved by the FDA.

J. Substantial Equivalence Information:

1. Predicate device name(s) and number(s):

cobas c Tina-quant Lipoprotein (a) Gen.2 assay is substantially equivalent to Lp(a)-Latex SEIKEN assay, Denka Seiken Co., Ltd. (k013359).

Preciset Lp(a) Gen.2 calibrator set is substantially equivalent to the Diazyme Lp(a) calibrator set, General Atomics (k082488).

PreciControl Lp(a) Gen.2 control is substantially equivalent to the Diazyme Lp(a) control set, General Atomics (k082488).

2. Comparison with predicate:

Similarities		
Characteristic	New Device cobas c 501 Tina.quant Lipoprotein(a) assay	Predicate Device Denka Seiken Lipoprotein(a) assay (K013359)
Intended Use	Same	Immunoturbidimetric assay for the quantitative <i>in vitro</i> determination of Lipoprotein(a) in human serum or plasma.
Principle	Same	Lp(a) in sample binds to specific anti-Lp(a) antibodies that are coated on latex particles. Lp(a) binding causes agglutination of the latex particles. The amount of agglutination is measured optically via sample turbidity and is directly proportional to the amount of Lp(a) in the sample.
Sample Type	Same	Serum, plasma

Differences		
Characteristic	New Device cobas c 501 Tina.quant Lipoprotein(a) assay	Predicate Device Denka Seiken Lipoprotein(a) assay (K013359)
Instrument	cobas c 501	Hitachi 917
Measuring Range	6-80 mg/dL	2-80 mg/dL

Similarities		
Characteristic	New Device Preciset Lp(a) Gen.2 Calibrator set	Predicate Device Diazyme Lp (a) calibrators (K082488)
Intended Use	Same	for use in calibration of Lp(a) assay
Form	Same	Lyophilized

Similarities		
Characteristic	New Device PeciControl Lp(a) Gen.2 Control set	Predicate Device Diazyme Lp (a) controls (K082488)
Intended Use	Same	For use in quality control of Lp(a) assay.
Form	Same	Lyophilized

K. Standard/Guidance Document Referenced (if applicable):

Standard Title CLSI EP05-A2 – Evaluation of Precision Performance of Quantitative Measurement Methods

Standard Title CLSI EP6-A – Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach

Standard Title CLSI EP07-A2 – Interference Testing in Clinical Chemistry

Standard Title CLSI EP17-A – Protocols for Determination of Limits of Detection and Limits of Quantitation

L. Test Principle:

The Roche cobas c Tina-quant Lipoprotein (a) Gen.2 test system is based on a latex enhanced immunoturbidimetric assay. Human lipoprotein (a) agglutinates with latex particles coated with anti-Lp(a) antibodies (rabbit). The precipitate is determined turbidimetrically.

M. Performance Characteristics (if/when applicable):

All studies were performed on the cobas c 501 Analyzer

1. Analytical performance:

a. *Precision/Reproducibility:*

Assay within lot precision was determined according to CLSI EP5-A2 with a study that measured pooled human sera samples (7.0, 16.1, 30.9 and 79.4 mg/dL) and plasma-based control samples using one lot of reagent in two aliquots per run and 2 runs per day for 21 days.

The 20 day results for the single site are summarized below as sample overall mean Lp(a) concentration in mg/dL, computed SDs and %CVs for within run precision and total precision between runs and between days.

Within Run Precision	Mean (mg/dL)	SD (mg/dL)	CV (%)
Lp(a) Control level L	20.3	0.3	1.4
Lp(a) Control level H	61.5	0.6	1.0
Human serum 1	7.0	0.4	5.4
Human serum 2	16.1	1.0	6.2
Human serum 3	30.9	0.7	2.4
Human serum 4	79.4	0.7	0.9

Between Day Precision	Mean (mg/dL)	SD (mg/dL)	CV (%)
Lp(a) Control level L	20.3	0.3	1.6
Lp(a) Control level H	61.5	0.7	1.1
Human serum 1	7.0	0.5	7.6

Human serum 2	16.1	1.0	6.4
Human serum 3	30.9	0.9	2.9
Human serum 4	79.4	0.9	1.1

b. *Linearity/assay reportable range:*

Linearity of this Lp(a) assay for pooled human sera and pooled human plasma samples was assessed according to CLSI EP6- A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach, in one run using one lot of reagents with each sample tested in triplicate. Two separate dilution series of 9 samples each from high to low concentration Lp(a) were prepared by adding 0.9% NaCl to pooled human sera and to pooled human plasma that contained Lp(a) at a concentration above the measuring range. This yielded linearity samples with Lp(a) levels that spanned a range from 1.3 to 95 mg/dL.

A polynomial evaluation of linearity was used for the data analysis that included unweighted regression models. Nonlinearity did not deviate more than 10% from linearity for serum samples. Nonlinearity did not deviate more than 2% from linearity for plasma samples.

A linear regression line and second and third order polynomials were fitted to the data. Sponsor evaluated results and significance of the second and third order polynomials by comparing to in-house acceptance criteria for deviation from linearity.

The fitted linear models are:

$$\text{Serum: } y = 0.0008 + 1.0000x$$

$$\text{Plasma: } y = -0.2896 + 1.0039x$$

where $y = \text{Lp(a)}$ (mg/dL) and $x = \text{theoretical concentration}$.

The sponsor demonstrated linearity across the claimed measuring range of the device from 6.0 mg/dL to 80 mg/dL (from assay LoQ to upper limit).

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Preciset Lp(a) Gen.2 calibrators are a set of five lyophilized pooled plasma standards that are value assigned to an in-house primary reference standard known as Standard Human Lp(a) and secondary in house standard via a commercially available method. For routine calibrator lots, samples are assayed in triplicate and value assigned according to the secondary in house reference material and checked per value assigned controls.

PreciControl Lp(a) Gen.2 controls are lyophilized pooled human plasma and set to low (20 mg/dL) and high (48 mg/dL) Lp(a) levels. These Lp(a) controls

are value assigned to the secondary in-house reference material by assaying in triplicate on the cobas c 501 Analyzer.

Stability:

Reagent, calibrator and control stability is based on shelf-life and open on board stability studies and includes studies with storage at 35°C for 150 hrs to mimic transport. The proposed protocol and acceptance criteria were reviewed and found to be adequate.

d. Detection limit:

LoB, LoD and LoQ studies were performed based upon CLSI EP-17A, Protocols for Determination of Limits of Detection and Limits of Quantitation (LoQ).

LoB Test Protocol

One (1) blank sample was tested on two (2) cobas c 501 analyzers with three (3) reagent lots for six runs of 5 replicates each run yielding 60 measurements per lot.

LoB was defined as the concentration at which there is a 95% probability that the sample is analyte-free and was calculated as the 95th percentile of 60 runs which was the average of the 57th and the 58th value.

LoD Test Protocol

Five samples with low analyte concentration (up to 4X LOB) were measured on two (2) cobas c 501 analyzers with three (3) reagent lots for six runs of singlicates for each run yielding 60 measurements per lot.

LoD was calculated using equation 3 from CLSI Document EP17-A (page 17)
 $LOD = c\beta SDs$.

LoQ Test Protocol

A low level sample set of six was measured in singlicate using 3 reagent lots on two (2) cobas c 501 analyzers for 3 days with 2 runs per day. The LoQ sample was observed to have $\leq 19.9\%$ total error between the measured value and the calculated value.

LoB = 3.0 mg/dL

LoD = 4.0 mg/dL

LoQ = 6.0 mg/dL

e. Analytical specificity:

i. Interference from endogenous substances/cross reactants.

Testing was performed to determine whether the presence of endogenous substances such as hemoglobin, lipids (using intralipid as a mimic), rheumatoid factor, conjugated and unconjugated bilirubin may interfere with assay results.

Two human serum samples were used with Lp(a) concentrations of 20 mg/dL and 55 mg/dL. Each sample level was spiked with varying levels of interferent for a total of 11 dilution steps per interferent (8 for RF, 5 for plasminogen and apolipoprotein B). The two sets of samples were tested in triplicate and median values used to calculate % recovery (measured concentration compared to Lp(a) concentration with zero interferent). Observed maximum interference was 5% at the following levels of interferent:

Endogenous Substance/Cross Reactant	Highest Tested Concentration
Hemoglobin	1000 mg/dL
Intralipid	2000 mg/dL
Bilirubin (conj)	60 mg/dL
Bilirubin (unconj)	60 mg/dL
Rheumatoid Factor	1200 IU/mL
Plasminogen	150 mg/dL
Apolipoprotein B	200 mg/dL

ii. Interference from common drugs

Two human serum samples were used with Lp(a) concentrations of 20 mg/dL and 45 mg/dL. Each sample pool was divided into 17 aliquots. One aliquot was not spiked with any drugs and was used as the reference sample. The remaining samples were spiked with the respective amount of drug. Then the concentration of Lp(a) was determined in triplicate and compared to the reference aliquot. Lp(a) recovery was within $\pm 5\%$ for the tested analyte concentrations.

No interference was found at therapeutic concentration of any of the following drugs: acetylcysteine, ampicillin-Na, ascorbic acid, cyclosporine, cefoxitin, heparin, levodopa, methyldopa, metronidazole, doxycycline, acetylsalicylic acid, rifampicin, acetaminophen, ibuprofen, theophylline.

f. Assay cut-off:
Not applicable

g. Additional studies
Dilution study to validate re-run function.

One sample each at the lower end, middle and the upper end of the extended

measuring range was diluted manually and automatically (1:3). The manually and automatically diluted and the neat samples were measured with 3-fold dilutions. All results were within 10%.

High dose hook effect studies.

Two samples with high concentrations of Lp(a) (higher than the measuring range claim of 80 mg/dL) were diluted with native serum with an Lp(a) concentration of about 1-2 mg/dL Lp(a). These samples are diluted in 11 steps. Each dilution was measured in triplicate.

No hook effect was seen up to 190 mg/dL.

2. Comparison studies:

a. *Method comparison with predicate device:*

To demonstrate substantial equivalence, a method comparison study was performed on 159 human serum samples ranging from 6 to 79.9 mg/dL Lp(a) were tested by the Roche cobas c 501 Tina-quant Lp(a) assay (expressed as mg/dL) and predicate on the Hitachi 917 (expressed as mg/dL). The method comparison study was performed according to CLSI EP9-A2 guideline. Samples were tested in singlicate and no spiked or diluted samples were included.

Lp(a) Passing Bablok Regression (n = 159) with 95% CI indicated in parentheses.

slope = 0.967 (0.948 – .983)
y-intercept = -0.105 (-0.392 to -0.218)
 $r^2 = 0.995$

The sponsor provided additional method comparison data to support that Roche Tina-Quant Lp(a) assay was not sensitive to the size heterogeneity of protein (a) isoforms. Experiments were done using samples with Lp(a) concentrations that covered the measuring range and compared to an ELISA method that was standardized via Western Blot analysis to known protein(a) isoforms.

Lp(a) Passing Bablok Regression:

slope = 0.981
y-intercept = -0.551
 $r^2 = 0.9950$

b. *Matrix comparison:*

The following sample types can be used for the cobas c Tina-quant Lipoprotein (a) Gen.2 assay: Serum, Gel separated Serum, Lithium heparin plasma, K2EDTA Plasma, K3EDTA Plasma. To demonstrate that gel separation serum, lithium heparin plasma, K2EDTA plasma, and K3EDTA plasma are supported for use with this assay, matrix comparison studies were run using serum as the comparator. Samples were tested in singlicate.

Lp(a) Matrix Passing Bablok Regression:

Matrix	slope	intercept	r²	Sample range (mg/dL)
Gel separation serum	0.997	0.0393	1.00	6.6 – 77.4
Lithium Heparin Plasma	0.979	-0.0660	0.999	6.6 – 77.4
K2EDTA Plasma	0.974	0.249	0.999	6.6 – 77.4
K3EDTA Plasma	0.944	0.106	1.00	6.6 – 77.4

3. Clinical studies:
 - a. *Clinical Sensitivity:*
Not applicable
 - b. *Clinical specificity:*
Not applicable
 - c. Other clinical supportive data (when a. and b. are not applicable):
Not applicable
4. Clinical cut-off:
Not applicable
5. Expected values/Reference range:

The sponsor provides the following information in the package insert.

The defined threshold of Lp(a) concentration at which individuals can be classified as being at increased risk varies greatly among studies, ranging from 20 mg/dL to 40 mg/dL. A Lp(a) concentration of 30 mg/dL, corresponding to the 75th percentile in a male Caucasian reference population is widely used as cut-off

point or threshold value.^{1,2}

The European Atherosclerosis Society recommends screening for elevated Lp(a) in those at intermediate or high CVD/CHD risk and defines a desirable Lp(a) level $\leq 50\text{mg/dL}$.³

Different Lp(a) levels can be found in different racial/ethnicity groups.^{4,5}

Since Lp(a) levels are largely influenced by hereditary factors, they vary with ethnic populations. Therefore, reference ranges have not been established for this assay for different ethnic populations or disease states. The sponsor states that each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.

¹ Marcovina SM, Koschinsky, ML. A Critical Evaluation of the Role of Lp(a) in Cardiovascular Disease: Can Lp(a) Be Useful in Risk Assessment? *Semin Vasc Med* 2002 Aug 2(3):335-344.

² Shai, I, Rimm EB, Hankinson SE, et al. Lipoprotein (a) and Coronary Heart Disease Among Women: Beyond a Cholesterol Carrier? *Eur Heart J* 2005; 26:1633-1639.

³ Nordestgaard BG, Chapman MJ, Ray K, et al. Lipoprotein (a) Levels and Small Apolipoprotein (a) as a cardiovascular risk factor: current status. *Eur Heart J* 2010, 31:241-249.

⁴ Wu HD, Berglund L, Dimayuga C, et al. High Lipoprotein (a) Levels and Small Apolipoprotein (a) Sizes Are Associated With Endothelial Dysfunction in a Multiethnic Cohort. *J Am Coll Cardiol* 2004, 43:1828-1833.

⁵ Virani SS, Brautbar A, Davis BC, et al. Associations Between Lipoprotein (a) Levels and Cardiovascular Outcomes in Black and White Subjects. The Atherosclerosis Risk in Communities (ARIC). *Circulation* 2012, 125:241-249.