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

A. 510(k) Number:

K161691

B. Purpose for Submission:

New Device

C. Measurand:

LDL Cholesterol (LDL)

D. Type of Test:

Quantitative colorimetric assay

E. Applicant:

Randox Laboratories Limited

F. Proprietary and Established Names:

Direct LDL Cholesterol (LDL)

G. Regulatory Information:

Product Code	Regulation	Classification	Panel
MRR	21 CFR §862.1475, Lipoprotein test system	Class I, meets the limitation of exemption 21 CFR §862.9(c)(4)	Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See Indications for Use below.

2. Indication(s) for use:

   For the quantitative in vitro determination of LDL-cholesterol concentration in human plasma and serum. Lipoprotein measurements are used in the diagnosis and treatment of lipid disorders (such as diabetes mellitus), atherosclerosis and various liver and renal diseases.

   This in vitro diagnostic device is intended for prescription use only.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   For use with the RX Daytona Plus Chemistry analyzer

I. Device Description:

The Randox Direct LDL Cholesterol (LDL) reagent consists of two solutions, reagent 1 (R1) and reagent 2 (R2). R1 is supplied in 4 x 20 mL vials in liquid ready-to-use form and contains PIPES Buffer (Piperazine-1, 4-bis (2-ethanesulfonic acid)), HDAOS (N-(2-hydroxy-3-sulfopropyl)-3), 5-dimethoxylaniline sodium salt, Cholesterol Esterase, Cholesterol Oxidase, Catalase, Ascorbate Oxidase and surfactant. R2 is supplied in 4 x 9 mL vials in liquid ready-to-use form and contains PIPES Buffer, 4-Amino antipyrine, peroxidase, surfactant and sodium azide.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Randox Laboratories Ltd., Direct LDL Cholesterol reagent

2. Predicate 510(k) number(s):

   K982529

3. Comparison with predicate:

Similarities		
Item	New Device	Predicate Device (K982529)
Intended Use	For the quantitative in vitro determination of LDL-cholesterol concentration in human plasma and serum.	Same
Assay Protocol	Quantitative colorimetric assay	Same
Storage (Unopened)	Reagents are stable up to the expiry date when stored unopened at +2 to +8°C	Same
Sample Type	Serum Lithium heparinized plasma	Same

Differences		
Item	New Device	Predicate Device (K982529)
Measuring Range	21 to 740 mg/dl	7.3 to 859 mg/dl

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

Clinical and Laboratory Standards Institute (CLSI) EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition

CLSI EP06-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline

CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline-Second Edition

CLSI EP9-A2: Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline-Second Edition

CLSI EP07-A2 Interference Testing in Clinical Chemistry; Approved Guideline-Second Edition

L. Test Principle:

The assay consists of 2 distinct reaction steps:

1. Elimination of chylomicron, VLDL-cholesterol and HDL cholesterol by Cholesterol Esterase, Cholesterol Oxidase and subsequently Catalase.

2. Specific measurement of LDL-cholesterol after release of LDL-cholesterol by detergents in Reagent 2. The intensity of the quinoneimine dye produced is directly proportional to the LDL-cholesterol concentration when measured at 600 nm. In the second reaction catalase is inhibited by sodium azide in Enzyme Reagent 2.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

All performance studies were performed on the Randox RX Daytona Plus analyzer.

a. Precision/Reproducibility:

Precision of the Direct LDL Cholesterol (LDL) test system was determined in accordance with the CLSI EP5-A2 guideline. The precision study was performed utilizing 2 reagent lots, 2 RX Daytona Plus analyzers using serum based control material, one unaltered human serum sample in the normal range and three human serum samples that were spiked with LDL cholesterol concentrations or diluted to achieve concentrations spanning medically relevant concentrations. Two replicates of each of the control and serum samples were tested on two separate runs per day for 20 days, leading to the generation of 80 data points for each sample. The mean, standard deviation (SD), and % coefficient of variation (CV) calculated for within-run and total imprecision for each lot yielded similar results. The results from one representative lot are shown below:

Sample	Mean (mg/dL)	Within-run		Total	
		SD	%CV	SD	%CV
QC 1	92.0	2.7	3.0	5.4	5.9
QC 2	135.9	3.8	2.8	6.2	4.6
QC 3	186.7	5.5	2.9	8.2	4.4
Serum pool 1	65.0	1.7	2.6	3.8	5.9
Serum pool 2	154.0	4.4	2.9	7.8	5.0
Serum pool 3	200.1	5.8	2.9	10.0	5.0
Serum pool 4	343.7	10.3	3.0	18.0	5.3

b. *Linearity/assay reportable range:*

Linearity of the Direct LDL Cholesterol (LDL) test system was determined in accordance with the CLSI EP6-A guideline. Linearity studies were performed using two lots of reagent on one RX Daytona Plus analyzer. For both studies, samples spanning the linear range of the assay were prepared by inter-mixing a high serum sample spiked with LDL Cholesterol (850 mg/dl) with a low concentration serum sample diluted with 0.9% saline (21 mg/dl) to generate a total of 11 samples. Each sample was assayed in 5 replicates. The results of the analysis showed that within the measured range, the results did not deviate by more than 10% from linearity. The linear regression analyses for one representative lot are summarized below:

$y = 0.99x + 6.34$; $r = 0.997$

The reportable range of the LDL assay is 21-740 mg/dL.

Samples with concentrations greater than 740 mg/dL are re-run automatically by the RX Daytona Plus using the established high re-run condition.

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

Traceability:
Refer to the previously cleared Randox Direct HDL-C/LDL-C Calibrator (K122126). The reagent system has not been tested or certified by the Cholesterol Reference Method Laboratory Network (CRMLN) and this information is included in the package insert.

Calibrator:
The calibrator recommended for use with the Direct LDL Cholesterol (LDL) reagent is the previously cleared Randox Direct HDL-C/LDL-C Calibrator (K122126).

Controls:
The controls recommend for use with the Direct LDL Cholesterol (LDL) reagent are the previously cleared Randox Lipid Control Sera; Level 1, Level 2 and Level 3 (K022591).

Stability:
The reagent shelf life stability claim is 18 months at 2 - 8°C. The reagent onboard (in use and refrigerated) stability claim is 28 days.

d. *Detection limit:*

Detection limit studies have been carried out in accordance with the CLSI EP17-A2 guideline. Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantification (LoQ) studies were performed on two lots of reagents tested on one

RX Daytona Plus analyzer. Each lot was analyzed separately.

The LoB pool consisted of artificial serum base matrix with no LDL added. The LoB study samples were tested in 20 replicates over 3 days for a total of 60 replicate results. Nonparametric analysis was used for the LoB determination. The LoB was determined as the 95th percentile of all values. The claim is based on the lot with the highest determined LoB.

The LoD pool consisted of 4 low level sample pools prepared by diluting patient sera. The LoD study samples were tested in 20 replicates over 3 days for a total of 60 replicate results per pool. The LoD was determined using the following equation: $LoD = LoB + c_{\beta}SD_s$ where SD_s is the estimated standard deviation of the sample distribution at a low level and c_{β} is derived from the 95th percentile of the standard Gaussian distribution. The claim is based on the lot with the highest determined LoD.

The LoQ pools consisted of 4 separate pools prepared by diluting patient sera. The LoQ is the lowest concentration that can be detected with ≤ 20%CV. The claim is based on the lot with the highest determined LoQ.

Results for one representative lot are summarized in the following table:

Analyte	LoB	LoD	LoQ
LDL (mg/dL)	1.94	3.19	16.1

e. *Analytical specificity:*

Interference studies have been carried out in accordance with the CLSI EP7-A2 guideline. Interference testing was performed at 2 different concentrations of LDL (96.75 mg/dL and 193.5 mg/dL) using one RX Daytona Plus analyzer and two reagent lots. Pooled human serum samples with added potential interferents were tested in replicates of 10, and compared to a sample without interferent. The sponsor defined no significant interference as < 10 % difference from the control sample.

Results are summarized in the table below:

Interferent	Concentration at which no significant interference was observed
Hemoglobin	1000 mg/dL
Total Bilirubin	60mg/dL
Conjugate Bilirubin	60 mg/dL
Triglycerides	500 mg/dL
Intralipid	500 mg/dL
Ascorbic acid	6 mg/dL

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was carried out in accordance with the CLSI guideline EP9-A2. 139 patient serum samples, comprised of 127 unaltered patient serum samples and 12 spiked patient serum samples spanning the measuring range 22.45 to 735.68 mg/dL, were tested in singlicate on two lots of the Direct LDL Cholesterol (LDL) reagent on one RX Daytona plus analyzer and using the predicate device on one RX Imola system across 3 working days. The results from the proposed test system were compared to the results from the predicate device. Both lots had similar results. The linear regression analysis of one representative lot yielded the following results:

$y = 1.01x – 1.45,\ r = 0.998$

b. Matrix comparison:

Matrix comparison studies for the Direct LDL Cholesterol (LDL) reagent were performed on one RX Daytona plus system and two lots of reagents. Both serum and lithium heparin plasma samples were tested in singlicate. Patient samples were drawn in matched pairs–the first sample was a serum sample (x) and the second sample was a lithium heparin plasma sample (y). A total of 70 matched patient sample pairs were analyzed spanning the range of 25.45 – 665.64 mg/dL. The samples were comprised of 63 unaltered patient serum/plasma samples, 6 spiked patient serum/plasma sample and 1 diluted patient serum/plasma samples. Both lots had similar results. The linear regression analysis of one representative lot yielded the following results:

$y = 1.01x – 2.81,\ r = 0.998$

Based on the study, lithium heparin plasma is an acceptable matrix for the candidate assay.

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 following expected values are provided in the product insert based on the literature[1].

Reference Range

Analyte	Expected Values
LDL Cholesterol[1]	< 100 mg/dL optimal 130 – 159 mg/dL borderline high 160 – 189 mg/dL high >190 mg/dL very high

(1) Third Report of the National Cholesterol Education Programme (NCEP) Expert Panel on Detection, Evaluation and treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). NIH Publication No. 02-5215 September 2002.

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.