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

A. 510(k) Number:

k150654

B. Purpose for Submission:

New submission

C. Measurand:

Total Cholesterol

D. Type of Test:

Quantitative colorimetric assay

E. Applicant:

Randox Laboratories Limited

F. Proprietary and Established Names:

Cholesterol

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
CHH	Class I, meets the limitation of exemption 21 CFR §862.9(c)(4)	21 CFR §862.1175 Cholesterol (total) Test System	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 Cholesterol in serum and plasma. Cholesterol measurements are used in the diagnosis and treatments of disorders involving excess cholesterol in the blood and lipid and lipoprotein metabolism disorders.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   For use with the RX Daytona Plus analyzer (k131554)

I. Device Description:

The Randox Cholesterol test system is a one reagent system. The reagent (R1) is supplied in liquid ready-to-use form and contains 4-aminoantipyrine (0.23 mmol/L), Phenol 6.0 mmol/L, Peroxidase (≥ 0.50 U/ml), cholesterol esterase (≥0.20 U/mL) cholesterol oxidase (≥0.10 U/mL) and Sodium Azide (0.09%).

Materials required but not provided within the assay kit: Randox Assayed Multisera Level 2 and 3 (k942458), Randox Calibration Serum Level 3 (k053153) and RX series Saline.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Randox Laboratories Ltd, Cholesterol enzymatic endpoint

2. Predicate 510(k) number(s):
   k923504
3. Comparison with predicate:

Similarities and Differences		
Item	Proposed Device	Predicate device (k923504)
Intended Use	The Cholesterol test system is a device intended for the quantitative in vitro determination of Cholesterol in serum and plasma. Cholesterol measurements are used in the diagnosis and treatment of lipid lipoprotein metabolism disorders and atherosclerosis	Same
Sample Type	Serum and Plasma (Li Heparin and K_2-EDTA)	Same
Calibration Frequency	Every 28 days, with a change of reagent lot or as indicated by quality control procedures	Same
Measuring Range	25 – 618mg/dL	0.55 – 750mg/dL

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

- CLSI EP5-A2: *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline –Second Edition*
- 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 (Interim Revision)*
- CLSI EP6-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*

L. Test Principle:

Cholesterol esters in serum are completely hydrolyzed by cholesterol esterase to free cholesterol and free fatty acids. The cholesterol liberated by the esterase, plus any endogenous free cholesterol, are oxidized by cholesterol oxidase to yield hydrogen peroxide (H_2O_2), which then reacts oxidatively with phenol and 4-aminoantipyrine (AAP) in a reaction catalyzed by peroxidase, producing a red colored quinoneimine complex. The amount of colored complex formed, determined by quantitatively measuring the increase in absorbance at 510 nm/700 nm, is directly proportional to the cholesterol concentration in the sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Precision of the Cholesterol test system was evaluated according to CLSI EP5-A2. Two reagent lots and 2 RX Daytona Plus analyzers were utilized in the study. A native patient serum pool, altered serum sample pools, control pools and a calibrator serum sample were all evaluated. Two replicates of the 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 results of one representative lot are shown below:

Sample	**Mean (mg/dL)**	**Within-run**		**Total**	
		SD	**%CV**	**SD**	**%CV**
Native Patient Pool	32.4	1.17	3.6	3.35	10.3
Serum Pool 1	177	3.56	2.0	4.82	2.7
Control Pool 1	192	3.33	1.7	4.68	2.4
Serum Pool 2	228	4.04	1.8	6.20	2.7
Serum Pool 3	272	3.84	1.4	7.35	2.7
Calibrator Serum	285	4.22	1.5	5.59	2.0
Control Pool 2	310	5.52	1.8	7.22	2.3
Serum Pool 4	592	6.76	1.1	11.3	1.9

   b. Linearity/assay reportable range:

   A linearity study was performed following CLSI EP6-A by evaluating a dilution

series of serum samples containing the cholesterol analyte. Samples were prepared by mixing a high spiked serum sample with a low diluted serum sample to obtain 11 samples with concentrations spanning the claimed measuring range, 25 to 618 mg/dL. Each level was analyzed in replicates of five using two lots of Randox Cholesterol reagent on one RX Daytona Plus analyzer system. The results of the linear regression analysis of one representative lot are shown below:

y = 1.00x - 5.49; r = 0.999

The RX Daytona Plus analyzer is capable of auto diluting with a 1:15 dilution factor when measuring samples > 619 mg/dL, and an auto-dilution study was conducted against a manual dilution and all results were within a pre-specified acceptance criteria.

The reportable range of the assay is 25 - 618 mg/dL.

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

Traceability:

The controls and calibrator to be used with this assay are manufactured by Randox Laboratories. Calibrator (Randox Calibration Serum Level 3) and controls (Randox Assayed Multisera Level 2 and Level 3) were previously cleared in k053153 and k942458 respectively. Randox Calibration Serum Level 3 is traceable to Cholesterol reference material NIST 1952a.

Stability Studies:

Stability of the Cholesterol kit is based on real-time stability study data of three kit lots. The stability study protocol and acceptance criteria was reviewed and found to be acceptable. The real time stability study supports a shelf life stability of 24 months when materials are stored at 2-8ºC. Once opened, the kit is stable for 28 days at 2-8ºC.

d. Detection limit:

A detection limit study was performed according to CLSI EP17-A2 guideline. Limit of Blank (LoB) was determined by running 4 blank samples (serum based matrix free of analyte) in 20 replicates across 3 days giving 60 determinations in total on the RX Daytona Plus analyzer, and LoB was the value at the 95th percentile. The Limit of Detection (LoD) was determined by evaluating 4 diluted patient serum sample pools in 20 replicates across 3 days for a total of 60 data points on a RX Daytona Plus analyzer. Limit of Quantitation (LoQ) was determined by running 4 diluted patient serum sample pools in 12 replicates across 5 days yielding a total of 60 results. Sponsor defines the LoQ as the lowest analyte concentration where the %CV is ≤ 20%.

Analyte	LoB	LoD	LoQ
Cholesterol	3.1 mg/dL	6.31 mg/dL	23.2 mg/dL

The claimed measuring range is 25 - 619 mg/dL for the Randox Cholesterol Assay.

e. Analytical specificity:

Interference testing was performed in accordance with CLSI EP-7A2 with 2 different concentrations of cholesterol (150 mg/dL and 250 mg/dL) using one RX Daytona Plus analyzer and one reagent lot. Samples with added potential interferents were tested in replicates of 10, and the mean recoveries were compared to samples without interferent. The sponsor defined no significant interference as ≤10 % difference from the control sample.

Summary of interference study showing highest concentration that does not interfere:

Interferent	**Concentration at which no interference was observed**
Hemoglobin	750 mg/dL
Total Bilirubin	60 mg/dL
Bilirubin (conjugated)	60 mg/dL
Intralipid	1000 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 performed in accordance with CLSI EP9-A2. A total of 107 serum patient samples spanning the range of 25 to 599.5 mg/dL were analyzed with the candidate cholesterol assay on the Randox Daytona Plus system versus the predicate device. Of the 107 samples, 9 samples were diluted or spiked. All samples were analyzed in singlicate. Results of the linear regression are presented below:

Slope (95% CI)	**Intercept (95% CI)**	**R**
1.00 (0.98 to 1.01)	-4.77 (-7.99 to -1.57)	0.997

b. Matrix comparison:

A matrix comparison study was conducted using matched sets of serum and plasma (lithium-heparin and K_2EDTA plasma) samples which spanned the reportable range of the assay. The samples (6 diluted and 4 spiked) were analyzed on the RX Daytona Plus analyzer. The details and results of the study using simple linear regression analysis are as follows:

Sample Type	n	Intercept	Slope (95% CI)	R	Sample range tested

Serum vs. lithium Heparin	54	-6.54 (-12.01 to -1.07)	1.01 (0.99 to 1.03)	0.997	25 to 613 mg/dL
Serum versus K_2-EDTA plasma	51	2.73 (-1.39 to 6.86)	0.99 (0.97 to 1.00)	0.998	29 to 603 mg/dL

Based on the study data, the sponsor claims that serum, lithium heparin and K^2-EDTA plasma samples are acceptable for use with the Randox cholesterol 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[1]:
< 200 mg/dL – Desirable blood cholesterol
200-239- Borderline High Blood Cholesterol
≥ 240 mg/dL –High Blood Cholesterol

[1]Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA Publication, Vol. 285, No. 19, P2486-2497; 2001.

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.