

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

**A. 510(k) Number:**

k120202

**B. Purpose for Submission:**

New device

**C. Measurand:**

Total Cholesterol

**D. Type of Test:**

Quantitative, Enzymatic Esterase – Oxidase Cholesterol

**E. Applicant:**

Vital Diagnostics (Manufacturing) Pty Ltd

**F. Proprietary and Established Names:**

Eon Cholesterol Reagent

**G. Regulatory Information:**

Product Code	Classification	Regulation Section	Panel
CHH	Class I, (meets limitations of exemption per 21 CFR 862.9(c)(4))	862.1175	Clinical Chemistry

**H. Intended Use:**

1. Intended use(s):

Refer to indications for use below

2. Indication(s) for use:

Eon Cholesterol Reagent is an in vitro diagnostic device intended for the quantitative

determination of total cholesterol in human plasma and serum using the Eon 100 Clinical Chemistry Analyzer. Cholesterol measurements are used in the diagnosis and treatment of disorders involving excess cholesterol in the blood and lipid and lipoprotein metabolism disorders.

3. Special conditions for use statement(s):

For in vitro diagnostic use

For prescription use

3. Special instrument requirements:

Eon 100 Clinical Chemistry Analyzer (previously cleared in k100060)

**I. Device Description:**

The Eon Cholesterol Reagent kit includes 4 x 23 mL bottles that contain the following:

- 0.25 mmol/L 4-aminoantipyrine
- 10.0 mmol/L Hydroxybenzoic acid (HBA)
- 200 U/L cholesterol oxidase (microbial)
- 500 U/L cholesterol esterase (microbial)
- 600 U/L peroxidase (horseradish)

As well as, buffer, surfactants, and other ingredients including sodium azide

Handle and dispose of all human source materials as though capable of transmitting infectious agents using the universal precautions recommended by the Centers for Disease Control and Prevention (CDC).

**J. Substantial Equivalence Information:**

1. Predicate device name(s):

Roche Cholesterol (CHOL) Reagent

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

k952127

3. Comparison with predicate:

Item	Eon Cholesterol Reagent (Candidate Device)	Roche Cholesterol (CHOL) Reagent (Predicate - k952127)
Intended Use	The Cholesterol Reagent is an in vitro diagnostic device intended for the quantitative determination of total cholesterol in human plasma and serum	Same
Indications for Use	Cholesterol measurements are used in the diagnosis and treatment of disorders involving excess cholesterol in the blood and lipid and lipoprotein metabolism disorders.	Same
Methodology	Enzymatic (esterase/oxidase end-point)	Same
Patient Sample Type	Serum and Plasma	Same
Assay Range	6 – 775 mg/dL	3 – 800 mg/dL
On-board stability	28 days	Same
Limit of Detection	2 mg/dL	3 mg/dL
Reagent configuration	Single part liquid	Same

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

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

CLSI EP05-A2: Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline – Second Edition

CLSI EP09-A2-IR: Method Comparison and Bias Estimation using Patient Samples; Approved Guideline – Second Edition (Interim Revision)

CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline

**L. Test Principle:**

Free cholesterol and cholesterol produced from the enzymatic hydrolysis of cholesterol esters are oxidized in the presence of cholesterol oxidase. The resulting hydrogen peroxide reacts with Hydroxybenzoic acid (HBA) and 4-aminoantipyrine to produce a red quinoneimine dye in the presence of peroxidase. The red quinoneimine dye absorbs at 505 nm. The final

absorbance at this wavelength is proportional to the concentration of total cholesterol in the sample.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

a. *Precision/Reproducibility:*

Four levels of control serum were tested on the Eon 100 analyzer two times per run, two runs per day for 20 days (n = 80). The results are summarized in the tables below:

Sample 1 Mean 120.6 mg/dL Cholesterol	Within Run	Total
Standard Deviation, mg/dL	1.6	2.3
Coefficient of Variation, %	1.3	1.9

Sample 2 Mean 185.6 mg/dL Cholesterol	Within Run	Total
Standard Deviation, mg/dL	2.6	3.4
Coefficient of Variation, %	1.4	1.8

Sample 3 Mean 249.2 mg/dL Cholesterol	Within Run	Total
Standard Deviation, mg/dL	2.4	3.8
Coefficient of Variation, %	1.0	1.5

Sample 4 Mean 413.2 mg/dL Cholesterol	Within Run	Total
Standard Deviation, mg/dL	4.2	6.2
Coefficient of Variation, %	1.0	1.5

b. *Linearity/assay reportable range:*

Linearity studies were carried out using serial dilutions of a commercially available serum cholesterol concentrate. The range tested was 5.0 – 832.0 mg/dL. A polynomial fit analysis did not indicate statistically significant non linearity. The linear regression analysis is provided below:

Claimed Measuring Range	Intercept (95%CI) (mg/dL)	Slope (95% CI)
6 – 775 mg/dL	-0.7 (-5.4 – 4.0)	0.996 (0.989 – 1.006)

Based on the linearity data, the measuring range claim 6 – 775 mg/dL is supported.

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

Reagent Traceability

A Cholesterol Reference Method Laboratory Network (CRMLN) Certificate of Traceability for the Eon Cholesterol Reagent was provided.

Reagent Stability

Closed vial/Shelf life

Accelerated heat stress stability studies have concluded that the reagent is stable for 21 months when stored at 2 – 8 °C. Initial shelf-life claims will be for 18 months; however, real-time studies are on-going and the final claim will be reflected by real-time results. Closed vial/Shelf life protocols and acceptance criteria were reviewed and found to be acceptable.

Open vial/On-board Stability

Open vial/On-board stability protocols and acceptance criteria were reviewed and found to be acceptable. The Eon Cholesterol Reagent demonstrated on-board stability of 28 days.

Eon calibrators, as referenced in the package insert, were cleared in k110394 under the name ATAC Serum Calibrator (Direct Bilirubin and Iron)

Eon controls, as referenced in the package insert, were cleared in k111063 under the name Vital Serum Controls

*d. Detection limit:*

Protocols for the determination of the limit of blank (LoB) and the limit of detection (LoD) were performed in accordance with the recommendations in the CLSI Guideline EP17-A. Testing was carried out using true blanks and low level samples (total 60 each) on two Eon 100 analyzers. The calculated LoB of 0.70 mg/dL and LoD of 1.5 mg/dL support a LoB and LoD claim of 1 and 2 mg/dL, respectively. The measuring range of the assay is 6 to 775 mg/dL.

e. *Analytical specificity:*

Interference studies were performed by using serum pools containing cholesterol (2 levels) with individual interferents at a range of concentrations. The sera were assayed for cholesterol ( $n \geq 3$  replicates) and the mean result calculated. Interference was considered to be significant by the sponsor if the analyte recovery exceeded  $\pm 10\%$ . The results were obtained on the Eon 100 analyzer.

Interferent	Level 1		Level 2		Interference Claim (mg/dL)
	Cholesterol Conc. (mg/dL)	No interference at this level of interferent (mg/dL)	Cholesterol Conc. (mg/dL)	No interference at this level of interferent (mg/dL)	
Hemoglobin	75	500	230	1000	No interference up to 500
Lipemia	66	1000	208	1000	No interference up to 1000
Bilirubin, conjugated	143	15	242	7.5	No interference up to 7.5
Bilirubin, unconjugated	140	7.5	235	7.5	No interference up to 7.5
Ascorbic acid	144	5	229	10	No interference up to 5
Gentisic acid	164	117 ( $\mu\text{mol/L}$ )	254	117 ( $\mu\text{mol/L}$ )	No interference up to 117 ( $\mu\text{mol/L}$ )
Dopamine	153	5.9 ( $\mu\text{mol/L}$ )	251	5.9 ( $\mu\text{mol/L}$ )	No interference up to 5.9 ( $\mu\text{mol/L}$ )

f. *Assay cut-off:*

- Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

Studies were carried out according to CLSI EP09-A2-IR. Serum samples (n=101) were assayed in parallel by both the test (Y) and predicate (X) methods. The results were analyzed by using both Least squares and Deming regression. The range tested 10 – 750 mg/dL. Altered samples were included in the study.

Linear Regression	Range (Predicate)	n	Slope	Intercept	R	Standard error est.
Least squares	10 – 750 (mg/dL)	10	0.995 (0.979 – 1.011)	3.422 (-0.630 – 7.474)	0.9968	10.294
Deming		1	0.998 (0.982 – 1.012)	2.722 (-1.33 – 6.777)		

*b. Matrix comparison:*

Parallel drawn (matched) samples from 68 individuals were collected as serum and anti-coagulated plasma (Lithium heparin. The paired samples for each individual were assayed in parallel with Eon cholesterol reagent on the Eon 100 analyzer. The results were analyzed by using both Least squares and Deming regression. The range tested 14 - 680 mg/dL. Altered samples were included in the study.

Linear Regression	Range (serum)	n	Slope	Intercept	R	Standard error est.
Least squares	14 - 680 (mg/dL)	68	0.965 (0.943 – 0.987)	5.389 (0.362 – 10.416)	0.9956	9.926
Deming			0.969 (0.947 – 0.992)	4.576 (-0.456 – 9.608)		

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:

For cholesterol, the National Cholesterol Education Program (NCEP) recommends classifying patient results as being desirable, borderline or high based on the following cut-off thresholds established by the Adult Treatment Panel (ATP) III:

Desirable: < 200 mg/dL  
Borderline: 200 – 239 mg/dL  
High:  $\geq$  240 mg/dL

**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.