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 **Title:** Cholesterol

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| **Author:** | **Effective Date:***Note: The Effective Date is assigned after all approval signatures are obtained* | **Supersedes Procedure #** |
| S. Baker |  | New |

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| **Revised By:** | **Date Revised** | **Effective (adopted) Date:***Note: The Effective Date is assigned after all approval signatures are obtained* |
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**REVISION HISTORY**

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| **Procedure #** | **Revision Date** | **Reason for Revision** |
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# TITLE:

1. Purpose

Cholesterol is a steroid with a secondary hydroxyl group in the C3 position. It is synthesized in many types of tissue, but particularly in the liver and intestinal wall. Approximately three quarters of cholesterol are newly synthesized and a quarter originates from dietary intake. Cholesterol assays are used for screening for atherosclerotic risk in the diagnosis and treatment of disorders involving elevated cholesterol levels as well as lipid and lipoprotein metabolic disorders.

Cholesterol analysis was first reported by Liebemann in 1885 followed by Burchard in 1889. In the Liebemann-Buchard reaction, cholesterol forms a blue-green dye from polymeric unsaturated carbohydrates in an acetic acid/acetic anhydride/concentrated sulfuric acid medium. The Abell and Kendall method is specific for cholesterol, but is technically complex and requires the use of corrosive reagents. In 1974, Roeschlau and Allain described the first fully enzymatic method. This method is based on the determination of Δ4-cholestenone after enzymatic cleavage of the cholesterol ester by cholesterol esterase, conversion of cholesterol by cholesterol oxidase, and subsequent measurement by the Trinder reaction of the hydrogen peroxide formed. Optimization of ester cleavage (>99.5%) allows standardization using primary and secondary and a direct comparison with the CBC and NIST reference methods. Nonfasting sample results may be slightly lower than fasting results.

The Roche cholesterol assay meets the 1992 National Institutes of Health (NIH) goal of less than or equal to 3% for both precision and bias.

1. TeST PRINCIPLE

 Enzymatic, colorimetric method.

Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids. Cholesterol oxidase then catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. Un the presence of peroxidase, the hydrogen peroxide formed effect the oxidative coupling of phenol and 4-aminoantipyrine to form a red quinone-imine dye.

CE

 cholesterol esters +H20 cholesterol + RCOOH

.

CHOD

cholesterol + O2 cholest-4-en-3-one + H2O2

POD

2 H2O2 + 4-AAP + Phenol quinone-imine dye + 4 H20

The color intensity of the dye formed is directly proportional to the cholesterol concentration. It is determined by measuring the increase in absorbance at 512 nm.

1. SCOPE

In Vitro diagnostic test for the quantitative determination of total cholesterol concentration in the human serum and plasma on COBAS INTEGRA systems

1. RESPONSIBILITIES

 Example:

|  |  |
| --- | --- |
| **Roles** | **Responsibilities** |
| Quality Assurance | Supports the process including provide leadership and/or assistance in support of the process.Review and approval of procedure (site dependent). |
| Medical Director | Supports the development of the document.Review and approval of the document. |
| Management | Review and approve the document.Ensure that procedure is followed. |
| Laboratory Technical staff | Follows procedure. |

1. **ACRONYMS/DEFINITIONS**

Example:

|  |  |
| --- | --- |
| URMC | University of Rochester Medical Center |
| SW | Strong West |
| HH | Highland Hospital |
| RR | Ridgeland Road Laboratory |
| SMH | Strong Memorial Hospital |
| CHOL | Cholesterol |

1. **SPECIMENS**

For specimen collection and preparation only use suitable tubes or collection containers. Only the specimens listed below were tested and found acceptable.

Serum

Plasma: Li-heparin, K2- and K3- EDTA plasma

Refer to SW.CP.GL.jad.0101 for sample stability.

1. **QUALITY CONTROL**

Analyze quality control materials as indicated on the Roche Integra analyzer set up form SW.CP.GL.frm.0101

1. **SPECIAL SAFETY PRECAUTIONS**

Exercise the normal precautions required for handling all laboratory reagents and biohazardous patient samples. Refer to Safety data sheets. Disposal of all waste material should be in accordance with local guidelines. Refer to Safety procedure SW.CP.GL.adm.0005

**VIII. MATERIALS**

**A. Equipment**

Roche Integra 400 Plus analyzer

 Data Innovations Middleware

 Bio-Rad Unity Real Time QC Application

**B. Supplies**

Roche Sample cups

Falcon tubes

Pipets

Pipet tips

**C. Reagents**

 Cholesterol Gen 2. (Ref # 03039773190) – ready for use

 Components:

R – PIPESa buffer: 225 mmol/L, pH 6.8;Mg2+: 10 mmol/L; sodium cholate: 0.6 mmol/L; 4-aminoantipyrine: ≥0.45 mmol/L; phenol: ≥12.6 mmol/L; fatty alcohol polyglycol ether: 3%; cholesterol esterase (Pseudomonas spec.): ≥25 ukat/L (≥1.5 U/mL); cholesterol oxidase (E. coli): ≥7.5 ukat/L (≥0.45 U/mL); peroxidase (horseradish): ≥12.5 ukat/L (≥0.75 U/mL); stabilizers; preservative

a) PIPES = Piperazine-1, 4-bis(2-ethanesulfonic acid

R is position B

Shelf life at 2-8°C see expiration date on cobas c pack label

On-board in use at 10-15°C 8 weeks

D. Calibrator

Calibrator C.f.a.s.

 Use deionized water as zero calibrator.

Calibration mode Linear regression

Calibration replicate Duplicate recommended

Calibration interval Each lot and as required following quality control procedures

Traceability: This method has been standardized by ID-MSb and also according to Abell-Kendall.

This complies with the requirements of the National Institutes of Standards and Technology (NIST)

b) Isotope dilution – mass spectrometry

1. **PROCEDURE**

Refer to general Integra 400 PLUS analyzer operating procedure SW.CP.GL.lab.0101

Test Definition:

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| --- | --- |
| Measuring mode | Absorbance |
| Abs. calculation mode | Endpoint |
| Reaction mode | R-S |
| Reaction direction | Increase |
| Wavelength A/B | 512/659 nm |
| Calc. first/last | 17/98 |
| Unit | mmol/L |

Pipetting Parameters:

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| --- | --- | --- |
|  |  | Diluent (H2O) |
| R | 47 µL | 73 uL |
| Sample | 2 µL | 20 µL |
|  |  |  |
| Total volume | 142 µL |  |

**IX. LIMITATIONS**

Drugs: No interference was found at therapeutic concentrations using common drug panels. 17,18

Acetaminophen intoxications are frequently treated with N-acetylcysteine. N-acetylcysteine at therapeutic concentration when used as an antidote and the acetaminophen metabolite N-acetyl-p-benzoquinone imine (NAPQI) independently may cause falsely low cholesterol results.

Venipuncture should be performed prior to the administration of metamizole. Venipuncture immediately after or during the administration of metamizole may lead to falsely low results.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.11

For diagnostic purposes, the results should always be assessed in conjunction with the patient’s medical history, clinical examination and other findings.

Refer to SW.CP.GL.jad.0102 for the chart indicating at what Roche H, I, L indice level the test is affected if any.

**X. CALCULATIONS**

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample.

**XI. MEASURING RANGE AND DILUTIONS**

Refer to the Roche Range Chart for the measuring range and manual dilution guidelines (SW.CP.GL.jad.0104).

**XII. INTERPRETATION**

Refer to Reference Range guide for age appropriate reference ranges and critical value levels (SW.CP.GL.jad.0103).

**XIIII. RESULT REPORTING**

Results are generally reported via the DI Middleware-refer to procedure SW.CP.GL.lab.0103.

**XIV. TRAINING**

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| **Role** | **Training Needed** |
| Management | Read procedure |
| Employees | Read procedure |

**XV. REFERENCES**



