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**Title:** HDL - 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

High density lipoproteins (HDL) are responsible for the reverse transport of cholesterol from the peripheral cells to the liver. In the liver, cholesterol is transformed to bile acids which are then excreted into the intestine via the biliary tract.

Monitoring of the HDL-cholesterol in serum or plasma is of clinical relevance as the HDL-cholesterol concentration is important in the assessment of the atherosclerotic risk. Elevated HDL-cholesterol concentrations protect against coronary heart disease (CHD), whereas reduced HDL-cholesterol concentrations, particularly in conjunction with elevated triglycerides, increase cardiovascular risk.

A variety of methods are available to determine HDL-cholesterol, including ultracentrifugation (reference method in combination with cholesterol measurement by the Abell-Kendall method), electrophoresis, HPLC, precipitation and direct methods. Of these, the direct methods are used routinely. Roche HDLC4 cholesterol oxidase (CHOD) and peroxidase to form a colored pigment that is measured optically. The HDLC4 assay meets the 1998 National Institutes of Health (NIH)/National Cholesterol Education Program (NCEP) goals for precision and accuracy.

1. TeST PRINCIPLE

Homogeneous enzymatic colorimetric test.

Non-HDL lipoproteins such as LDL, VLDL, and chylomicrons are combined with polyanions and a detergent forming a water-soluble complex. In this complex the enzymatic reaction of CHER and CHOD towards non-HDL lipoproteins is blocked

Finally only HDL-particles can react with CHER and CHOD. The concentration of HDL-cholesterol is determined enzymatically by CHER and CHOD.

Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by CHER.

CHER

HDL-cholesterol esters +H20 HDL-cholesterol + RCOOH

In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ-cholestenone and hydrogen peroxide.

CHOO

HDL-cholesterol + O2 Δ-cholestenone + H2O2

In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-amino-antipyrine and EMSE to form a dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically.

Peroxidase

2 H2O2 + 4-amino-antipyrine + EMSE + H + H2) color pigment + 5 H2O

1. SCOPE

In Vitro diagnostic test for the quantitative determination of the HDL-cholesterol concentration in the human serum and plasma on Roche/Hitachi cobas c 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 |
| HDL | HDL-cholesterol |
| 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**

HDL-Cholesterol Gen 4. (Ref # 07528566190) – ready for use

Components:

R1 - TAPSOb buffer: 62.1 mmol/L, pH7.77; polyanion: 1.25g/L; EMSE: 1.08 mmol/L; ascorbate oxidase (cucurbita):≥50 ukat/L; peroxidase (horseradish): ≥166.7 ukat/L; detergent; BSA: 2.0 g/L; preservative

SR – Bis-Trisc buffer: 20.1 mmol/L, pH6.70; cholesterol esterase (microorganism): ≥7.5 ukat/L; cholesterol oxidase (recombinant E. coli): ≥7.17 ukat/L; cholesterol oxidase (microorganism): ≥76.7 ukat/L; peroxidase (horseradish): ≥333 ukat/L; 4-amino-antipyrine: 1.48 mmol/L; BSA 3.0 g/L; detergents; preservative

b) 2-Hydroxy-N-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid

c) Bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane

R1 is position B and SR is in position C

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

On-board in use at 10-15°C 12 weeks

D. Calibrator

Calibrator C.f.a.s. Lipids

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 against the CDC reference method (ultracentrifugation method)5 The standardization meets the requirements of the “HDL Cholesterol Method Evaluation Protocol for Manufacturers” of the US National Reference System for Cholesterol, CRMLN (Cholesterol Reference Method Laboratory Network), November 19949

1. **PROCEDURE**

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

Test Definition:

|  |  |
| --- | --- |
| Measuring mode | Absorbance |
| Abs. calculation mode | Endpoint |
| Reaction mode | R1-S-SR |
| Reaction direction | Increase |
| Wavelength A/B | 583/800 nm |
| Calc. first/last | 33/69 |
| Unit | mmol/L |

Pipetting Parameters:

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| --- | --- | --- |
|  |  | Diluent (H2O) |
| R1 | 120 µL |  |
| Sample | 2.5 µL | 7 µL |
| SR | 40 µL |  |
| Total volume | 169.5 µL |  |

**IX. LIMITATIONS**

Drugs: No interference was found at therapeutic concentrations using common drug panels. 9,10

Statins (Simvastatin) and fibrates (Bezafibrate) tested at therapeutic concentration ranges did not interfere.

N-acetylcysteine: No significant interference up to a N-acetylcysteine concentration of 450 mg/L (2.76 mm/L).

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 HDL-cholesterol results.

Metamizole: 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**



