#

 **Title:** Triglycerides

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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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| **Approval Signature** | **Approval Date** |
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**REVISION HISTORY**

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

1. Purpose

Triglycerides are esters of the trihydric alcohol glycerol with 3 long-chain fatty acids. They are partly synthesized in the liver and partly ingested in food.

The determination of triglycerides is utilized in the diagnosis and treatment of patients having diabetes mellitus, nephrosis, liver obstruction, lipid metabolism disorder and numerous other endocrine diseases.

The enzymatic triglycerides assay as described by Eggstein and Kreutz still required saponification with potassium hydroxide. Numerous attempts were subsequently made to replace alkaline saponification by enzymatic hydrolysis with lipase. Bucolo and David tested a lipase/protease mixture; Wahlefeld used an esterase from the liver in combination with a particularly effective lipase from Rhizopus arrhizus for hydrolysis.

This method is based on the work by Wahlefeld using a lipoprotein lipase from microorganisms for the rapid and complete hydrolysis of triglycerides to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced then reacts with 4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase to form a red dyestuff (Trinder endpoint reaction). The color intensity of the red dyestuff formed is directly proportional to the triglyceride concentration and can be measured photometrically.

1. TeST PRINCIPLE

 Enzymatic, colorimetric method.

LPL

 Triglycerides + 3 H20 glycerol + 3 RCOOH

.

GK

 glycerol + ATP glycerol-3-phosphate + ATP

Mg++

GPO

Glycerol-3-phosphate + O2 dihydroxyacetone phosphate + H2O2

peroxidase

H2O2 + 4-aminophenazone + 4-chlorophenol 4-(pbenzoquinone-monoimino)-phenazone + 2 H2O + HCl

1. SCOPE

In Vitro diagnostic test for the quantitative determination of the triglycerides 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, EDTA plasma

EDTA tubes that are less than ½ full may cause a negative bias for triglycerides results

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**

 Triglycerides (Ref # 20767107322) – ready for use

 Components:

R – PIPESa buffer: 50 mmol/L, pH 6.8;Mg2+: 40 mmol/L; sodium cholate: 0.2 mmol/L; ATP: ≥1.4 mmol/L; 4-aminophenazone: ≥0.13 mmol/L; 4-chlorophenol: 4.7 mmol/L; LPL (microbial): ≥83ukat/L; GK (microbial): ≥ 3 ukat/L; GPO (microbial): ≥ 41 ukat/L; POD (horseradish): ≥1.6 ukat/L; preservative; stabilizers

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-MSa

a) Isotope dilution – mass spectrometry

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 | R-S |
| Reaction direction | Increase |
| Wavelength A/B | 512/659 nm |
| Calc. first/last | 17/42 |
| Unit | mmol/L |

Pipetting Parameters:

|  |  |  |
| --- | --- | --- |
|  |  | Diluent (H2O) |
| R | 120 µL |  |
| Sample | 2 µL | 28 µL |
|  |  |  |
| Total volume | 150 µL |  |

**IX. LIMITATIONS**

Drugs: No interference was found at therapeutic concentrations using common drug panels. 12,13 Exceptions: Ca-Dobesilate, L-α-Methyldopa, Levodopa, and Phenylbutazone cause artificially low triglycerides values at the tested drug level. Dicynone (

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**



