**Title:** Ferritin

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| **Author:** | **Effective Date:***Note: The Effective Date is assigned after all approval signatures are obtained* | **Supersedes Procedure #** |
| Sue 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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# TITLE:

1. Purpose

Ferritin is a macromolecule with a molecular weight of at least440 kD (depending on the iron content) and consist of a protein shell (apoferritin) of 23 subunits and an iron core containing an average of approximately 2050 Fe3+ ions (in liver and spleen ferritin).1

Ferritin tends to form oligomers, and when it is present in excess in the cells of the storage organs there is a tendency for condensation to semicrystalline hemosiderin to occur in the lysosomes.

At least 20 isoferritins can be distinguished with the aid of isoelectric focusing.2 The microheterogeneity is due to differences in the contents of the acidic H and weakly basic L subunits. The basic isoferritins are responsible for the long-term iron storage function, and are found mainly in the liver, spleen, and bone marrow. 1,3

Acidic isoferritins are found mainly in the myocardium, placenta, and tumor tissue. They have a lower iron content and presumably function as intermediaries for the transfer of iron in various syntheses.4,5,6

The determination of ferritin is a suitable method for ascertaining the iron metabolism situation. Determination of ferritin at the beginning of therapy provides a representative measure of the body’s iron reserves. A storage deficiency is the reticulo-endothelial system (RES) can be detected at a very early stage.

Clinically, a threshold value of 20 µg/L (ng/mL) has proved useful in the detection of prelatent iron deficiency. This value provides a reliable indication of exhaustion of the iron reserves that can be mobilized for hemoglobin synthesis. Latent iron deficiency is defined as a fall below the 12 µg/L (ng/mL) ferritin threshold. These two values necessitate no further laboratory elucidation, even when the blood picture is still morphologically normal. If the depressed ferritin level is accompanied by hypochromic microcytal anemia, then manifest iron deficiency is present.1

When the ferritin level is elevated and the possibility of a distribution disorder can be ruled out, this is a manifestation of iron overloading in the body. 400 µg/L (ng/mL) ferritin is used as the Threshold value. Elevated ferritin values are also encountered with the following tumors: acute leukemia, Hodgkin’s disease and carcinoma of the lung, colon, liver and prostate. The determination of ferritin has proved to be of value in liver metastasis. Studies indicate that 76% of all patients with liver metastasis have ferritin values above 400 µg/L (ng/mL). Reasons for the elevated values could be cell necrosis, blocked erythropoiesis or increased synthesis in tumor tissue.

Two monoclonal mouse antibodies – M-4.184 and M-3.170 – are used to form the sandwich complex in the assay.

1. TEST PRINCIPLE

Sandwich principle. Total duration of assay: 18 minutes.

* 1st incubation: 10 uL of sample, a biotinylated monoclonal ferritin-specific antibody, and a monoclonal ferritin-specific antibody labeled with a ruthenium complexa) form a sandwich complex.
* 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
* The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
* Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.
1. SCOPE

Immunoassay for the in vitro quantitative determination of ferritin in human serum and plasma.

The electrochemiluminescence immune assay “ECLIA” is intended for use on Elecsys and cobas e immunoassay analyzers.

1. RESPONSIBILITIES

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| **Roles** | **Responsibilities** |
| Quality Assurance | Supports the process including provide leadership and/or assistance in support of the process.Review and approval of procedure  |
| 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**

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| URMC | University of Rochester Medical Center |
| VB12 | Vitamin B12 |
| SW | Strong West |
| RR | Ridgeland Road Laboratory |
| SMH | Strong Memorial Hospital |

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 collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, Na-heparin, K3-EDTA, and sodium citrate plasma. When sodium citrate is used, the results must be corrected by + 10%

Centrifuge samples containing precipitates before performing the assy. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators, and controls are at 20-25°C prior to measurement. Due to possible evaporation effects, samples, calibrators, and controls on the analyzers should be analyzed/measured within 2 hours.

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

1. **QUALITY CONTROL**

Analyze quality control materials as indicated on the Roche e411 analyzer set up form SW.CP.GL.frm.0102

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 cobas e 411 analyzer

 Data Innovations Middleware

 Bio-Rad Unity Real Time QC Application

**B. Supplies**

Roche Sample cups

Falcon tubes

Pipets

Pipet tips

**C. Reagents**

Elecsys Ferritin - (Ref # 03737551 160) – ready for use

 Components:

The reagent pack is labeled as FERR.

M – Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 – Anti-Ferritin-Ab~biotin (gray cap), 1 bottle, 10 mL: Biotinylated monoclonal anti-ferritin antibody (mouse) 3.0 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.

R2 – Anti-Ferritin-AB~Ru(bpy)32+ (black cap), 1 bottle, 10 mL: Monoclonal anti-ferritin antibody (mouse) labeled with ruthenium complex 6.0 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

Store the Elecsys reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Shelf life at 2-8°C see expiration date

After opening at 2-8°C 12 weeks

On-board 6 weeks onboard

D. Calibrator

Calibrator Ferritin CalSet

Traceability: This method has been standardized against the Ferritin assay (REF 11820982). The Ferritin assay (REF 11820982) has been standardized against the Enzymun-Test Ferritin method. This in turn has been standardized against the 1st International Standard (IS) NIBSC (National Institute for Biological Standards and Control) “Reagent for Ferritin (human liver)” 80/602

Recovery studies, including a published study9, to assess traceability of the Elecsys Ferritin assay to more recent international standards (2nd IS 80/578 and 3rd IS 94/572) have been conducted, with results showing very good agreement.

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

*Calibration frequency*: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

• after 2 months (8 weeks) when using the same reagent lot

• after 7 days (when using the same reagent kit on the analyzer)

• as required: e.g. quality control findings outside the defined limits

1. **PROCEDURE**

Refer to general cobas e411 analyzer operating procedure SW.CP.GL.lab.0102

**IX. LIMITATIONS**

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. >5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 2500 IU/mL.

There is no high-dose hook effect at ferritin concentrations of up to 100000 ug/L (ng/mL).

In vitro tests were performed on 19 commonly used pharmaceuticals and in addition on human erythropoietin. No interference with the assay was found.

Iron2+ and iron3+-ions at therapeutic concentrations do not interfere with the Elecsys Ferritin assay.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

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

**X. CALCULATIONS**

COBAS e411 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).

**XII. RESULT REPORTING**

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

**XIII. TRAINING**

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

**IVX. REFERENCES**



