**Title:** Folate

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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

Nutritional and macrocytic anemias can be caused by a deficiency of folate. This deficiency can result for diets devoid of raw fruits, vegetables or other foods rich in folic acid, as may be the case with chronic alcoholics, drug addicts, the elderly or persons of low socioeconomic status, etc. In addition, low serum folate during pregnancy has been associated with neural tube defects in the fetus.1Dietary deficiency and malabsorption are the major causes of folate deficiency in humans.2 Folate is necessary for normal metabolism, DNA synthesis and red blood cell regeneration. Untreated deficiencies may lead to megaloblastic anemia; it is advisable to determine the concentration of both vitamin B12 and folate in order to properly diagnose the etiology of anemia. Radioassays were first reported for folate in 19733,4,5,6

The majority utilize 125i-folate radiolabeled tracers and natural binding proteins (milk binding protein, folate binding protein). The various commercial assays differ in their free versus bound separation techniques and choice of specimen pretreatment. The Elecsys Folate assay employs a competitive test principle using natural folate binding protein (FBP) specific for folate. Folate in the sample competes with the added folate (labeled with biotin) for the binding sites on FBP (labeled with ruthenium complexa))

1. Tris(2,2’-bipyridyl)ruthenium(II)-complex(Ru(bpy)32+)
2. TEST PRINCIPLE

Competition principle. Total duration of assay: 27 minutes.

* 1st incubation: 25 uL of sample with the folate pretreatment reagents 1 and 2, bound folate is released from endogenous folate binding proteins.
* 2nd incubation: By incubating the pretreated sample with the ruthenium labeled folate binding protein, a folate complex is formed, the amount of which is dependent upon the analyte concentration in the sample.
* 3rd incubation: After addition of streptavidin-coated microparticles and folate labeled with biotin, the unbound sites of the ruthenium labeled folate binding protein become occupied, with formation of a ruthenium labeled folate binding protein-folate biotin complex. The entire 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

Binding assay for the in vitro quantitative determination of folate in human serum. The binding assay is intended for use on Elecsys and cobas e immunoassay analyzers. Folic acid measurements are used in the diagnosis and treatment of anemias.

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.

**NOTE: Hemolysis may significantly increase folate values due to high concentrations of folate in red blood cells. Therefore, hemolyzed samples are not suitable for use in this assay. Samples for folate determinations should be collected from fasting persons.**

Serum: Stable for 2 hours at 15-25°C, 2 days at 2-8°C, 4 weeks at (-15)-(-25)°C. Freeze only once. Protect from light. Store the samples at 2-8°C if they cannot be measured immediately.

Samples should not subsequently be altered with additives (biocides, anti-oxidants, or substances possibly changing the pH of the sample) in order to avoid erroneous folate recovery.

Centrifuge samples containing precipitates before performing the assy. Do not use heat-inactivated samples.

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.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

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 Folate III - (Ref # 07559992160) – ready for use

Components:

The reagent pack (M, R1, R2) and the pretreatment reagents (PT1, PT2) are labeled as FOL III.

PT1 – Pretreatment reagent 1 (white cap), 1 bottle, 4 mL: Sodium 2-mercaptpethanesulfonate (MESNA) 40 g/L, pH5.5.

PT2 – Pretreatment reagent 2 (gray cap), 1 bottle, 5 mL: Sodium hydroxide 25 g/L.

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

R1 – Folate binding protein~Ru(bpy)32+ (gray cap), 1 bottle, 9 mL: Ruthenium labeled folate binding protein 75 ug/L; human serum albumin (stabilizer); borate/phosphate/citrate buffer 70 mmol/L, pH5.5; preservative.

R2 – Folate~biotin (black cap), 1 bottle, 8 mL: Biotinylated folate 17 ug/mL; biotin 120 ug/L; human serum albumin (stabilizer); borate buffer 100 mmol/L, pH 9.0; 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 8 weeks

On-board 2 weeks onboard or 4 weeks when stored alternately in the refrigerator and on the analyzer, with the total time onboard the analyzers not exceeding 10 X 8 hours.

D. Calibrator

Calibrator Folate III CalSet

Traceability: This method has been standardized against the WHO International Standard NIBSC code: 03/178

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 1 month (28 days) 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 1000 IU/mL and samples from dialysis patients.

In vitro tests were performed on 16 commonly used pharmaceuticals and in addition on human erythropoietin. No interference with the assay was found.

It is contraindicated to measure samples of patients receiving therapy with certain pharmaceuticals, e.g. methotrexate or leucovorin, because of the cross-reactivity of folate binding protein with these compounds.

Samples with extremely high total protein concentrations (hyperproteinemia) are not suitable for use in this assay. Hyperproteinemia may be caused by, but not limited to, the following conditions: Lymphoma9, bone marrow disorders such as multiple myeloma, monoclonal gammopathy of undermined significance (MGUS), Waldenstrom macroglobulinemia, plasmocytoma10,11,12,13, Amyloidosis14,15,16,17. Respective samples may lead to the formation of protein gel in the assay cup, which may cause a run abort. The critical total protein concentration is dependent upon the individual sample composition,

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**



