**Title:** Vitamin B12

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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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**REVISION HISTORY**

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# TITLE:

1. Purpose

Vitamin B12, also referred to as cobalamin, is a complex organometallic compound in which a cobalt atom is situated within a corrin ring. It is a water-soluble vitamin which is synthesized by microorganisms. It cannot be synthesized in the human body and is seldom found in products of plant origin. Main sources of vitamin B12 are meat, fish, eggs, and dairy products.1 The uptake in the gastrointestinal tract depends on intrinsic factor, which is synthesized by the gastric parietal cells, and on the “cubam receptor” in the distal ileum. The most frequent cause of severe vitamin B12 deficiency is a lack of intrinsic factor due to autoimmune atrophic gastritis. The disease is historically called “pernicious anemia: even though many patients present with mainly neurologic manifestations. Examples of other causes for vitamin B12deficiency are malabsorption due to gastrectomy, inflammatory bowel disease or dietary deficiency, e.g. in strict vegetarians (vegans).2 Vitamin B12 is the cofactor for two enzymes, methionine synthase and methylmalonyl CoA mutase.2,3 Methionine synthase, located in the cytoplasm, requires vitamin B12 in the form of methylcobalamin and catalyzes the conversion of homocysteine to methionine, an essential amino acid. During the step a methyl group is transferred from methyltetrahydrofolate to the amino acid.3 This enzyme links the methylation pathway through synthesis of the methyl donor S-Adenosyl methionine and the pathway in which purine and pyrimidine are synthesized via generation of tetrahydrofolate.3 In the form of 5’-deoxyadenosylcobalamin, vitamin B12 is also required for the mitochondrial enzyme methylmalonyl CoA mutase, which converts methylmalonyl CoA to succinyl CoA. This step in the oxidation of odd-chain fatty acids and catabolism of ketogenic amino acids.3 Thus, vitamin B12 is important for DNA synthesis, regenerating methionine for protein synthesis and methylation, as well as for the development and initial myelination of the central nervous system (CNS) and for the maintenance of normal CNS function.2,3

Vitamin B12 deficiencies are common in wealthier countries principally among the elderly and the most prevalent in poorer populations. In general the prevalence increases with age.4,5

Vitamin B12 deficiency impacts red blood cell synthesis, resulting in megaloblastic anemia due to abnormal DNA synthesis.3 In addition it impairs neurological function, in particular demyelination of nerves in part due to abnormal methylation, leading to peripheral neuropathy, dementia, poor cognitive performance, and depression.3 Other effects of vitamin B12 deficiency or depletion are increased risk of neural tube defects, osteoporosis, cerebrovascular and cardiovascular diseases.3 Early diagnosis is essential, because of the latent nature of this disorder and the risk of permanent neurological damage.3,5

Generally, the primary test performed to confirm the diagnosis of vitamin B12 deficiency is measurement of serum vitamin B12 level.2 Recent publications suggest that in addition the following biomarkers should be measured to improve the specificity of diagnosis: folate, methylmalonic acid (MMA), homocysteine, and holotranscobalamin.2,5,6,7

The Elecsys Vitamin B12 II assay employs a competitive test principle using intrinsic factor specific for vitamin B12. Vitamin B12 in the sample competes with the added vitamin B12 labeled with biotin for the binding sites on the ruthenium-labeled intrinsic factor complexa).

a)Tris(2,2’-bipyridyl)ruthenium(II)-complex(Ru(bpy)32+)

1. TEST PRINCIPLE

Competition principle. Total duration of assay: 27 minutes.

* 1st incubation: By incubation the sample (15 uL) with the vitamin B12 pretreatment 1 and pretreatment 2, bound vitamin B12 is released.
* 2nd incubation: By incubating the pretreated sample with the ruthenium labeled intrinsic factor, a vitamin B12-binding protein 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 vitamin B12 labeled with biotin, the still vacant sites of the ruthenium labeled intrinsic factor become occupied, with formation of a ruthenium labeled intrinsic factor vitamin B12 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 vitamin B12 in human serum and plasma. Measurements obtained by this device are used in the diagnosis and treatment of anemias of gastrointestinal malabsorption.

The electrochemiluminescence immunoassay “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

Plasma: Na-heparin, Li-heparin, K2- and K3- EDTA plasma. Li-heparin plasma tubes containing separating gel can be used.

Centrifuge samples containing precipitates before performing the assy. Do not use any hemolyzed samples because samples showing visible signs of hemolysis will have falsely low results. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.

Vitamin B12 determinations should be performed on serum or plasma samples from fasting patients.

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.

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 Vitamin B12 II - (Ref # 07212771160) – ready for use

 Components:

The reagent pack (M, R1, R2) and the pretreatment reagents (PT1, PT2) are labeled as B12 II.

PT1 - Pretreatment reagent 1 (white cap), 1 bottle, 4 mL: Bithiothreitol 1.028 g/L; stabilizer, pH 5.5

PT2 – Pretreatment reagent 2 (gray cap), 1 bottle, 4 mL: Sodium hydroxide 40 g/L; sodium cyanide 2.205 g/L.

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

R1 – Intrinsic factor~Ru(bpy)32+ (gray cap), 1 bottle, 10 mL: Ruthenium labeled recombinant porcine intrinsic factor 4ug/L; cobinamide dicyanide 15 ug/L; stabilizer; human serum albumin; phosphate buffer, pH 5.5; preservative.

R2 – Vitamin B12~biotin (black cap), 1 bottle, 8.5 mL: Biotinylated vitamin B12 25 ug/L; biotin 3 ug/L; phosphate buffer, pH 7.0; 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 12 weeks

On-board 5 weeks or 60 days when stored alternatively in the refrigerator and on the analyzer, with the total time onboard on the analyzer not exceeding 10 x 8 hours

D. Calibrator

Calibrator Vitamin B12 CalSet

Traceability: This method has been standardized against the Elecsys Vitamin B12 assay. Accuracy to WHO Standard 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 1500 IU/mL.

In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

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.

Because intrinsic factor is typically used as the binding protein in serum vitamin B12 assays, anti-intrinsic factor antibodies (which are common in pernicious anemia) can lead to elevated vitamin B12 measurement values.2,11,12 The Elecsys Vitamin B12 II assay is designed to avoid interference due to anti-intrinsic factor antibodies.13

Samples with extremely high total protein concentrations (hyperproteinemia) are not suitable for use in this assay. Hyperproteinemia may be cause by, but not limited to, the following conditions: Lymphoma,14 bone marrow disorders such as multiple myeloma, monoclonal gammopathy of undetermined significance (MGUS), Waldenstrom macroglobulinemia, plasmocytoma,15,16,17,18 Amyloidosis.19,20,21,22 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 concentration.

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

Note: The presence of immunoglobulin-vitamin B12 complexes may cause unexpectedly high values of vitamin B12.23,24

**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**



