HEALTH HOLDING

HAFER ALBATIN HEALTH CLUSTER MATERNITY AND CHILDREN HOSPITAL

Department:	Laboratory and Blood Bank (Hormone)		
Document:	Internal Policy and Procedure		
Title:	Analysis of Vitamin B12 Level		
Applies To:	All Laboratory Staff		
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1. PURPOSE:

1.1 To illustrate the necessary steps required for performing Vitamin B12 Assay By-COBASe411.

2. DEFINITONS:

2.1 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.

3. POLICY:

3.1 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 catalyses the conversion of homocysteine to methionine, an essential amino acid. During this step a methyl group is transferred from methyltetrahydrofolate to the amino acid. 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 is a step in the oxidation of oddchain 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.

3.2 Vitamin B12 deficiency impacts red blood cell synthesis, resulting in megaloblastic anaemia due to abnormal DNA synthesis. 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. Other effects of vitamin B12 deficiency or depletion are increased risk of neural tube defects, osteoporosis, cerebrovascular and cardiovascular diseases. Early diagnosis is essential, because of the latent nature of this disorder and the risk of permanent neurological damage.

3.3 Generally, the primary test performed to confirm the diagnosis of vitamin B12 deficiency is measurement of serum vitamin B12 level. 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.

4. PROCEDURE:

4.1 **Principle :** Competition principle

4.2 **Specimen collection and preparation:** Serum collected using standard sampling tubes or tubes Na–heparin, Li–heparin, K2–EDTA and K3–EDTA plasma. Li–heparin plasma tubes containing separating gel can be used. Stable for 2 hours at 15°–25°C, 48 hours at 2°–8°C, 56 days at –20°C.

4.3 **Method:** See policy of loading sample on machine (Ref: Operative Manuals' of COBAS e411).

4.4 **Calculation:** The analyser automatically calculates the analyte concentration of each sample in pg/mL.
4.5 **Status:** Stat and Routine
4.6 **Reference ranges:** 197-771 pg/mL
4.7 **Limitations- interference:**
4.7.1 Samples showing visible signs of haemolysis may cause interference. Haemoglobin concentrations > 2 g/L (> 0.124 mmol/L) may lead to elevated results. The assay is unaffected by icterus (bilirubin < 1129 µmol/L or < 66 mg/dL), lipemia (Intralipid < 400 mg/dL) and biotin (< 287 nmol/L or < 70 ng/mL).
4.7.2 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.
4.7.3 For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings
4.8 **Measuring range:** 50.0–2000 pg/m
4.8.1 Values below the Limit of Blank are reported as < 50.0 pg/mL .
4.8.2 Values above the measuring range are reported as > 2000 pg/mL.

5. MATERIALS AND EQUIPMENT:

5.1 **Reagent:** For preparation see package insert
5.1.1 PT1 Pretreatment reagent 1 (white cap), 1 bottle, 4 mL: Dithiothreitol 1.028 g/L; stabilizer, pH 5.5
5.1.2 PT2 Pretreatment reagent 2 (gray cap), 1 bottle, 4 mL: Sodium hydroxide 40 g/L; sodium cyanide 2.205 g/L.
5.1.3 M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
5.1.4 R1 Intrinsic factor~Ru(bpy) (gray cap), 1 bottle, 10 mL: Ruthenium labeled recombinant porcine intrinsic factor 4 µg/L; cobinamide dicyanide 15 µg/L; stabilizer; human serum albumin; phosphate buffer, pH 5.5; preservative.
5.1.5 R2 Vitamin B12~biotin (black cap), 1 bottle, 8.5 mL: Biotinylated vitamin B12 25 µg/L; biotin 3 µg/L; phosphate buffer, pH 7.0; preservative.
5.2 **Calibration:**
5.2.1 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.
5.2.2 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).
5.2.3 Calibration interval may be extended based on acceptable verification of calibration by the laboratory
5.2.4 Renewed calibration is recommended as follows:
5.2.4.1 After 8 weeks when using the same reagent lot.
5.2.4.2 After 7 days when using the same reagent kit on the analyser.
5.2.4.3 As required: e.g. quality control findings outside the defined limits.
5.3 **Quality control:**
5.3.1 For quality control, use Preci Control varia. In addition, other suitable control material can be used.
5.3.2 Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

6. RESPONSIBILITIES:

6.1 Hormone shift on charge is responsible for, running calibration, control and samples of total vit B12.
6.2 Hormone staff are responsible for running total vit B12 samples every morning.

7. APPENDICES:

7.1 N/A

8. REFERENCES:

8.1 Operator's manual for the analyser
8.2 Company Leaflets of reagents

9. APPROVALS:

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