

Detection of Vitamin B12 in Human Serum and Plasma with Roche e801

Test Name:	Immunoassay for the in vitro quantitative determination of vitamin B12 in human serum and plasma.
Method Name:	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 complex.
Results:	Technical Range: 150-2000 pg/mL Reportable Range: 177-1033 pg/mL
Reference Ranges:	211-911 pg/mL
Clinical Significance:	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. 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 B12 deficiency are malabsorption due to gastrectomy, inflammatory bowel disease or dietary deficiency, e.g. in strict vegetarians (vegans).

Vitamin B12 is the cofactor for two enzymes, methionine synthase and methylmalonyl CoA mutase. 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 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. 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 odd chain fatty acids and catabolism of ketogenic amino acids. 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.

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

Vitamin B12 deficiency impacts red blood cell synthesis, resulting in megaloblastic anemia 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.

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.

Submission Criteria:

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 and K₂-EDTA plasma

Do not use fluoride plasma

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, therefore not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Storage and Stability: 56 days at -20°C
48 hours at 2-8°C

Rejection Criteria:

Rejection criteria include but are not limited to:

1. Specimens containing fibrin or clots.
2. Excessive platelet clumping
3. Leaking specimens
4. Substandard mixing or collection
5. Expired or improperly stored collection tubes.
6. Improperly filled tubes based on collection tube manufacturer's guidelines.
7. Contaminated specimens (IV fluid, foreign particles, etc.)
8. Specimens not analyzed within the appropriate time frame.
9. Samples not shipped at appropriate temperature.
10. Samples without 2 proper identifiers or samples having identifiers that do not match the electronic or paper lab requisition.

Authorization: Diagnostic testing can only be performed with approval from an authorized provider/agency.

Turn Around Time: 1 day.

Instructions for Serum Specimen Submission

General Information

The detection of vitamin B12 in human serum and plasma is performed using a Roche cobas i58 analyzer. However, serum specimens are preferred.

Specimens must be collected and stored at 2-8 °C to be analyzed within 48 hours and -20°C if to be analyzed within 56 days. Please be aware that storing specimens at $\leq -70^{\circ}\text{C}$ ($\leq -94^{\circ}\text{F}$) is not permissible.

Specimens MUST be received at Reditus Laboratories within 2 days of collection.

Collection Instructions for Serum Specimen

1. Do not use expired collection tubes. Store collection tubes as per manufacturers recommendations. Use standard venipuncture practices for collecting samples. Filled gold top serum tubes are preferred.
2. Ensure that the patient's name, date-of-birth, and time/date of collection are recorded on the specimen tube along with the name or initials of the individual collecting the sample.
3. Complete all the demographic information on a sample requisition form through the approved electronic submission process
4. Refrigerate the specimen between 2-8°C (36-46°F) and ship or courier the specimen(s) within 48 hours.
5. The specimen(s) *must* be received at the laboratory **no later than** 48 hours *from the time of collection*.
 - a. **Avoid shipping specimens over weekends or holidays** as they may not be received at the laboratory and cold-packs will not maintain the required 2-8°C (36-46°F) specimen temperature.
 - b. Ensure that specimens shipped by commercial carrier are shipped with **overnight delivery**. If shipping on a Friday for Saturday delivery, ***you must include Saturday Delivery*** during shipment, or the specimens will not be received until the following non-holiday business day. Failure to receive specimens within 24 hours of shipment will result in specimens being rejected from testing.
6. For any questions pertaining to sample collection, storage, or shipping, please contact the Reditus Laboratories using the below contact information.

Instructions for Specimen Transport

1. **Messenger/Courier by ground transport.** Place specimen(s) into a biohazard labeled bag and seal securely. Place the test requisition(s) on the outside of the biohazard labeled bag. Place the sealed biohazard bag and test requisition(s) inside the shipping container. Place cold packs, which have been frozen for at least 24 hours, in the leak-proof outer container. The shipping container must be rigid, such as a Styrofoam cooler, and labeled with the UN 3373 Biological Substance Category B marking. Close securely.
2. **Commercial carrier by ground/air transport.** Place the specimen(s) inside a biohazard labeled bag and seal securely. Place the test requisition(s) on the outside of the biohazard labeled bag. Place the sealed bag and completed test requisitions(s) inside the outer shipping container. Place cold packs, which have been frozen for at least 24 hours, in the leak-proof outer container. Label the outer shipping container with Reditus Laboratories address listed below. Complete the return address section to include the name of the person shipping the package, business name and address, and a business phone number. The shipping container must include the UN3373 Biological Substance Category B marking.
3. *Ship specimens by overnight delivery* to the attention of Clinical Chemistry at Reditus Laboratories. This can be accomplished by use of local courier, shipping corporations or U.S. Postal Service.
 - a. **If specimens are shipped on a Friday for Saturday delivery, you must include/indicate Saturday delivery** during shipment, or the specimens will not be received until the following non-holiday business day. Failure to receive specimens within 24 hours of shipment will result in specimens being rejected from testing.

4. The specimen(s) must be received at the laboratory **no later than** 48 hours *from the time of collection* and 24 hours from the time of shipment. Do not ship specimens over weekends or holidays as they will not be received, and cold-packs will not maintain the required 2-8°C (36-46°F) specimen temperature.

NOTE: Testing may be delayed, or specimens may be considered UNSATISFACTORY if the above instructions are not followed or the requisition form is not filled out completely. If there are any questions about specimen collection, handling, or shipping please contact the Reditus Laboratories to speak with laboratory personnel.

Ship specimens by a local courier or overnight by commercial carrier to the designated laboratories indicated below.

Send to: Reditus Laboratories
200 Enterprise Drive
Pekin, IL 61554

Phone: (469) 498-0222

Website: <https://www.redituslabs.com/>