MAYO CLINIC LABORATORIES

Test Definition: B12

Vitamin B12 Assay, Serum

Overview

Useful For

Investigation of macrocytic anemia

Workup of deficiencies seen in megaloblastic anemias

Testing Algorithm

For more information see Vitamin B12 Deficiency Evaluation.

Special Instructions

- Vitamin B12 Deficiency Evaluation

Method Name

Immunoenzymatic Assay

NY State Available

Yes

Specimen

Specimen Type

Serum

Ordering Guidance

Ask patients if they have received a vitamin B12 injection or radiolabeled vitamin B12 injection within the last 2 weeks. Patient results will not reflect deficiency or malabsorption after recent B12 injection. If patient has received such an injection within the past 2 weeks, **this test should not be ordered**.

This test provides a measurement of serum vitamin B12 level only. For a more comprehensive workup, order ACASM / Pernicious Anemia Cascade, Serum, which initiates testing with measurement of vitamin B12. Depending on the vitamin B12 concentration, testing for intrinsic factor blocking antibody, gastrin, and methylmalonic acid may be added.

Necessary Information

Ask patients if they have received a vitamin B12 injection within the last 2 weeks. Patient results will not reflect deficiency or malabsorption after recent B12 injection. If patient has received an injection within the past 2 weeks, this test **should not be ordered**.

Specimen Required

Patient Preparation: This test should not be performed on patients who have received a vitamin B12 injection or radiolabeled vitamin B12 injection within the previous 2 weeks.

Collection Container/Tube:
Preferred: Serum gel
Acceptable: Red top
Submission Container/Tube: Plastic vial
Specimen Volume: 0.6 mL
Collection Instructions:
1. Serum gel tubes should be centrifuged within 2 hours of collection.
2. Red-top tubes should be centrifuged, and the serum aliquoted into a plastic vial within 2 hours of collection.

Forms
If not ordering electronically, complete, print, and send a Benign Hematology Test Request (T755) with the specimen

Specimen Minimum Volume
0.5 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	OK

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Serum	Refrigerated (preferred)	7 days	
	Frozen	90 days	

Clinical & Interpretive

Clinical Information
Vitamin B12 (cobalamin) is necessary for hematopoiesis and normal neuronal function. In humans, it is obtained only from animal proteins and requires intrinsic factor (IF) for absorption. The body uses its vitamin B12 stores very economically, reabsorbing vitamin B12 from the ileum and returning it to the liver; very little is excreted.

Vitamin B12 deficiency may be due to lack of IF secretion by gastric mucosa (eg, gastrectomy, gastric atrophy) or intestinal malabsorption (eg, ileal resection, small intestinal diseases).

Vitamin B12 deficiency frequently causes macrocytic anemia, glossitis, peripheral neuropathy, weakness, hyperreflexia, ataxia, loss of proprioception, poor coordination, and affective behavioral changes. These manifestations may occur in any combination; many patients have the neurologic defects without macrocytic anemia.

Pernicious anemia is a macrocytic anemia caused by vitamin B12 deficiency that is due to a lack of IF secretion by gastric mucosa.

Serum methylmalonic acid and homocysteine levels are also elevated in vitamin B12 deficiency states.

Reference Values

180-914 ng/L

Interpretation

A serum vitamin B12 level less than 180 ng/L may cause megaloblastic anemia and peripheral neuropathies.

Vitamin B12 levels less than 150 ng/L are considered evidence of vitamin B12 deficiency. Follow-up with a test for antibodies to intrinsic factor (IFBA / Intrinsic Factor Blocking Antibody, Serum) is recommended to identify this potential cause of vitamin B12 malabsorption. For specimens without antibodies and the patient is symptomatic, follow-up testing for vitamin B12 tissue deficiency may be indicated. Consider analysis of methylmalonic acid (MMAS / Methylmalonic Acid, Quantitative, Serum) and/or homocysteine (HCYSP / Homocysteine, Total, Plasma).

Patients with serum vitamin B12 levels between 150 and 400 ng/L are considered borderline deficient and should be evaluated further by functional tests for vitamin B12 deficiency. Plasma homocysteine measurement (HCYSP / Homocysteine, Total, Plasma) is a good screening test where a normal level effectively excludes vitamin B12 and folate deficiency in an asymptomatic patient. However, the test is not specific, and many situations can cause an increased level. In contrast, an increased serum methylmalonic acid (MMAS / Methylmalonic Acid, Quantitative, Serum) level is more specific for cellular-level B12 deficiency and is not increased by folate deficiency.

In patients being evaluated for vitamin B12 deficiency who have intrinsic factor blocking antibodies (IFBA), false elevations of vitamin B12 may occur due to IFBA interference, potentially obscuring a physiological deficiency of vitamin B12. If observed vitamin B12 concentrations are discordant with clinical presentation, measurement of methylmalonic acid (MMAS / Methylmalonic Acid, Quantitative, Serum) should be considered.

For more information see Vitamin B12 Deficiency Evaluation.

Cautions

Patients who have received a vitamin B12 injection or radiolabeled vitamin B12 injection within the previous 2 weeks may have high serum vitamin B12 levels, which can interfere with this assay leading to falsely elevated results.

Many other conditions are known to cause an increase or decrease in the serum vitamin B12 concentration and should be considered in the interpretation of the assay results, including:

Increased serum vitamin B12	Decreased serum vitamin B12
Ingestion of vitamin C	Pregnancy
Ingestion of estrogens	Aspirin
Ingestion of vitamin A	Anticonvulsants
Hepatocellular injury	Colchicine
Myeloproliferative disorder	Ethanol ingestion
Uremia	Contraceptive hormones
	Smoking
	Hemodialysis
	Multiple myeloma

The evaluation of macrocytic anemia requires measurement of both vitamin B12 and folate levels; ideally, they should be measured simultaneously.

Some patients exposed to animal antigens, either in the environment or as part of treatment or imaging procedure, may have circulating anti-animal antibodies present. These antibodies may interfere with the assay reagents to produce unreliable results.

Clinical Reference

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3. Klee GG. Cobalamin and folate evaluation: measurement of methylmalonic acid and homocysteine vs vitamin B12 and folate. Clin Chem. 2000;46(8 Pt 2):1277-1283
4. Allen LH, Miller JW, de Groot L, et al. Biomarkers of nutrition for development (BOND): Vitamin B-12 review. J Nutr. 2018;148(suppl_4):1995S–2027S. doi:10.1093/jn/nxy201
5. Wolffenbuttel BHR, Wouters HJCM, Heiner-Fokkema MR, van der Klauw MM. The many faces of cobalamin (vitamin B12) deficiency. Mayo Clin Proc Innov Qual Outcomes. 2019;3(2):200-214. Published 2019 May 27. doi:10.1016/j.mayocpiqo.2019.03.002
6. Hannibal L, Lysne V, Bjorke-Monsen AL, et al. Biomarkers and algorithms for the diagnosis of vitamin B12 deficiency [published correction appears in Front Mol Biosci. 2017 Aug 08;4:53]. Front Mol Biosci. 2016;3:27. doi:10.3389/fmolb.2016.00027
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9. Bizzaro N, Antico A. Diagnosis and classification of pernicious anemia. Autoimmun Rev. 2014;13(4-5):565-568
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Performance

Method Description

The instrument used is a Beckman Coulter DXI 800. The Access Vitamin B12 assay is a competitive-binding immunoenzymatic assay. The sample is added to a reaction vessel along with alkaline potassium cyanide and dithiothreitol. This treatment denatures B12 binding proteins and converts all forms of vitamin B12 to the cyanocobalamin form. After neutralization, intrinsic factor-alkaline phosphatase conjugate and paramagnetic particles coated with goat-antimouse IgG:mouse monoclonal anti-intrinsic factor are added to the sample. Vitamin B12 in the sample binds to the intrinsic factor conjugate, preventing the conjugate from binding to the solid phase anti-intrinsic factor. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field, while unbound materials are washed away. A chemiluminescent substrate is added to the vessel, and the light generated by the reaction is measured with a luminometer. The light production is inversely proportional to the concentration of vitamin B12 in the sample. The amount of analyte in the sample is determined by means of a stored, multipoint calibration curve.(Package insert: Access Vitamin B12. Beckman Coulter, Inc; 04/2020)

PDF Report
No

Day(s) Performed
Monday through Saturday

Report Available
1 to 3 days

Specimen Retention Time
2 weeks

Performing Laboratory Location
Rochester

Fees & Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

Test Classification
This test has been cleared, approved, or is exempt by the US Food and Drug Administration and is used per manufacturer's instructions. Performance characteristics were verified by Mayo Clinic in a manner consistent with CLIA requirements.

CPT Code Information
82607

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
B12	Vitamin B12 Assay, S	2132-9

Result ID	Test Result Name	Result LOINC® Value
B12	Vitamin B12 Assay, S	2132-9