

Overview

Useful For

Investigation of macrocytic anemia

Workup of deficiencies seen in megaloblastic anemias

Testing Algorithm

For information see [Vitamin B12 Deficiency Evaluation](#).

Special Instructions

- [Vitamin B12 Deficiency Evaluation](#)

Method Name

Immunoenzymatic Assay

NY State Available

No

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.

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.

Supplies: Sarstedt Aliquot Tube, 5 mL (T914)

Collection Container/Tube:

Preferred: Serum gel

Acceptable: Red top

Submission Container/Tube: Plastic vial

Specimen Volume: 0.6 mL Serum

Collection Instructions:

1. Within 2 hours of collection, centrifuge the specimen.
2. For red-top tubes aliquot the serum into a plastic vial.

Forms

If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:

[-Kidney Transplant Test Request](#)

[-Benign Hematology Test Request \(T755\)](#)

Specimen Minimum Volume

Serum: 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 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 to 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 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 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 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.

Table. Conditions known to affect vitamin B12 concentration

| 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.

In rare cases, some individuals can develop antibodies to mice or other animals (often referred to as human anti-mouse antibodies [HAMA] or heterophile antibodies), which may cause interference in some immunoassays. Caution should be used in interpretation of results, and the laboratory should be alerted if the result does not correlate with the clinical presentation.

Clinical Reference

1. Babior BM. The megaloblastic anemias. In: Williams WJ, Beutler E, Lichtman MA et al, eds. Hematology. 5th ed. McGraw-Hill; 1995:471-490
2. Roberts NB, Taylor A, Sodi R. Vitamins and trace elements. In: Rifai N, Horvath AR, Wittwer CT, eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 6th ed. Elsevier; 2018:chap 37
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. Front Mol Biosci. 2016;3:27. Published 2016 Jun 27. doi:10.3389/fmolb.2016.00027
7. Green R, Kinsella LJ. Current concepts in the diagnosis of cobalamin deficiency. Neurology. 1995;45(8):1435-1440
8. Lahner E, Annibale B. Pernicious anemia: new insights from a gastroenterological point of view. World J Gastroenterol. 2009;15(41):5121-5128
9. Bizzaro N, Antico A. Diagnosis and classification of pernicious anemia. Autoimmun Rev. 2014;13(4-5):565-568
10. Toh BH. Pathophysiology and laboratory diagnosis of pernicious anemia. Immunol Res. 2017;65(1):326-330

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 photon 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 Friday, Sunday

Report Available

1 to 3 days

Specimen Retention Time

14 days

Performing Laboratory Location

Mayo Clinic Jacksonville Clinical Lab

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 |