MAYO CLINIC LABORATORIES

Test Definition: FB12

Vitamin B12 and Folate, Serum

Overview

Useful For

Investigation of macrocytic anemia

Workup of deficiencies seen in megaloblastic anemias

Investigation of suspected folate deficiency

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
B12	Vitamin B12 Assay, S	Yes	Yes
FOL	Folate, S	Yes	Yes

Testing Algorithm

For more information, see Vitamin B12 Deficiency Evaluation.

Special Instructions

- Vitamin B12 Deficiency Evaluation

Method Name

B12: Immunoenzymatic Assay

FOL: Competitive Binding Receptor Assay

NY State Available

No

Specimen

Specimen Type

Serum

Specimen Required

Patient Preparation:

1. Patient should be fasting for 8 hours.
2. **Do not order** on patients who have recently received methotrexate or other folic acid antagonists.

Collection Container/Tube:

Preferred: Red top

Acceptable: Serum gel

Submission Container/Tube: Plastic vial

Specimen Volume: 1 mL
Collection Instructions: Centrifuge and aliquot serum into a plastic vial.

Forms
If not ordering electronically, complete, print, and send a Benign Hematology Test Request Form (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
B12:
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.

Folate:
The term folate refers to all derivatives of folic acid. For practical purposes, serum folate is almost entirely in the form of

N-(5)-methyl tetrahydrofolate.(4)

Approximately 20% of the folate absorbed daily is derived from dietary sources; the remainder is synthesized by intestinal microorganisms. Serum folate levels typically fall within a few days after dietary folate intake is reduced and may be low in the presence of normal tissue stores. RBC folate levels are less subject to short-term dietary changes.

Significant folate deficiency is characteristically associated with macrocytosis and megaloblastic anemia. Lower than normal serum folate also has been reported in patients with neuropsychiatric disorders, in pregnant women whose fetuses have neural tube defects, and in women who have recently had spontaneous abortions.(5) Folate deficiency is most commonly due to insufficient dietary intake and is most frequently encountered in pregnant women or in alcoholics.

Other causes of low serum folate concentration include:
-Excessive utilization (eg, liver disease, hemolytic disorders, and malignancies)
-Rare inborn errors of metabolism (eg, dihydrofolate reductase deficiency, forminotransferase deficiency, 5,10-methylenetetra-hydrofolate reductase deficiency, and tetrahydrofolate methyltransferase deficiency)

Reference Values

VITAMIN B12
180-914 ng/L

FOLATE
> or =4.0 mcg/L
<4.0 mcg/L suggests folate deficiency

Interpretation

B12:

Concentration of vitamin B12 <180 ng/L may cause megaloblastic anemia and/or peripheral neuropathies.

Vitamin B12 concentrations <150 ng/L are considered evidence of vitamin B12 deficiency.

Vitamin B12 concentrations between 150 ng/L and 300 ng/L are considered borderline.

Follow-up testing for antibodies to intrinsic factor (IF) (IFBA / Intrinsic Factor Blocking Antibody, Serum) is recommended to identify this potential cause of vitamin B12 malabsorption.

For specimens without antibodies, follow-up testing of vitamin B12 tissue deficiency by measuring methylmalonic acid (MMA) (MMAS / Methylmalonic Acid [MMA], Quantitative, Serum) and/or homocysteine (HCYSP / Homocysteine, Total, Plasma) may be indicated if the patient is symptomatic.

A normal serum concentration of vitamin B12 does not rule out tissue deficiency of vitamin B12. The most sensitive test for vitamin B12 deficiency at the cellular level is the assay for MMA. If clinical symptoms suggest deficiency, measurement of MMA and homocysteine should be considered, even if serum vitamin B12 concentrations are normal.

Folate:

Serum folate is a relatively nonspecific test.(4) Low serum folate levels may be seen in the absence of deficiency and normal levels may be seen in patients with macrocytic anemia, dementia, neuropsychiatric disorders, and pregnancy disorders.

Results <4 mcg/L are suggestive of folate deficiency. The cut-off is based on consensus and was derived from the US NHANES III data.(5)

Evaluation of macrocytic anemias commonly requires measurement of the serum concentration of both vitamin B12 and folate; ideally they should be measured at the same point in time.

Additional testing with homocysteine and MMA determinations may help distinguish between B12 and folate deficiency states. In folate deficiency, homocysteine levels are elevated and MMA levels are normal. In vitamin B12 deficiency, both homocysteine levels and MMA levels are elevated.

For more information, see Vitamin B12 Deficiency Evaluation.

Cautions

B12:

Patients taking vitamin B12 supplementation may have misleading results.

Many other conditions are known to cause an increase or decrease in the serum vitamin B12 concentration including:

Increased Serum B12	Decreased Serum 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 who have been 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.

Folate:

Patients with combined deficiency of folate and iron may not demonstrate the erythrocyte macrocytosis that is typical of folate deficiency anemia. In these patients, however, the red cell distribution width (RDW) will typically be elevated.

Nonfasting specimens yield falsely elevated results.

Recent folic acid administration or dietary folate intake could result in normal or elevated values and possibly mask an

underlying folate deficiency.

Patients taking folate may have misleading results.

Folates other than *N*-(5)-methyltetrahydrofolate and folic acid antagonists (such as methotrexate) may, under some circumstances, be present in serum and will also be measured by this method.

Serum folate measurement is preferred over RBC folate measurement due to considerable analytic variability (coefficient of variation; CV) of assays. Both results give the same interpretation (internal Mayo study), therefore RBC folate quantitation is not recommended. Additional serum testing with homocysteine and methylmalonic acid (MMA) determinations may help distinguish between vitamin B12 and folate deficiency states. In folate deficiency, homocysteine levels are elevated and MMA levels are normal. In vitamin B12 deficiency, the analytic variability (CV) of both serum and RBC folate assays is considerable. Homocysteine and MMA levels are alternate determinates of folate deficiency.

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Supportive Data

See Individual Unit Codes

Clinical Reference

1. Babior BM: The megaloblastic anemias. In Hematology. Fifth edition. Edited by WJ Williams, E Beutler, MA Lichtman, et al. New York, McGraw-Hill Book Company, 1995, pp 471-490
2. Shenkin A, Baines M, Fell GS, et al: In Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. Edited by CA Burtis, ER Ashwood, DE Bruns. St. Louis, Elsevier, Inc., 2006, pp 1100-1105
3. Klee GG: Cobalamin and folate evaluation: measurement of methylmalonic acid and homocysteine vs vitamin B12 and folate. Clin Chem 2000 August;46(8 Pt 2):1277-1283
4. Fairbanks VF, Klee GG: Biochemical aspects of hematology. In Tietz Textbook of Clinical Chemistry. Edited by CA Burtis, ER Ashwood. Philadelphia, WB Saunders Company, 1999, pp 1690-1698
5. George L, Mills JL, Johansson AL, et al: Plasma folate levels and risk of spontaneous abortion. JAMA 2002 October 16;288:1867-1873
6. Benoist BD: Conclusions of a WHO Technical Consultation on folate and vitamin B12 deficiencies. Food and Nutrition Bulletin 2008(volume 29, number 2) S238-S244

Performance

Method Description

B12:

The instrument used is a Beckman Coulter DXI 800. The Access Vitamin B12 assay is a competitive-binding

immunoenzymatic assay. A 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. The chemiluminescent substrate Lumi-Phos 530 is added to the vessel and 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.(Beckman Coulter Assay Manual 2009, Beckman Coulter Inc, Fullerton, CA)

Folate:
The instrument used is a Beckman Coulter DXI 800. The Access Folate assay is a competitive-binding receptor assay. A serum sample is treated to release folate from endogenous binding proteins. After neutralization of the reaction mixture, folate-binding protein, mouse antifolate-binding protein, folic acid-alkaline phosphatase conjugate, and goat antimouse capture antibody coupled to paramagnetic particles are added to the reaction vessel. Folate in the sample competes with the folic acid-alkaline phosphatase conjugate for binding sites on a limited amount of folate-binding protein. Resulting complexes bind to the solid phase via mouse antifolate binding protein. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field, while unbound materials are washed away. The chemiluminescent substrate Lumi-Phos 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is inversely proportional to the concentration of folate in the sample. The amount of analyte in the sample is determined from a stored, multipoint calibration curve. The assay is standardized to the World Health Organization (WHO) International Standard 03/178.(Beckman Coulter Assay Manual 2011, Beckman Coulter Inc., Fullerton, CA)

PDF Report
No

Day(s) Performed
Monday through Friday, Sunday

Report Available
1 to 3 days

Specimen Retention Time
14 days

Performing Laboratory Location
Jacksonville

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-Vitamin B12
82746-Folate

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
FB12	Vitamin B12 and Folate, S	96805-7

Result ID	Test Result Name	Result LOINC® Value
B12	Vitamin B12 Assay, S	2132-9
FOL	Folate, S	2284-8