

## Overview

### Useful For

Diagnosis of pernicious anemia

Diagnosis of vitamin B12 deficiency-associated neuropathy

### Reflex Tests

Test ID	Reporting Name	Available Separately	Always Performed
IFBPA	Intrinsic Factor Blocking Ab, S	Yes, (order IFBA)	No
MMAPA	Methylmalonic Acid, QN, S	Yes, (order MMAS)	No
GASTR	Gastrin, S	Yes, (order GAST)	No

### Testing Algorithm

If the vitamin B12 concentration is less than 150 ng/L, then the intrinsic factor blocking antibody (IFBA) test is performed. If IFBA result is negative or indeterminate, then the gastrin test is performed.

If the vitamin B12 concentration is 150 to 400 ng/L, then the methylmalonic acid (MMA) test is performed. If the MMA result is greater than 0.40 nmol/mL, then the IFBA test is performed.

See [Vitamin B12 Deficiency Evaluation](#) in Special Instructions.

### Special Instructions

- [Vitamin B12 Deficiency Evaluation](#)

### Method Name

Immunoenzymatic Assay

### NY State Available

Yes

## Specimen

### Specimen Type

Serum

### Specimen Required

#### Patient Preparation:

1. This test should not be ordered on patients who have received a vitamin B12 injection within the last 2 weeks.
2. Patient should be fasting for 8 hours.
3. If medically feasible, proton pump inhibitor (omeprazole, lansoprazole, dexlansoprazole, esomeprazole,

pantoprazole, and rabeprazole) therapy should be discontinued 1 week before measurement of serum gastrin levels.

4. Drugs that interfere with gastrointestinal motility (eg, opioids) should be discontinued for at least 2 weeks before serum gastrin testing.

**Container/Tube:**

**Preferred:** Red top

**Acceptable:** Serum gel

**Specimen Volume:** 4 mL

**Collection Instructions:**

1. Divide specimen into 3 plastic vials, 1 containing 1 mL (label as PAGAS), 1 containing 1.5 mL (label as PAMMA), and 1 containing 1.5 mL (label as B12PA).

2. Band specimens together.

**Forms**

If not ordering electronically, complete, print, and send a [Benign Hematology Test Request Form](#) (T755) with the specimen.

**Specimen Minimum Volume**

2.3 mL

**Reject Due To**

Gross hemolysis	Reject
Gross lipemia	OK
Gross icterus	Reject

**Specimen Stability Information**

Specimen Type	Temperature	Time	Special Container
Serum	Frozen (preferred)	30 days	
	Refrigerated	24 hours	

**Clinical and Interpretive**

**Clinical Information**

[Vitamin B12 deficiency can be caused by many factors, one of which is pernicious anemia, a condition resulting in deficient production of intrinsic factor in the parietal cells of the stomach. Intrinsic factor is a protein that is needed to assist in the absorption of vitamin B12 into the small intestine. Vitamin B12 is converted into adenosylcobalamin, which converts L-methylmalonic acid to succinyl coenzyme A; hence, a decrease in vitamin B12 absorption in the intestine can cause an excess of methylmalonic acid within the body.](#)

Vitamin B12 deficiency may present with any combination of the following: macrocytic anemia, glossitis (painful inflammation of the tongue), peripheral neuropathy, weakness, hyperreflexia, ataxia, loss of proprioception, poor coordination, and affective behavioral changes. These manifestations may occur in any combination; many patients present with neurologic symptoms without macrocytic anemia.

A group of tests is often required to establish the correct diagnosis as determination of vitamin B12 in serum does not detect all cases of vitamin B12 deficiency. Mayo Clinic's Department of Laboratory Medicine and Pathology offers a diagnostic algorithm to expedite the identification of patients with vitamin B12 deficiency. This algorithm takes into account the following facts:

- The most sensitive test for vitamin B12 deficiency at the cellular level is the assay for methylmalonic acid (MMA).
- Nearly half of the cases of pernicious anemia can be unambiguously identified if the serum test for intrinsic factor blocking antibody is positive (this is a simpler and less expensive test than the MMA).
- Serum gastrin is usually markedly increased in pernicious anemia (as a result of gastric atrophy) and this test can be used as a substitute for the more complicated and more expensive Schilling test of intestinal absorption of vitamin B12.

The algorithm is similar to that published,<sup>(1)</sup> except that the serum gastrin assay is performed in place of the Schilling test. Experience with both Mayo Clinic and Mayo Clinic Laboratories' cases has corroborated that this is a cost-effective alternative to the Schilling test.

In our experience, greater than 90% of laboratory test costs can be saved by using the algorithm rather than ordering all of the services for a patient suspected of having B12 deficiency. Furthermore, the substitution of the serum gastrin assay for the Schilling test offers 3 advantages:

1. It is an in vitro test that does not require administration of radioisotopes to patients
2. It can be performed on mailed-in specimens
3. It is much less expensive

Only those tests that are appropriate, as defined by the algorithm, will be performed.

### Reference Values

180-914 ng/L

### Interpretation

Vitamin B12 >400 ng/L	Results do not suggest B12 deficiency-no further testing.
Vitamin B12 150 to 400 ng/L	Borderline vitamin B12 level-methylmalonic acid (MMA) is performed. If MMA is >0.40 nmol/mL, then intrinsic factor blocking antibody (IFBA) is performed.
Vitamin B12 <150 ng/L	Vitamin B12 deficiency-IFBA is performed. If IFBA is negative or indeterminate, then gastrin is performed.
MMA < or =0.40 nmol/mL	This value implies that there is no vitamin B12 deficiency at the cellular level.
IFBA positive	Consistent with pernicious anemia, Graves disease, or Hashimoto thyroiditis.

Gastrin >200 pg/mL	Result consistent with pernicious anemia.
Gastrin <200 pg/mL	Result does not suggest pernicious anemia.

[See Vitamin B12 Deficiency Evaluation](#) in Special Instructions.

## Cautions

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

Some patients exposed to animal antigens, either in the environment or as part of treatment or imaging procedure, may have circulating antianimal antibodies present. These antibodies may interfere with the vitamin B 12 assay reagents to produce unreliable results.

Some patients with other autoimmune diseases may have positive intrinsic factor blocking antibody (IFBA) assays without suffering from pernicious anemia (PA). This is reported particularly in patients with autoimmune thyroid disease or type I diabetes mellitus. In the validation of this assay, 24 individuals with these autoimmune endocrine diseases were tested and all were IFBA negative. However, 5 of 15 of patients with rheumatoid arthritis were IFBA positive during the validation of this assay. The literature suggests such individuals may, in fact, be at risk of later development of PA.

Since the IFBA test is competitive binding assay, the risk of heterophile antibody interference is low. During validation, 24 human antimouse antibody positive specimens and 25 specimens with other heterophile antibodies were tested and all were IFBA negative. However, if the clinical picture does not agree with the IFBA test result, the laboratory should be consulted for advice.

Isolated serum gastrin levels can only be interpreted in fasting patients; nonfasting specimens are uninterpretable.

Artificial hypergastrinemia may be observed in fasting patients who have undergone procedures that result in temporary gastric distention or dysmotility (eg, after gastroscopy).

Renal failure prolongs the serum half-life of gastrin and is associated with increased serum gastrin levels.

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**Clinical Reference**

1. Green R, Kinsella LJ: Current concepts in the diagnosis of cobalamin deficiency. *Neurology*. 1995 Aug;45(8):1435-1440
2. Lahner E, Annibale B: Pernicious anemia: new insights from a gastroenterological point of view. *World J Gastroenterol*. 2009 Nov 7;15(41):5121-5128
3. Bizzaro N, Antico A: Diagnosis and classification of pernicious anemia. *Autoimmun Rev*. 2014 Apr-May;13(4-5):565-568
4. Toh BH: Pathophysiology and laboratory diagnosis of pernicious anemia. *Immunol Res*. 2017 Feb;65(1):326-330

**Performance****Method Description**

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 vitamin 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 anti-mouse 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. (Package insert: ACCESS Vitamin B12. Beckman Coulter, Inc; 04/2020)

**PDF Report**

No

**Day(s) and Time(s) Test Performed**

Monday through Friday; 6 a.m.-12 a.m.

Saturday; 6 a.m.-6 p.m.

**Analytic Time**

Same day/1 day

**Maximum Laboratory Time**

4 days

**Specimen Retention Time**

2 weeks/3 months

**Performing Laboratory Location**

Rochester

**Fees and 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 Regional Manager. For assistance, contact [Customer Service](#).

**Test Classification**

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

**CPT Code Information**

82607-Vitamin B12 assay

82941-Gastrin (if appropriate)

83921-MMA (if appropriate)

86340-IFBA (if appropriate)

**LOINC® Information**

Test ID	Test Order Name	Order LOINC Value
ACASM	Pernicious Anemia Cascade	2132-9

Result ID	Test Result Name	Result LOINC Value
B12PA	Vitamin B12 Assay, S	2132-9