Test Definition: ACASM

Pernicious Anemia Cascade

Overview

Useful For

Diagnosis of pernicious anemia
Diagnosis of vitamin B12 deficiency-associated neuropathy

Testing Algorithm

If the vitamin B12 concentration is less than 150 ng/L, then the intrinsic factor blocking antibody (IFBA) test is performed at an additional charge.
If IFBA result is negative or indeterminate, then the gastrin test is performed at an additional charge.
If the vitamin B12 concentration is 150 to 400 ng/L, then the methylmalonic acid (MMA) test is performed at an additional charge.
If the MMA result is greater than 0.40 nmol/mL, then the IFBA test is performed at an additional charge.
See Vitamin B12 Deficiency Evaluation in Special Instructions.

Special Instructions

- Vitamin B12 Deficiency Evaluation

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
IFBPA	Intrinsic Factor Blocking Ab, S	Yes	No
MMAPA	Methylmalonic Acid, QN, S	Yes	No
GASTR	Gastrin, S	Yes	No

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.**

Specimen Required

Patient Preparation:

1. 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.

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.

Reject Due To

Gross hemolysis	Reject
Gross lipemia	OK
Gross icterus	Reject

Specimen Minimum Volume

2.3 mL

Specimen Stability Information

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

Clinical & 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,(1) 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.

Cautions

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

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. The 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. 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

Specimen Retention Time

2 weeks/3 months

Performing Laboratory Location

Rochester

Fees & Codes

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 US Food and Drug Administration.

CPT Code Information

82607-Vitamin B12 assay
82941-Gastrin (if appropriate)
83921-MMA (if appropriate)
86340-IFBA (if appropriate)