MAYO CLINIC LABORATORIES

# Test Definition: ACASM

Pernicious Anemia Cascade, Serum

## 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 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.

If the IFBA test is negative or indeterminate, then the gastrin test is performed at an additional charge.

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.**

## Specimen Required

**Patient Preparation:**

**1. Fasting: 12 hours, required**

2. For 12 hours before specimen collection, patient **should not** take multivitamins or dietary supplements (eg, hair, skin, and nail supplements) containing biotin (vitamin B7).

3. For 1 week before specimen collection, if medically feasible, patient **should not** take proton pump inhibitors (omeprazole, lansoprazole, dexlansoprazole, esomeprazole, pantoprazole, and rabeprazole).

4. For at least 2 weeks before specimen collection, patient **should not** take or receive drugs that interfere with gastrointestinal motility (eg, opioids).

**Collection Container/Tube:**

**Preferred:** Serum gel

**Acceptable:** Red top

**Submission Container/Tube:** Plastic vial

**Specimen Volume:** 3 mL serum

**Collection Instructions:**

1. Centrifuge, divide specimen into 3 plastic vials:

Vial 1 (B12PA): 1 mL of serum

Vial 2 (PAMMA): 1.5 mL of serum

Vial 3 (PAGAS): 0.5 mL of serum

2. Band specimens together and send frozen.

## Forms

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

## Specimen Minimum Volume

Serum: 1.6 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 & 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. 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 accounts for 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 simpler and less expensive than 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 by Green,(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 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. |

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 |

In rare cases, some individuals can develop antibodies to mouse or other animal antibodies (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.

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 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 HAMA 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.

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

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

### Clinical Reference

1. Green R, Kinsella LJ. Current concepts in the diagnosis of cobalamin deficiency. Neurology. 1995;45(8):1435-1440
2. Lahner E, Annibale. Pernicious anemia: new insights from a gastroenterological point of view. World J Gastroenterol. 2009;15(41):5121-5128
3. Bizzaro N, Antico A. Diagnosis and classification of pernicious anemia. Autoimmun Rev. 2014;13(4-5):565-568
4. Toh BH. Pathophysiology and laboratory diagnosis of pernicious anemia. Immunol Res. 2017;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; 07/2025)

### PDF Report

No

### Day(s) Performed

Monday through Saturday

### Report Available

Same day/1 to 4 days

### Specimen Retention Time

2 weeks

**Performing Laboratory Location**

Mayo Clinic Laboratories - Rochester Superior Drive

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

### CPT Code Information

82607
82941-if appropriate
83921-if appropriate
86340-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 |