

Overview**Useful For**

Confirming the diagnosis of pernicious anemia

Testing Algorithm

[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: For patients receiving vitamin B12 injections wait a minimum of 2 weeks after last injection before obtaining specimen.

Container/Tube:

Preferred: Serum gel

Acceptable: Red top

Specimen Volume: 1 mL

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)	14 days	
	Frozen	14 days	

Clinical and Interpretive

Clinical Information

The cobalamins, also referred to as vitamin B12, are a group of closely related enzymatic cofactors involved in the conversion of methylmalonyl-coenzyme A to succinyl-coenzyme A and in the synthesis of methionine from homocysteine. Vitamin B12 deficiency can lead to megaloblastic anemia and neurological deficits. The latter may exist without anemia, or precede it. Adequate replacement therapy will generally improve or cure cobalamin deficiency. Unfortunately, many other conditions, which require different interventions, can mimic the symptoms and signs of vitamin B12 deficiency. Moreover, even when cobalamin deficiency has been established, clinical improvement may require different dosages or routes of vitamin B12 replacement, depending on the underlying cause. In particular, patients with pernicious anemia (PA), possibly the commonest type of cobalamin deficiency in developed countries, require either massive doses of oral vitamin B12 or parenteral replacement therapy. The reason is that in PA patients suffer from gastric mucosal atrophy, most likely caused by a destructive autoimmune process. This results in diminished or absent gastric acid, pepsin and intrinsic factor (IF) production. Gastric acid and pepsin are required for liberation of cobalamin from binding proteins, while IF binds the free vitamin B12, carries it to receptors on the ileal mucosa, and facilitates its absorption. Most PA patients have autoantibodies against gastric parietal cells or intrinsic factor, with the latter being very specific but only present in approximately 50% of cases. By contrast, parietal cell antibodies are found in approximately 90% of PA patients, but are also found in a significant proportion of patients with other autoimmune diseases, and in approximately 2.5% (4th decade of life) to approximately 10% (8th decade of life) of healthy individuals.

Reference Values

Negative

Interpretation

The aim of the work-up of patients with suspected vitamin B12 deficiency is to first confirm the presence of deficiency and then to establish its most likely etiology.

Measurement of serum vitamin B12, either preceded or followed by serum methylmalonic acid measurement, is the first step in diagnosing pernicious anemia (PA). If these tests support deficiency, then intrinsic factor blocking antibody (IFBA) testing is indicated to confirm PA as the etiology. A positive IFBA test supports very strongly a diagnosis of PA. Since the diagnostic sensitivity of IFBA testing for PA is only around 50%, an indeterminate or negative IFBA test does not exclude the diagnosis of PA. In these patients, either PA or another etiology, such as malnutrition, may be present. Measurement of serum gastrin levels will help in these cases. In patients with PA, fasting serum gastrin is elevated to more than 200 pg/mL in an attempted compensatory response to the achlorhydria seen in this condition.

For a detailed overview of the optimal testing strategies in PA diagnosis, see ACASM / Pernicious Anemia Cascade, Serum and associated [Vitamin B12 Deficiency Evaluation](#) in Special Instructions.

Cautions

Patients who have received a vitamin B12 injection within the last 2 weeks may have high serum vitamin B12 levels, which can interfere with this assay leading to false-positive results. All positive intrinsic factor blocking antibody (IFBA) results that have not been ordered through the ACASM / Pernicious Anemia Cascade reflex to vitamin B12 measurement. If this yields a level above 800 ng/L, a comment is appended to the report indicating a possible false-positive result.

Some patients with other autoimmune diseases may have positive 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 this is a 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.

Clinical Reference

1. Toh BH, Van Driel IR, Gleeson PA: Pernicious anemia. *N Engl J Med* 1997;337:1441-1448
2. Klee GG: Cobalamin and folate evaluation: measurement of methylmalonic acid and homocysteine vs vitamin B12 and folate. *Clin Chem* 2000;46:1277-1283
3. Ward PC: Modern approaches to the investigation of vitamin B12 deficiency. *Clin Lab Med* 2002;22:435-445
4. Stabler SP, Allen RH: Vitamin B12 deficiency as a worldwide problem. *Ann Rev Nutr* 2004;24:299-326
5. Roberts NB, Taylor A, Sodi R: Chapter 37: Vitamins and trace elements. In *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*. Sixth edition. Edited by N Rafai, AR Horvath, CT Wittwer. Elsevier, 2018, pp 639-718
6. Bizzaro N, Antico A: Diagnosis and classification of pernicious anemia. *Autoimmun Rev* 2014;13(4-5):565-568

Performance

Method Description

The Access Intrinsic Factor Antibody assay is a competitive binding immunoenzymatic assay. A sample is added to a reaction vessel along with intrinsic factor alkaline phosphatase conjugate and a protein blocking solution. Intrinsic factor antibody in the sample binds to the intrinsic factor conjugate. After incubation in a reaction vessel, paramagnetic particles coated with a mouse monoclonal, specific for the vitamin B12 binding site on intrinsic factor, is added to the reaction. Intrinsic factor conjugate that has not been blocked by sample anti-intrinsic factor binds to the monoclonal antibody on the solid phase. After an additional incubation in the reaction vessel, materials bound to the solid phase are held in a magnetic field, while unbound materials are washed away. Chemiluminescent substrate 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 intrinsic factor antibody in the sample expressed in AU/mL (Antibody Units/mL). The amount of analyte in the sample is determined from a stored calibration. (Instruction manual: Beckman Coulter Assay. Beckman Coulter Inc; 2019)

PDF Report

No

Day(s) Performed

Monday through Saturday

Report Available

Same day/1 day to 4 days

Specimen Retention Time

2 weeks

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 has been cleared, approved or is exempt by the U.S. 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

86340

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
IFBA	Intrinsic Factor Blocking Ab, S	31444-3

Result ID	Test Result Name	Result LOINC Value
IFBLA	Intrinsic Factor Blocking Ab, S	31444-3
CMT31	Comment	48767-8