



Test Ordering Guidelines for Suspected Vitamin B12 and Folate Deficiency

SYNOPSIS AND RELEVANCE

Clinicians frequently order vitamin B₁₂ and folate levels to evaluate anemia and neuropsychiatric symptoms. Vitamin B₁₂ deficiency is more prevalent than folate deficiency in the general population as well as in anemic and hospitalized patients.¹ Automated chemistry concentration assays, combined with clinical correlation, may confirm or rule out a deficiency of B₁₂ or folate.¹ However, laboratory cut-offs for B₁₂ and folate deficiencies lack sensitivity and specificity,¹ and there is overlap of signs and symptoms between these deficiencies and many other illnesses. Pathologists can add value by reviewing peripheral blood smears, recommending and interpreting additional tests, and avoiding unnecessary testing.

INSIGHTS

Whom to test:

- Patients with incidentally discovered or symptomatic anemia without alternative explanations; absence of macrocytic MCV does not exclude B₁₂ or folate deficiency
- Patients with new neurologic signs and symptoms of unclear etiologies

What tests to perform:

- CBC (includes MCV and RDW), reticulocyte count, LDH, haptoglobin, total and indirect bilirubin, review of peripheral blood smear
- Plasma/serum total B₁₂ concentration
- Plasma/serum total folate concentration only when history supports a deficient diet or malabsorption comorbidities

Subsequent testing:

- When B₁₂ or folate concentrations are borderline or discordant with clinical judgement:
 - Metabolites that accumulate due to deficiencies of B₁₂ (MMA, HCY) or folate (HCY)
 - To confirm pernicious anemia: IF autoantibody

Tests that are rarely indicated:

- Plasma/serum folate in patients with adequate diet and no GI malabsorption symptoms
- RBC folate
- Active B₁₂

BACKGROUND

Vitamins B₁₂ and folate are essential cofactors in enzymatic pathways essential for DNA synthesis and other cell functions.^{2,3} A deficiency of either vitamin can cause impaired hematopoiesis, or neuropsychiatric disorders, while B₁₂ deficiency is more likely to cause progressive, and potentially irreversible, demyelination of dorsolateral spinal column white matter and peripheral nerves.⁴

Clinicians typically order B₁₂ and folate levels together in various clinical situations. However, differences in the vitamins' content in foods, gastrointestinal (GI) absorption, and prevalence of deficiencies should direct selective test ordering patterns guided by the patient's history.¹

Review of Vitamin B₁₂

Causes of B₁₂ deficiency are grouped by mechanism and clinical severity. Pernicious anemia, due to autoimmune gastritis and antibodies to IF, is the most common cause of severe B₁₂ deficiency, with an estimated prevalence of approximately 4.0% in older European and African adults.⁵ Other causes of severe deficiency include bariatric

surgery, ileal resection, and inflammatory bowel disease.⁶ Causes of mild B₁₂ deficiency include malabsorption of protein bound B₁₂, atrophic gastritis, metformin, and vegetarian/vegan diets.⁶ Nitrous oxide inactivates B₁₂ without affecting blood levels and chronic abuse raises Hcy and MMA levels and can have serious hematologic and neurologic sequelae.²

B₁₂ deficiency causes ineffective hematopoiesis and shortens red cell survival due to desynchrony between nuclear and cytoplasmic maturation. Maintenance of neuronal myelination is dependent upon vitamin B₁₂ as well. Clinical consequences of B₁₂ deficiency range from asymptomatic anemia to progressive fatigue and jaundice.² Neurologic symptoms are protean. The most common is peripheral paresthesia,⁴ which can progress to ataxia and paraplegia.¹ Additional symptoms include cognitive decline and psychiatric disorders.⁵ Hematologic and neurologic signs and symptoms of B₁₂ deficiency frequently arise and progress independently of each other.^{1,2,5,6}

Review of Folate

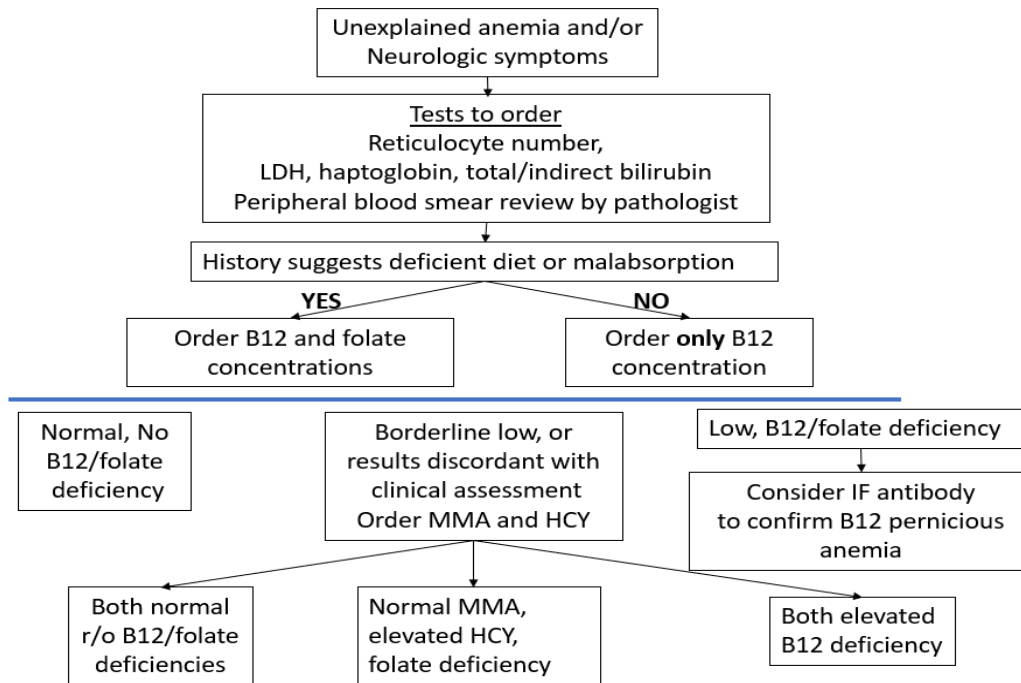
Insufficient dietary intake is the primary cause of folate deficiency, typically associated with alcohol use disorders and severe malnutrition.¹ Other causes include gastrointestinal malabsorption and increased folate turnover states, eg, pregnancy, chronic hemolytic anemias.³ The hematologic consequences of folate deficiency are identical to those of B₁₂ deficiency. Neuropsychiatric complications are similar to those that occur with B₁₂ deficiency, except peripheral neuropathies are rare.^{1,4}

Laboratory Investigation of B₁₂/Folate Deficiencies

Macrocytic anemia is a classic presentation of B₁₂ or folate deficiency.⁷ However, neither anemia nor macrocytosis is a sensitive screening test,² and a modest macrocytosis (mean cell volume [MCV] 100-115 fL) is not specific for B₁₂/folate deficiencies.¹ Peripheral blood smear findings may include hypersegmented neutrophils along with nucleated red cells, oval macrocytes and schistocytes, mimicking a microangiopathic hemolytic process.⁷ Bone marrow examination is not required to confirm B₁₂/folate deficiency anemia. However, if performed, findings of marked erythroid hyperplasia, megaloblastic maturation and atypia mimicking dysplasia may be misinterpreted as a hematologic clonal disorder.⁷

Automated serum/plasma B₁₂ and folate chemiluminescence assays are widely available. However, due to very low prevalence of folate deficiency from grain fortification, it is not necessary to measure folate in patients who have adequate diets and no evidence for malabsorption.¹ Many factors, both biologic and analytic, limit the sensitivities and specificities of B₁₂ and folate concentrations near the lower limit cut-offs.^{1,2} Whenever there is clinical uncertainty surrounding a low or "borderline-low normal" B₁₂/folate result, adjuvant tests of enzyme cofactor activities having higher sensitivities may be recommended,^{2,3,5} as shown in the following table.

Test \ Findings	B₁₂ Deficiency	Folate Deficiency	No B₁₂ or Folate Deficiency
Methylmalonic acid	Elevated	Normal	Normal
Homocysteine	Elevated	Elevated	Normal



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