

"A Pernicious Problem": K-Kase for K-A (Educational Exercise)

In the K-A mailing, we presented the following scenario and questions related to specimen K-03:

The patient is an 82-year-old woman who has been relatively well during most of her life. Over the past two years, however, her family has noticed some short-term memory loss and occasional confusion. She recently developed muscle weakness, primarily affecting her lower extremities. Before referring her to a neurologist, her primary care physician orders several routine laboratory tests including a complete blood count (which shows no evidence of anemia), a comprehensive metabolic panel (which shows no abnormalities), and a vitamin B₁₂ level.

Review the result your laboratory obtained for serum vitamin B₁₂ for specimen K-03. If the patient's physician were to call the lab to ask what additional testing might be able to definitively rule-out vitamin B₁₂ deficiency, what would be the best response?

- The patient's peripheral blood smear should be examined.
- The patient should have a Schilling test performed.
- The patient's specimen should be tested for methylmalonic acid.
- The patient's specimen should be tested for holo-transcobalamin.
- The patient has already been shown not to be vitamin B₁₂-deficient.

Vitamin B₁₂ is an essential nutrient produced only by bacteria, but found in most animal-derived foods. B₁₂ is a co-factor for the enzymatic reaction which converts homocysteine to methionine and also regenerates the form of folate required for DNA synthesis. The classical hematologic findings of B₁₂ deficiency are megaloblastic anemia with macroovalocytes and hypersegmented neutrophils. B₁₂ is also required for the conversion of methylmalonic acid to succinic acid, an important component of branched-chain amino acid catabolism. Therefore, B₁₂ deficiency also produces neurologic disturbances, including sensory (diminished positional and vibrational sensation) and motor (mild weakness and hyporeflexia) deficits. Psychological effects include confusion, depression, and paranoia ("megaloblastic madness").

Absorption of vitamin B₁₂ in the small intestine requires production of a protein called intrinsic factor (IF) in the stomach. After waltzing across the mucosa, the B₁₂-IF complex dissociates and B₁₂ changes partners to form a complex with plasma transcobalamin (TC). Only B₁₂ in this complex (known as holo-transcobalamin) can be utilized by peripheral tissues. Plasma B₁₂ carried by other proteins (haptocorrins) are not metabolically active.

B₁₂ deficiency afflicts an estimated 10-20% of Americans over 60 years of age. Predisposing factors include inadequate nutritional intake (vegans are particularly susceptible) and malabsorption caused by bowel resection, bacterial overgrowth, inflammatory bowel disease and the use of certain drugs (metformin, antacids, ethanol). The famous "pernicious anemia" is due to an autoimmune process affecting the stomach lining, which results in a lack of intrinsic factor production. This is also more common in the elderly.

So, vitamin B₁₂ deficiency was an appropriate consideration in this elderly woman with evidence of progressive dementia and muscle weakness. Almost 2000 laboratories reported B₁₂ results for specimen K-03; the all method mean was 240 pg/ml. Depending on the lower limit of their reference range, most labs should have considered this result to be either

slightly low or low normal. Over half of the laboratories reporting B₁₂ results responded to the question posed about the appropriate follow-up testing.

Should this patient's peripheral blood smear be examined?

This is not the best testing option to recommend. Negative results are inconclusive, and positive results are not specific for B₁₂ deficiency. Only approximately 6% of the respondents chose this answer.

While megaloblastic anemia generally is an early manifestation of B₁₂ deficiency, many patients develop neurologic deficits in advance of any hematologic findings. Biologically, B₁₂ and folate are closely linked, particularly in regards to the clinical development of megaloblastic anemia. One of the recognized perils of our current folate fortification of food (which has prevented many neural tube defects) is that the hematological signs of B₁₂ deficiency may be masked. It is clearly desirable to find and treat B₁₂ deficiency before the onset of irreversible neurologic deficits (which folate can not treat). This has raised two public health major questions: should B₁₂ also be supplemented, and/or should we screen more aggressively for B₁₂ deficiency?

Should the patient have a Schilling test performed?

This is not the best testing option to recommend. Negative results are inconclusive for B₁₂ deficiency, and further testing is complicated. Approximately 10% of the respondents chose this answer.

While not commonly performed today, the Schilling test (oral administration of labeled B₁₂ +/- IF along with injected, unlabeled B₁₂ to block hepatic uptake, followed by measurement of urinary excretion of labeled B₁₂) still has a role in the assessment of IF deficiency. Some have advocated the use of IF antibody assays as an alternative. Neither is effective at identifying problems with stomach acid production, which are more likely in elderly patients. Also, by virtue of the administered B₁₂, the Schilling test complicates further assessment of serum B₁₂ or related tests.

Should patient's specimen be tested for methylmalonic acid (MMA)?

MMA testing is a reasonable option to confirm the diagnosis of B₁₂ deficiency. Almost half of the respondents (47%) chose this answer.

MMA is a sensitive marker of B₁₂ deficiency but elevations can also be attributable to renal impairment, pregnancy, or thyroid disease. Homocysteine (HCY) elevations also occur in B₁₂ deficiency; the combination of normal MMA and HCY levels effectively excludes a diagnosis of B₁₂ deficiency. There is currently no commercially-available automated MMA assay that is FDA approved.

Should the patient's specimen be tested for holo-transcobalamin (hTC)?

hTC testing is also a reasonable test option. But only approximately 6% of the respondents chose this answer.

As hTC is the form of vitamin B₁₂ in the circulation correlating with absorption, hTC levels become abnormal long before total body B₁₂ deficiency (and long before total plasma or serum B₁₂ levels fall). The largest study to date indicates that hTC is a superior indicator of B₁₂ deficiency than total serum B₁₂, but hTC is still not highly specific. Like MMA, hTC can be also elevated in renal disease.

Has the patient already been shown not to be vitamin B₁₂-deficient?

Although one-third of the respondents chose this answer, further testing is probably recommended.

This exercise was designed to challenge your interpretation of a slightly low or low-normal vitamin B₁₂ level. The result is probably not low enough to definitively explain the patient's

condition but total B₁₂ measurements have limited predictive value, given the preceding discussion. Part of this may also be attributable to the low end inaccuracy and imprecision of B₁₂ assays. One must also be careful in interpreting published guidelines, as commercial assays are not well harmonized and reference ranges are not consistent among methods.

In summary, two-thirds of the respondents believed that further testing was indicated and, of these, most chose methylmalonic acid testing. Laboratories recommending this approach may wish to consider concurrent homocysteine testing as this increases the negative predictive value. As assays for methylmalonic acid and holotranscobalamin become more available, we may see a shift towards these newer tests.

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Read more about it! Johnson LE. Vitamin B₁₂. Merck Manual of Medicine (On-line Edition)
<http://www.merck.com/mmpe/sec01/ch004/ch004i.html?qt=b12&alt=sh>

NOTE: K-Kases are educational exercises using CAP proficiency testing specimens to mimic real-life laboratory situations. Participation is optional. Responses will not be graded and will not be included in the individual laboratory reports. Please give us feedback about whether you like this addition to the proficiency surveys and suggestions regarding how they can be improved.