Selenium

The definitive test for selenium deficiency remains the response to selenium supplementation.1 Of the available tests, blood glutathione peroxidase is one that has been shown to be a sensitive index of selenium nutrition.2

Hair selenium is also of value. It is correlated with selenium intake as well as with selenium concentrations in the liver, lung and renal cortex. However, the use of selenium-containing shampoos will falsely elevate hair selenium levels.3

Toenail selenium, while unaffected by dietary intake in the prior 3 months, is a reasonable measure of intake over the past 6 to 12 months.4 Blood selenium is of limited usefulness as, while it responds quickly to dietary intake,5 it tends to stay in the normal range except at the extremes of tissue selenium levels.5 Urinary selenium, although inadequate as an indicator of tissue nutrition, may be useful to confirm the results of blood selenium testing.7

Sodium

Serum sodium is commonly used as a measure of sodium nutrition. The principal cation of the extracellular fluid, any change in serum sodium is associated with a fluid shift into or out of the cell. Serum levels, however, do not reflect total body sodium content.8

Hair sodium does not reflect dietary intake or body stores.9

Thiamine

The most commonly used procedure for assessing thiamine nutrition has been the measurement of erythrocyte transketolase activity and its stimulation in vitro by the addition of thiamine pyrophosphate (the thiamine pyrophosphate effect). Because of the limitations of these two tests,10 both should be performed together, along with an assessment of thiamine intake.

If available, the erythrocyte thiamine diphosphate level may be superior to the measurement of erythrocyte transketolase activity.10

Vitamin A

While low concentrations of plasma or serum retinol are suggestive of frank deficiency, more subtle tests are needed to identify the presence of a marginal deficiency.2 Of these, the modified relative-dose-response test is proving to be highly reliable, while isotope dilution assay methods (which measure total body reserves) are currently being refined.11

Vitamin B6

Pyridoxal-5’phosphate (PLP) and pyridoxal (PL), its hydrolysis product and the ultimate transport form of B6, are the predominant vitamin B6 vitamins in the circulation; measurement of both (preferably in the erythrocytes rather than in the plasma) is recommended.12 The urinary pyridoxic acid level may give additional information to that obtained from a combination of pyridoxal phosphate and pyridoxal plasma levels, for example during pregnancy when plasma pyridoxal phosphate is decreased while plasma pyridoxal is increased.13

The activities of enzymes that are vitamin B6-dependent (such as transaminases) are of some value in assessing nutriture. However, they have a number of limitations.3

Xanthurenic acid and kynurenine excretion after a tryptophan load most likely represents an aberrant reaction to the load and has no documented clinical significance. In addition, pregnancy and contraceptive pills may render the test abnormal due to inhibition of kynureninase by estrogens.11

Vitamin B12

The diagnosis of vitamin B12 deficiency is often missed by routine laboratory tests, as many practitioners are unaware that the vitamin may be deficient despite normal serum vitamin B12 levels and the absence of a megaloblastic (large cell) anemia.18 Moreover, serum vitamin B12 levels are often normal when cerebrospinal fluid vitamin B12 is deficient.19 Most low serum cobalamin levels are not due to vitamin B12 deficiency but to gastric dysfunction in which protein-bound cobalamin is not digested; this can be corrected with oral administration of crystal cobalamin concentrate.17

One reason why serum, as well as intracellular vitamin B12 may be misleading, relates to the binding of the newly absorbed vitamin to transcobalamin II, a transport protein, to form holotranscobalamin II; thus this measure indicates the earliest stage of a negative B12 balance that is measurable from the serum.18 In the elderly, a decline of this transport protein results in higher serum B12 levels but lower intracellular B12 levels;17 therefore, although >150 pmol/L is usually considered as normal, older individuals with serum vitamin B12 of 150-250 pmol/L may also have a tissue B12 deficiency.19

Another problem with serum vitamin B12 assays is that it is commonly done using radioisotope dilution techniques, resulting in values that may be falsely increased due to the presence of inactive cobalamin analogues.20 The solution is...
to employ instead, a **microbiological assay**, since the microorganism whose growth is measured following feeding of the patient's serum, will be unaffected by the inactive analogues.\(^2\)

If patients have hematological, neuropsychiatric or gastrointestinal disorders supportive of cobalamin deficiency even though their serum cobalamin levels are normal, intracellular cobalamin deficiency can be diagnosed by testing for elevated serum methylmalonic acid and homocysteine levels.\(^2\) The serum methylmalonic acid assay appears especially useful in evaluating vitamin B12 status in hepatic disease as serum vitamin B12 may be increased.\(^2\)

Moreover, when evaluating for vitamin B12 deficiency, serum folate levels should be measured as, when both vitamins are low, deficiency of either may be primary. This is because megaloblastic intestinal cells may not absorb properly, causing a secondary deficiency of the other vitamin.\(^2\) Moreover, a low erythrocyte folate level may occur in primary vitamin B12 deficiency, because vitamin B12 is necessary to keep methylfolate in red cells.\(^2\)

The **Schilling test** is a popular test of B12 absorption although, because it only tests absorption of free vitamin B12, the test is often normal despite malabsorption of vitamin B12 from food resulting in low serum B12 levels.\(^2\)

The determination of **hypersegmentation of neutrophils** is a useful measure of vitamin B12 deficiency, and may sometimes uncover a deficiency which failed to depress serum vitamin B12.\(^2\) However, it is unreliable during pregnancy and fails to distinguish between vitamin B12 and folate deficiencies.\(^2\)

**Vitamin C**

There is to date no satisfactory assay technique for ascorbic acid status. In addition to physiologic status, many other factors influence the levels present at any particular time.\(^2\) Once collected, ascorbic acid readily oxidizes to dehydroascorbic acid; however, this can be prevented by acidifying the specimen immediately upon collection.

**Urinary ascorbic acid excretion** declines to undetectable levels during vitamin C depletion.\(^2\) By itself it is an inadequate measure of body reserves; however, it serves as the basis of an **ascorbic acid saturation test** in which a 24-hour urine is collected and analyzed prior to and following oral supplementation of 1 gram of ascorbic acid daily. Urinary ascorbic acid will rise until saturation is achieved. Normally, this should take one to two days; however, in borderline to frank scurvy, it takes 7 to 10 days to achieve saturation.\(^2\)

Among the blood vitamin C assays, **leukocyte ascorbic acid** is one of the better choices. As it responds less readily than serum ascorbic acid to recent dietary intake, it is probably more closely related to tissue stores.\(^2\) It is unreliable, however, as it may be normal despite other evidence of vitamin C deficiency.\(^2\) While serum or plasma ascorbic acid is not a particularly sensitive measure of vitamin C nutriture, low levels suggest inadequate intake and diminished reserves.\(^2\) Whole blood and erythrocyte ascorbic acid are even less sensitive indicators.\(^2\)

A **lingual ascorbic acid** test has been developed as a simple screening test for reduced tissue nutriture. A small amount of a dark blue chemical (N-2,6 dichlorphenolindophenol) is placed on the tongue, preferably under fasting conditions. The faster the color vanishes, the greater the tissue vitamin C concentration. While the results are consistent and significantly correlated with intradermal ascorbate levels,\(^2\) they are unrelated to either changes in vitamin C intake or with plasma or leukocyte concentrations,\(^2\) making the value of the test controversial.

**Vitamin D**

The diagnosis of inadequate tissue stores is made by testing the **plasma 25-hydroxyvitamin D** level, preferably in the middle of the winter when the lack of sunshine makes a deficiency more likely. Measurement of **plasma 1,25-dihydroxyvitamin D**, the activated form of the vitamin, provides an index of current biological action; however, it should be interpreted in the light of concurrent mineral nutriture and physiological state.\(^2\)

**Vitamin E**

While **adipose tissue vitamin E** is one of the best markers of vitamin E intake,\(^2\) **platelet toopherol** appears to be the best blood measure.\(^2\)

Since the vitamin is not associated with a protein carrier in the blood, but travels with the different lipoprotein fractions, **plasma and serum vitamin E** are closely correlated with total serum triglycerides.\(^2\) Therefore, when these levels are being measured, **tocopherol to triglyceride serum ratios** should be calculated to more accurately assess tocopherol status. The normal range of this ratio is 35 to 120.\(^2\)

**Vitamin K**

Subclinical deficiency, which may be substantial enough to cause adverse changes in bone metabolism, does not usually affect blood clotting parameters. An example is **prothrombin time** which can still be normal when the **prothrombin concentration** is only 50% of normal.\(^2\)

**Plasma phylloquinone** is considered to be a sensitive measure of vitamin K nutriture.\(^2\) Vitamin K acts as a cofactor in a reaction that converts specific glutamic acid residues present in vitamin D-dependent proteins to gamma-carboxylglutamic acid. When the vitamin is even mildly deficient, abnormal, des-gamma-carboxy forms of these proteins, referred to as **PIVKA** (protein induced by vitamin K deficiency or antagonism), are secreted in the plasma. **PIKVA concentration**, using a highly sensitive immunological method, is an excellent measure of vitamin K nutriture.\(^2\) **Undercarboxylated osteocalcin** is yet another sensitive measure for evaluating vitamin K status.\(^2\)

**Zinc**

Patients with **serum zinc** above 7.6 mmol/L (50 mg/dL) are considered unlikely to develop symptoms due to zinc deficiency.\(^2\) However, as there is no adequate laboratory test,\(^2\) the best way to determine whether an abnormality is due to a borderline or mild zinc deficiency is to perform a therapeutic trial.

Since **serum, salivary, urinary and hair zinc** are poorly correlated, a **zinc tolerance test** is perhaps the best available method of determining body zinc nutriture. After a fast, a baseline plasma level is drawn, and an oral loading dose of zinc sulfate 220 mg (50 mg elemental zinc) is given. Two hours later, plasma zinc is redrawn. A two or threelfold increase in plasma zinc is indicative of zinc inadequacy.\(^2\)
Of the various blood parameters, measures of zinc in leukocytes and platelets are more sensitive than in plasma, serum or erythrocytes. Sweat zinc is also a sensitive index of zinc status. While depressed hair zinc reliably indicates depletion (unless there is external contamination), normal or even high values do not rule out low body stores. A simple zinc taste test has been developed which suggests that there will be a favorable response to zinc supplementation using a test solution made by dissolving zinc sulfate 1 gm in 1 liter of distilled water (0.1% solution). If the subject tasting 5 to 10 ml notes either no taste or a dry and furry taste developing after a few minutes, zinc supplementation is suggested. Adequate zinc nutrition is suggested by an immediate taste which may be strong and unpleasant. This test, however, has not been well-validated and, in its present form, appears to be of limited value.

Other tests that appear to be of some value include measurement of neutrophil alkaline phosphatase activity, the enzyme 5'-nucleotidase, and erythrocyte metallothionein.

Other Laboratory Tests
Any of a wide variety of laboratory studies can be valuable in evaluating a particular patient. So many tests are available that they need to be chosen carefully. Certainly, tests measuring the functioning of the various body systems are sometimes indicated; these are well-discussed in standard medical texts. Stool analysis and tests for intestinal permeability and liver detoxification should also be considered for patients presenting with nonspecific complaints and an inconclusive physical examination.

References
42. Alpera DH. Zinc and deficiencies of taste and smell. Questions and Answers. JAMA 272(16):1233-4, 1994


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