Recommended Methods for the Measurement of Vitamin B_{12} Absorption

International Committee for Standardization in Hematology*

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Because vitamin B_{12} deficiency may arise from inadequate absorption, tests of the patient's ability to absorb orally administered radioactive vitamin B_{12} are widely used in the study of vitamin B_{12} deficiency.

The five methods in general use involve fecal, hepatic, urinary, plasma, or whole-body radioactivity measurements. Some of the salient features, advantages, and disadvantages of each test are shown in Table 1. Cooperation of the patient is required for the urinary and the usual fecal methods. The urine radioactivity test will be invalid when the glomerular filtration rate is substantially diminished (i.e., below 20 ml/min) or when bladder emptying is incomplete. Incomplete collection of urine may lead to classification of the patient as a malabsorber when in fact he is not. In general, loss of feces will result in a more serious error, since incomplete stool collection may result in failure to detect an existing malabsorption. The plasma radioactivity requires administration to the subject of a large amount of radioactivity, and more than one sample of plasma should be collected between 8 and 12 hr. Estimation of hepatic radioactivity does not require specimens from the patient, nor does whole-body counting. In the hepatic radioactivity method it is essential that identical counting geometry can be achieved at the time of the initial and final counting. From theoretical considerations, whole-body counting should be the best method for measuring directly retention of the radioactive vitamin.

Investigators have used varying quantities of radioactive vitamin B_{12} (most often 0.5–2 μg or 0.37–1.48 nmol). The fractional absorption is inversely related to the mass of vitamin B_{12} ingested. Consequently in order to compare results in different laboratories it is desirable to use a standard mass of vitamin B_{12} in oral dose. One microgram (0.74 nmol) of cyanocobalamin is recommended. It is not necessary to administer a standard amount of radioactivity; rather, one should use the minimum activity that will provide acceptable counting statistics. The generally preferred tracer is Co-57, because the radiation dose per microcurie is the lowest among the cobalt radionuclides, and it is quantified readily with high efficiency, using even simple gamma counting and recording apparatus (see Table 2). The vitamin should be administered in solution with 100–200 ml of water. The analog form of the vitamin may influence the fractional absorption, and because most published data relate to cyanocobalamin, we recommend its use. The cyanocobalamin should be obtained from a reputable company that performs quality control to ensure absence of other analogs that may influence the absorption. There is no assurance that other analogs of cobalamin (e.g., hydroxocobalamin) will behave in an identical way in the test. Before beginning any vitamin B_{12} absorption test it is essential to obtain pre-test radioactivity measurements relevant to the type of test to be applied (whole-body, urine, feces, plasma, or liver).

For patients who have been receiving vitamin B_{12} injections for therapy, it is advisable to wait 24 hr after the last injection before beginning the test of absorption.

The test dose of oral radioactive vitamin B_{12} should be administered after an overnight fast.

If the absorption of vitamin B_{12} is clearly lower than in healthy controls, it is recommended that a second absorption test be done giving intrinsic factor together with vitamin B_{12} as the oral dose. No generally accepted pure intrinsic factor is available commercially. Human and hog preparations of intrinsic factor have been used. The former is considered preferable. Because preparations of intrinsic factor commonly contain two kinds of vitamin-B_{12}-binding proteins, only one of which is intrinsic factor, the quantity of intrinsic-factor preparation administered should contain sufficient *intrinsic factor* to bind the oral dose of vitamin B_{12}. The manufacturer should ensure that the intrinsic-factor preparation has been shown to promote the absorption of radioactive vitamin B_{12} in patients with pernicious anemia or a total gastrectomy.

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TABLE 1. SURVEY OF ADVANTAGES AND DISADVANTAGES OF THE FIVE DIAGNOSTIC VITAMIN B_{12} ABSORPTION TESTS

Vitamin B_{12} absorption test	Fecal-excretion test	Urinary-excretion test	Blood-concentration test	Liver-radioactivity test	Whole-body retention test
Counting technique	In vitro	In vitro	In vitro	In vivo	In vivo
Counting sample	Feces	Urine	Blood	Liver	Whole body
Required time (days)	7–10	2–3	0.5	5–7	7–10
Quantitative test for B_{12} absorption	Directly	Indirectly	Indirectly	Indirectly	Directly
Possible errors introduced by patients, nurses, and technicians	Lost feces*	Lost urine	†	Lack of reproducibility of counting geometry	No
Disturbing diseases	—	Renal disease	—	Liver disease	—
Test influences B_{12} metabolism	No	Yes	No	No	No

* The effects of lost feces can be eliminated by administering an inabsorbable substance (e.g., $^{51}CrCl_3$) together with the oral radioactive B_{12} dose and correcting the observed excretion of radioactive B_{12} with the inverse fraction of the amount of inabsorbable substance excreted.

† False-normal results have been observed in some patients with proven pernicious anemia. It is theoretically possible that these occasional serious diagnostic errors are secondary to the elevation to the transcobalamin I (TC I) vitamin-B_{12}-binding protein in the serum of patients with pernicious anemia; vitamin B_{12} bound to TC I clears from the blood extremely slowly.

SELECTED METHOD: URINARY EXCRETION TEST

The urine radioactivity technique is chosen as a selected method because it is reliable, convenient, and widely used.

The following conditions, in addition to those mentioned above, are recommended with a view to standardization of the test.

1. A control urine must be collected during the 12 hr immediately preceding the oral dose. This will detect unexpected urinary radioactivity in a significant number of hospitalized patients.
2. One milligram (0.74 μmol) of nonradioactive *cyanocobalamin* should be administered subcutaneously or intramuscularly 1–2 hr after the oral dose of radioactive vitamin B_{12}.
3. A light breakfast containing no vitamin B_{12} (e.g., toast and jelly) may be given 2 hr after the start of the test. Other meals may be given as usual during the day.
4. All urine voided in the 24 hr after starting the test should be collected and saved. If the test is begun at 8 a.m., the urine collection is closed by having the patient void into the collection vessel at 8 a.m. the following day.
5. Urine sample and standard should be counted in an identical geometry. It is preferable to count generous volumes of urine, as this will improve the counting statistics and permit smaller quantities of radioactivity to be administered. The commonly used amount of radioactivity is 0.1 μCi. It must be ensured that

TABLE 2. CHARACTERISTICS OF RADIONUCLIDES OF COBALT USED TO LABEL VITAMIN B_{12} (CYANOCOBALAMIN)

	Co isotopes available for labeling vitamin B_{12} with high specific activity		
	Co-57	Co-58	Co-60
$T_{1/2}$	272 days	71 days	5.24 yr
Major radiations	γ	β^+ and γ	β^- and γ
Energies of major γ emission(s)	122 keV	0.81 MeV	1.33 MeV
		0.51 MeV	1.17 MeV
Radiation dose to liver of 70-kg man per μCi retained in body*	270 mrad	300 mrad	5700 mrad

* Assumes 90% of body's vitamin B_{12} is in liver, mean residence time of retained vitamin B_{12} in body is 667 days (i.e., 0.15% excretion/day), physical dosimetry data from MIRD Pamphlet No. 11 (W. S. Synder, N. R. Ford, G. G. Warner, and E. B. Watson, MIRD Pamphlet No. 11, Society of Nuclear Medicine, 475 Park Ave. S., N.Y., NY 10016). *For urinary excretion* test, a typical administration is 0.1 μCi, typical absorption is 70% (for normal), typical initial retention of absorbed vitamin is 70% following parenteral administration of 1 mg vitamin B_{12}; therefore, typical radiation dose to liver using Co-57 is $270 \times 0.1 \times 0.7 \times 0.7 = 13$ mrad. *For whole-body counting test*, a typical administration is 1 μCi; typical absorption is 70% (for normal), typical initial retention of absorbed vitamin is 100%; therefore, typical radiation dose to liver is $270 \times 1 \times 0.7 \times 1 = 189$ mrad.

adequate mixing of the entire 24-hr urine collection has occurred before preparation of the sample. If smaller samples are used, the sample must be counted for sufficient time to achieve counting statistics to a standard deviation of 2% or less.

6. If the result of the first absorption test is low, it is not necessary to wait more than 24 hr before repeating the test with intrinsic factor.

RECOMMENDED DIRECT METHOD: WHOLE-BODY RETENTION TEST

A reference method as defined by ICSH should provide sufficiently accurate and precise laboratory data for its use to access the validity of other laboratory methods. No reference method is yet available for this determination. However, theoretical considerations and practical experience show that whole-body counting following oral administration of radionuclide (provided that fecal excretion is complete) can provide an accurate measurement of retention and hence absorption. For this reason the direct method is recommended until an unequivocal reference method becomes available.

Urinary excretion of vitamin B_{12} need not be considered, since it is insignificant unless the patient is receiving large parenteral doses of vitamin B_{12} in the first 24–72 hr after the oral administration of radioactive vitamin. The method causes the patient the minimum of inconvenience and requires minimum cooperation, but it requires withholding therapeutic injections of vitamin B_{12} until the test is completed.

This direct method is not necessarily feasible in routine practice. It requires the availability of more complicated apparatus, specifically a whole-body counter offering adequate sensitivity, stability of background, and uniformity of counting efficiency throughout the body. A shadow-shield whole-body counter used in the "scanning mode" is one favorable compromise between price and performance. Cobalt-58 is the preferred radionuclide; radiation doses to the patient are needlessly high with Co-60, and inaccuracies associated with radiation attenuation in the body may be large if Co-57 is used under suboptimal conditions of whole-body counting. The test cannot be completed in less than 7 days, and may be impracticable as a routine test. The necessity of withholding therapy from the patient during the early days of the study is a major clinical limitation of the whole-body retention method.

The following conditions are recommended.

1. An initial measurement of the patient is made to measure natural body radioactivity and to detect any residual radionuclide from previous investigations or therapy. Subsequent counting rates are corrected appropriately for these contributions.
2. The patient is measured again at least 30 min after administration of the oral dose of labeled vitamin B_{12} as described earlier. When fecal excretion of the unabsorbed vitamin B_{12} is complete, usually after 7 or more days, the patient is measured again, giving retention directly as a percentage of the initial counting rate. The counting rates are usually related to a standard.
3. As in the urinary excretion test, when a low value has been obtained for the absorption, the test is repeated after giving intrinsic factor.

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