

EFFECTIVE DATE	N P Analytical Laboratories	METHOD CODE
REVISED: 08/21/24	LABORATORY TEST METHOD SUMMARY	B12
REPLACES: 12/30/20	B12 Activity	PAGE 1 OF 1
KEY WORDS: microvitamin, Cyanocobalamin, Cobalamin, vitamin B12		

1. **SCOPE AND PURPOSE:**

This method measures total (bound and free) cyanocobalamin (vitamin B₁₂) activity in plant and animal tissues, foods, feeds, ingredients, premixes, veterinary and pharmaceutical products. Expected vitamin B₁₂ form should be specified because extraction procedures vary.

2. **PRINCIPLE:**

- 2.1. This is an Autoturb turbidimetric microbiological method based on the observation that *Lactobacillus leichmanii* ATCC 7830 requires vitamin B₁₂ for growth. A basal medium, nutritionally complete in all respects except for vitamin B₁₂, is used as a diluent for the standards and samples. Following incubation, the growth response of the bacterial cultures is measured as percent light transmittance on the Autoturb. A dose-response line is constructed and the sample concentrations are calculated.
- 2.2. Total B₁₂ activity: Using a 5 g sample the lowest confidence level of this method is 0.005 ppm total vitamin B₁₂ activity.
- 2.3. Free vitamin B₁₂ activity: Using a 2 g sample the lowest confidence level of this method is 0.031 ppm free vitamin B₁₂ activity.
- 2.4. Known Interferences: Vitamin B₁₂ is decomposed in aqueous solutions containing thiamine and ascorbic acid unless a stabilizer, such as a trace amount of iron, is present, or a suitable vehicle, such as equal parts of propylene glycol and glycerol, is employed.
- 2.5. There is no assurance that matrices other than those listed can be assayed using this method.

3. **PRECISION:**

Records of method precision based on Method Validation and/or known control summaries are located in the QA Master file for this test method. Assay precision may vary with test matrix and analyte level. Terms used to describe method precision are defined in NPSOP3000, *Validation of Quantitative Chemical Tests*.

4. **REFERENCES:**

- 4.1. Official Methods of Analysis of the AOAC Method 960.46.
- 4.2. Augustin, Jorg et. al., **1985**. *Methods of Vitamin Assay*, pp. 43, 50, 57, and 502-507, John Wiley & Sons, Inc., New York, NY.
- 4.3. Glick, D., **1954**. *Methods of Biochemical Analysis*, Vol. I, pp. 81-87; Interscience Publishers, Inc., New York, NY.
- 4.4. Kavanagh, F. W., **1963**. *Analytical Microbiology*, Vol. I, pp. 48, 551-565, Academic Press, New York, San Francisco, London.
- 4.5. Shaw, W. H. C. and Bessell, C. J., **1960**. *The Determination of Vitamin B₁₂*, *Analyst*, Vol. 85, pp. 397-403.