

EFFECTIVE DATE	NP Analytical Laboratories	METHOD CODE
REVISED: 12/06/24	LABORATORY TEST METHOD SUMMARY	FOA
REPLACES: 11/30/17	Folic Acid Activity	PAGE 1 OF 1
KEY WORDS: Folic Acid, microvitamin		

1. SCOPE AND PURPOSE:

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

2. PRINCIPLE:

- 2.1. This is an Autoturb turbidimetric microbiological method based on the observation that *Enterococcus hirae* ATCC 8043 requires folic acid for growth. A basal medium nutritionally complete in all respects except for folic acid is used as the diluent for extracts from standards and samples. The mixture is incubated, and 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. Using a 4 g sample, the lowest confidence level of this method is 0.125 ppm total folic acid activity.
- 2.3. Using a 5 g sample, the lowest confidence level for free forms is 0.20 ppm folic acid activity.
- 2.4. Known Interferences: Sample extracts high in turbidity or color interfere with the Autoturb reader.
- 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 International, Methods 2004.05 and 944.12.
- 4.2. Augustin, Jorg et. al., **1985**. *Methods of Vitamin Assays*, pp. 43 and 452-462, John Wiley & Sons, Inc., New York, NY.
- 4.3. JAOAC, Vol. 48, No. 6, pp. 1230-1235, **1965**, *Microbiological Assays of Folic Acid Activity in Foods: Changes in Results with Variation of Experimental Conditions and Methods*, Flynn, Laura M.
- 4.4. Hurdle, A. D. F.; Barton, D.; Searles, I. H. Am., J. Clinical Nutr., **1968**, *A Method for Measuring Folate in Food and Its Application to a Hospital Diet*, Vol. 21, pp. 1202-1207.
- 4.5. Kavanagh, F. W., **1963**. *Analytical Microbiology*, Vol. I, pp. 431-488, Academic Press, New York, San Francisco, London.
- 4.6. Flynn, L. M., **1966**. AOAC Collaborative Microbiological Assay of Folic Acid (unpublished)