Appendix C: References for Analytical Methods for Biochemical Indicators

Detailed laboratory method information is provided on the NHANES web site for each survey cycle:

- http://wwwn.cdc.gov/nchs/nhanes/

Additional Useful Analytical Method References:

Water-Soluble Vitamins and Metabolites

Camara JE, Pritchett JS, Daniels YC, Bedner M, Nelson MA, Lowenthal MS, Fazili Z, Pfeiffer CM, Phinney KW, Sharpless KE, Sander LC, Lippa KA, Yen JH, Kuszak AJ, Wise SA. Development of an improved Standard Reference Material for folate vitamers in human serum. Anal Bioanal Chem. 2023;415(5):809–821.

Fazili Z, Pfeiffer CM. Measurement of folates in serum and conventionally prepared whole blood lysates: Application of an automated 96-well plate isotope-dilution tandem MS method. Clin Chem. 2004;50:2378–2381.

Fazili Z, Pfeiffer CM, Zhang M, Jain RB. Erythrocyte folate extraction and quantitative determination by LC/MS/MS: Comparison of results with microbiologic assay. Clin Chem. 2005;51(12):2318–2325.

Fazili Z, Pfeiffer CM, Zhang M. Comparison of serum folate species analyzed by LC-MS/MS with total folate measured by microbiologic assay and BioRad radioassay. Clin Chem. 2007;53(4):781–784.

Fazili Z, Pfeiffer CM, Zhang M, Jain RB, Koontz D. Influence of 5,10-methylenetetrahydrofolate reductase polymorphism on whole blood folate concentrations measured by LC-MS/MS, microbiologic assay and BioRad radioassay. Clin Chem. 2008;54:197–201.

Fazili Z, Whitehead RD Jr, Paladugula N, Pfeiffer CM. A high-throughput LC-MS/MS method suitable for population biomonitoring measures five serum folate vitamers and one oxidation product. Anal Bioanal Chem. 2013;405:4549–4560.

Fazili Z and Pfeiffer CM. Accounting for an isobaric interference allows correct determination of folate vitamers in serum by isotope dilution-liquid chromatography-tandem mass spectrometry. J Nutr. 2013;143:108–113.

Fazili Z, Sternberg MR, Paladugula N, Pfeiffer CM. Two international Round Robin studies showed good comparability of 5-methyltetrahydrofolate, but poor comparability of folic acid measured in serum by different HPLC-MS/MS methods. J Nutr. 2017;147:1815–1825.

Gunter EW, Bowman BA, Caudill SP, Twite DB, Adams MJ, Sampson EJ. Results of an international round robin for serum folate and whole-blood folate. Clin Chem. 1996;42:1689–1694.

Life Sciences Research Office. Assessment of folate methodology used in the Third National Health and Nutrition Survey (NHANES 1988–1994). Washington, D.C.: Center for Food Safety and Applied Nutrition, Food and Drug Administration, Department of Health and Human Services; 1994.

McCoy LF, Bowen MB, Xu M, Chen H, Schleicher RL. Improved HPLC assay for measuring serum vitamin C with 1-methyluric acid used as an electrochemically active internal standard. Clin Chem. 2005;51:1062–1064.

Mineva EM, Zhang M, Rabinowitz DJ, Phinney KW, Pfeiffer CM. An LC-MS/MS method for serum methylmalonic acid suitable for monitoring vitamin B12 status in population surveys. Anal Bioanal Chem. 2015;407:2955–2964.

Pfeiffer CM, Huff DL, Gunter EW. Rapid and accurate HPLC assay for total plasma homocysteine and cysteine in a clinical lab setting. Clin Chem. 1999;45(2):290–292.

Pfeiffer CM, Twite D, Shih J, Holets-McCormack SR, Gunter EW. Method comparison for total plasma homocysteine between the Abbott IMx analyzer and an HPLC assay with internal standardization. Clin Chem. 1999;45(1):152–153.

Pfeiffer CM, Huff DL, Smith SJ, Miller DT, Gunter EW. Comparison of plasma total homocysteine measurements in 14 laboratories: an international study. Clin Chem. 1999;45(8):1261–1268.

Pfeiffer CM, Smith SJ, Miller DT, Gunter EW. Comparison of serum and plasma methylmalonic acid measurements in 13 laboratories: an international study. Clin Chem. 1999;45:2236–2242.

Pfeiffer CM, Caudill SP, Gunter EW, Bowman BA, Jacques PF, Selhub J, et al. Discussion of critical issues related to the comparison of homocysteine values between the Third National Health and Nutrition Examination Survey (NHANES) and NHANES 1999+. J Nutr. 2000;130:2850–2854.

Pfeiffer CM, Fazili Z, McCoy LF, Gunter EW. Determination of folate vitamers in human serum by stable-isotope dilution tandem mass spectrometry and comparison to radioassay and microbiologic assay. Clin Chem. 2004;50(2):423–432.

Pfeiffer CM, Fazili Z, Zhang M. Folate analytical methodology. In: Bailey LB, editor. Folate in Health and Disease. 2nd ed. CRC Press. Taylor & Francis Group. 2010. p. 517–574.

Pfeiffer CM, Zhang M, Lacher DA, Molloy AM, Tamura T, Yetley EA, Picciano M-F, Johnson CL. Comparison of serum and red blood cell folate microbiologic assays for national population surveys. J Nutr. 2011;141:1402–1409.

Pfeiffer CM, Sternberg MR, Hamner HC, Crider KS, Lacher DA, Rogers LM, Bailey RL, Yetley EA. Applying inappropriate cutpoints leads to misinterpretation of folate status in the US population. Am J Clin Nutr. 2016;104:1607–1615.

Rybak ME, Pfeiffer CM. Clinical analysis of vitamin B6: Determination of pyridoxal 5'-phosphate and 4-pyridoxic acid in human serum by reversed-phase high-performance liquid chromatography with chlorite postcolumn derivatization. Anal Biochem. 2004;333:336–344.

Rybak ME, Jain RB, Pfeiffer CM. Clinical vitamin B_6 analysis: an inter-laboratory comparison of pyridoxal 5'-phosphate measurements in serum. Clin Chem. 2005;51:1223–1231.

Rybak ME, Pfeiffer CM. A simplified protein precipitation and filtration procedure for determining serum vitamin B6 by high-performance liquid chromatography. Anal Biochem. 2009;388:175–177.

Satterfield MB, Sniegoski LT, Sharpless KE, Welch MJ, Hornikova A, Zhang N-F, Pfeiffer CM, Fazili Z, Zhang M, Nelson B. Development of a new standard reference material: SRM 1955 (homocysteine and folate in human serum). Anal Bioanal Chem. 2006;385(3):612–622.

Stamm RA, Fazili Z, Pfeiffer CM. Addition of exogenous γ-glutamyl hydrolase eliminates the need for lengthy incubation of whole blood lysate for quantitation of folate vitamers by high performance liquid chromatography-tandem mass spectrometry. Curr Dev Nutr. 2017;2(1):1–9.

Zhang M, Sternberg MR, Pfeiffer CM. Harmonizing the calibrator and microorganism used in the folate microbiologic assay leads to improvements in serum and whole blood folate comparability as shown in a CDC Round Robin study. J Nutr. 2018;148:807–817.

Fat-Soluble Vitamins and (Micro)Nutrients

Chaudhary-Webb M, Schleicher RL, Erhardt JG, Pendergrast EC, Pfeiffer CM. An HPLC ultraviolet method using low sample volume and protein precipitation for the measurement of retinol in human serum suitable for laboratories in low-and middle-income countries. J Appl Lab Med. 2019;4(1):101–107.

Mineva EM, Schleicher RL, Chaudhary-Webb, M, May KL, Botelho JC, Vesper HW, Pfeiffer CM. A candidate reference measurement procedure for quantifying serum concentrations of 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 using isotope-dilution liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem. 2015;407(19):5615–5624.

Mineva EM, Sternberg MR, Pfeiffer CM, Momin SS, Maw KL, Schleicher RL. Quality specifications and their daily application to evaluate the accuracy of reference measurements for serum concentrations of 25-hydroxyvitamin D_3 and 25-hydroxyvitamin D_2. Clin Chim Acta. 2018;487:241–249.

Phinney KW, Bedner M, Tai S-C, Vamathevan VV, Sander LC, Sharpless KE, Wise SA, Yen JH, Schleicher RL, Chaudhary-Webb M, Pfeiffer CM, Betz JM, Coates PM, Picciano MF. Development and certification of a Standard Reference Material for vitamin D metabolites in human serum. Anal Chem. 2012;84:956–962.

Schleicher RL, Encisco SE, Chaudhary-Webb M, Paliakov E, Pfeiffer CM. Isotope dilution ultra performance liquid chromatography-tandem mass spectrometry method for simultaneous measurement of 25-hydroxyvitamin D2, 25-hydroxyvitamin D3 and 3-epi-25-hydroxyvitamin D3 in human serum. Clin Chim Acta. 2011;412:1594–1599.

Sowell AL, Huff DL, Yeager PR, Caudill SP, Gunter EW. Retinol, alpha-tocopherol, lutein/zeaxanthin, beta-cryptoxanthin, lycopene, alpha-carotene, trans-beta-carotene, and four retinyl esters in serum determined simultaneously by reversed-phase HPLC with multi-wavelength detection. Clin Chem. 1994;40:411–416.

Iron-Status Indicators

Looker AC, Gunter EW, Johnson CL. Methods to assess iron status in various NHANES surveys. Nutr Rev. 1995;53:246–254.

Lyle AN, Budd JR, Kennerley V, Smith BN, Danilenko U, Pfeiffer CM, Vesper HW. Assessment of WHO 07/202 reference material and serum pools for commutability and suitability as calibrators for soluble transferrin receptor assays. Clin Chem Lab Med. 2023;61(10):1719–1729.

Pfeiffer CM, Cook JD, Mei Z, Cogswell ME, Looker AC, Lacher DA. Evaluation of an automated soluble transferrin receptor (sTfR) assay on the Roche Hitachi analyzer and its comparison to two ELISA assays. Clin Chim Acta. 2007;382:112–116.

Pfeiffer CM, Looker AC. Laboratory methodologies for indicators of iron status: strengths, limitations and analytical challenges. Am J Clin Nutr. 2017;106(Suppl):1606S–1614S.

Mei Z, Addo Y, Jefferds M, Sharma A, Flores-Ayala R, Pfeiffer CM, Brittenham GM. Comparison of current WHO guidelines with physiologically based serum ferritin thresholds for iron deficiency in healthy young children and non-pregnant women. J Nutr. 2023;153:771–780.

Thorpe SJ, Heath A, Blackmore S, Lee A, Hamilton M, O'Broin S, Nelson BC, Pfeiffer CM. An international standard for serum vitamin B12 and serum folate: International collaborative study to evaluate a batch of lyophilized serum for B12 and folate content. Clin Chem Lab Med. 2007;45:380–386.

Trace Elements

Caldwell KL, Maxwell CB, Makhmudov A, Pino S, Braverman LE, Jones RL, et al. Use of inductively coupled plasma mass spectrometry to measure urinary iodine in NHANES 2000: comparison with previous method. Clin Chem. 2003;49:1019–1021.

Phytoestrogens

Valentin-Blasini L, Blount BC, Rogers HS, Needham LL. HPLC-MS/MS method for the measurement of seven phytoestrogens in human serum and urine. J Expo Anal Environ Epidemiol. 2000;10:799–807.

Kuklenyik Z, Ye X, Reich JA, Needham LL, Calafat AM. Automated online and off-line solid-phase extraction methods for measuring isoflavones and lignans in urine. J Chromatogr Sci. 2004;42:495–500.

Parker DL, Rybak ME, Pfeiffer CM. Phytoestrogen biomonitoring: an extractionless LC-MS/MS method for measuring urinary isoflavones and lignans using atmospheric pressure photoionization (APPI). Anal Bioanal Chem. 2012;402:1123–1136.

Rybak ME, Parker DL, Pfeiffer CM. Determination of urinary phytoestrogens by HPLC-MS/MS: a comparison of atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI). J Chromatogr B. 2008;861:145–150.

Caffeine and Metabolites

Rybak ME, Pao C-I, Pfeiffer CM. Determination of urine caffeine and its metabolites by use of high-performance liquid chromatography-tandem mass spectrometry: estimating dietary caffeine exposure and metabolic phenotyping in population studies. Anal Bioanal Chem. 2014;406:771–784.

Acrylamide Hemoglobin Adducts

Vesper HW, Ospina M, Meyers T, Ingham L, Smith A, Gray JG, Myers GL. Automated method for measuring globin adducts of acrylamide and glycidamide at optimized Edman reaction conditions. Rapid Commun Mass Spectrom. 2006;20:959–964.

Trans Fatty Acids

Kuiper HC, Wei N, McGunigale SL, Vesper HW. Quantitation of trans-fatty acids in human blood via isotope dilution-gas chromatography-negative chemical ionization-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2018;1076:35–43.