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Red Blood Cell Folate Testing

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Author
Peter L. Perrotta, MD, FCAP
Department of Pathology, Anatomy and Laboratory Medicine, West Virginia University Health System

Editors
Richard W. Brown, MD, FCAP*, Curtis A. Hanson, MD, FCAP; Barbara Blond, MBA; Thomas Long, MS
*Senior Editor, Department of Pathology, Memorial Hermann Health System, Houston, TX

Update Editors
Jeffrey Vos, MD, FCAP, Barbara Blond, MBA

SYNOPSIS AND RELEVANCE

Testing of serum folate levels is the preferred method for detecting nutritional folate deficiency. Adherence to using serum folate levels for this purpose can:

1. Ensure that serum folate testing is a first line test for detecting folate deficiency.
2. Reduce the utilization of less useful markers of folate deficiency like the red blood cell (RBC) folate test.
3. Impact patient care by ensuring that the most clinically useful tests are used to diagnose folate deficiency.

OBJECTIVES

1. Define which testing is most useful to clinicians who are assessing the folate status of patients.
2. Understand the clinical and laboratory technical issues that impact tests used to detect nutritional folate deficiency.
3. Recognize ways to facilitate the ordering of tests used to diagnose folate deficiency.

BACKGROUND

Folate levels are frequently performed on patients with suspected nutritional deficiency or anemia, in particular, macrocytic anemias. Concerns over the relationships between folate deficiency and neural tube defects led to a Food and Drug Administration (FDA) mandate to fortify foods with folic acid in the 1990s. Folate deficiency is suspected in patients with reduced hemoglobin and hematocrit who have an elevated mean cell volume (MCV). Folate levels can be measured in serum and in erythrocytes (RBC folate). Approximately 95% of folate is located within erythrocytes, so historically, RBC folate has been thought to better assess longer-term folate storage, whereas serum folate levels may reflect more recent dietary folate intake. While RBC folate levels may better estimate longer-term storage levels, RBC levels generally add no diagnostic value to a serum folate level in detecting folate deficiency. Vitamin B12 levels should also be tested in patients with a megaloblastic anemia because folic acid supplements may correct the hematologic changes in B12 deficiency but do not halt the progression of neurologic disease.

Folate levels are typically measured by a sensitive competitive binding protein assay, however, folate results can vary between laboratories depending on the test system and methodology used. One reason serum folate levels are preferred is that RBC folate levels demonstrate higher analytical variation than serum folate levels because of the additional pre-treatment steps (ie, red cell lysis to release folate) needed to perform the RBC folate test. Many laboratories send out RBC folate tests because of these technical issues. MTHFR mutations may also alter the distribution of folate in red cells and may cause further analytic variability using the RBC technique. RBC folate levels may also be affected by RBC oxygen saturation and hemoglobin content.

Furthermore, a number of studies have demonstrated that RBC and serum folate testing provide equivalent data and that RBC folate testing is not necessary. Thus, routine testing of both serum and RBC folate is discouraged. Serum folate levels are much more widely performed by laboratories of all sizes, whereas RBC folate testing is referred to outside laboratories in most cases. Widespread folic acid supplementation has dramatically reduced the incidence of folate deficiency in the United States, further calling into question

the utility of folate testing in general. For patients who are supplemented with folate, serum levels may better reflect responses to folate therapy. Other than rare, unusual circumstances, such as suspicion of a hereditary folate-related enzyme or transport protein deficiency, there are no additional indications for RBC folate testing outside of those for serum folate testing.

The routine use of serum folate levels to detect nutritional folate deficiency may increase the value of this testing for providers and patients by:

- Establishing an accurate and timely diagnosis of patients with nutritional folate deficiencies.
- Improving the performance of the diagnostic strategy used at your institution to detect folate deficiency.
- Facilitating the evaluation of patients with folate deficiency for your health care professionals in a collaborative manner.
- Enhancing the training and education of providers who routinely test patients for folate vitamin deficiencies.
- Ensuring that your health information technology services support the decision making of health care providers who utilize nutritional folate testing by optimizing the electronic test ordering and laboratory test resulting systems.
- Impact testing costs for patients being evaluated for folate deficiencies.

INSIGHTS

1. Folate deficiency is relatively rare in the United States because of widespread supplementation of foods with folate.
2. Serum folate levels are preferred over RBC folate for assessing the folate status of patients.

INTERVENTIONS

Interventions that are useful for RBC folate test ordering follow below.

Investigate how providers order RBC folate levels: Providers may at times order an RBC folate level when they had intended to order a serum folate level. Evaluate your test ordering system (electronic and/or paper) to determine if there is ambiguity in the names of these tests. Ensure that the serum folate test is more prominently displayed.

Restrict the ability to order RBC folate in electronic and/or paper ordering systems: Most electronic ordering systems can facilitate the ordering of the most useful diagnostic tests. For example, screen “pop-ups” can be used to alert the provider that they are ordering an RBC folate instead of a serum folate level, or when they order both of these tests at the same time. The “pop-up” screen can be designed so that the provider can easily cancel the RBC folate order.

RBC folate levels can also be removed from electronic “order sets”. Prior to discontinuing RBC folate testing, more stringent gatekeeping may be implemented by requiring approval from the laboratory director or clinical chemist before performing the test or sending it to a reference laboratory. This may also be facilitated by using a “hard stop” notification process in the electronic ordering system.

Provide information to providers and/or services regarding the utility of RBC folate levels:
Targeted educational opportunities can update providers when a test has become antiquated or a more useful test is available that can replace an older test. In many cases providers are aware that a test has little clinical utility but have become accustomed to ordering that test.

Remove RBC folate from the laboratory test menu: The most effective way to reduce RBC folate levels is to remove the ability to order the test. This will require interactions with hospital leadership and providers who frequently order nutritional testing. This project could be reviewed by a laboratory test utilization group, or other appropriate institutional committee. RBC folate levels can be eliminated from both electronic and paper ordering systems. The laboratory should notify providers when significant changes are made to the test ordering menu to avoid confusion and frustration.

INTERVENTION ANALYSIS

It is relatively straightforward to assess RBC folate ordering practices depending on your goals for this project (**see Appendix A**).

- Determine the total number of serum and RBC folate levels performed over a period (eg, 3-12 months depending on test volume). These should be relatively easy to extract from your information systems.

- Consider calculating the ratio of the number of serum to RBC folate levels performed over this period (ie, # serum folate/# RBC folates). This ratio would serve as a baseline indicator of folate test utilization and helps to standardize calculations based on your test volume.
- You may identify patients who had both serum and RBC folate tested at the same time or within or short period. This may be considered a form of redundant testing and eliminating redundant testing can reduce both laboratory and patient costs.
- If interested, you could determine the incidence of folate deficiency in your practice. The incidence of folate deficiency in the United States is quite low.
- Identify individual providers and groups of providers (ie, services) that order most of the RBC folate levels performed.
- Implement interventions to reduce the number of RBC folate tests ordered; these interventions should be focused on providers and services that order the highest numbers of RBC folate tests.
- After interventions have been put in place, follow-up studies can be performed to assess the success of the interventions used to reduce the number of RBC folate orders (**see Appendix B**).
    - Recalculate the ratio of serum to RBC folate levels for a period of time. Depending on the laboratory's test volume, only 1-2 months of data may be needed to determine if the changes were effective. An increase in this ratio indicates there is an increase in serum folate tests and/or a decrease in RBC folate tests.

APPENDIX A: METHODS TO DETERMINE INTERVENTION IMPACT

12 MONTH PERIOD IS ASSUMED, SHORTER PERIODS CAN BE USED

RBC and Serum Testing Volume	Value	Result
# Serum folate levels performed over 12 months (Total)	A1	
# RBC folate levels performed over past 12 months	A2	
Standardized Indicator of Folate Test Utilization		
Calculate ratio serum to RBC folate levels = **A1/A2 =A3**	A3	
Redundant Testing		
# Patients having serum and RBC folate tested at same time	A4	
Folate Deficiency Incidence Based on RBC Folate Level		
# RBC folate levels below 140 ng/mL (CDC definition)	A5	
Calculate % deficient RBC folates = **100*(A5/A2) = A6**	A6	
Folate Deficiency Incidence Based on Serum Folate Level		
Serum folate lower reference limit (eg, 7.0 ng/mL)	A7	
# Serum folate levels below lower limit	A8	
Calculate % of deficient serum folate levels = **100*(A8/A1) = A9**	A9	
Providers Who Ordered Highest # of RBC Folates (top 5) Indicate the provider and # of tests	Provider	# Tests
Provider #1 (#RBC folates)		
Provider #2 (#RBC folates)		
Provider #3 (#RBC folates)		
Provider #4 (#RBC folates)		
Provider #5 (#RBC folates)		
Services That Ordered Highest # of RBC Folates (top 5) Indicate the service and # of tests	Service	# Tests
Service #1 (#RBC folates)		
Service #2 (#RBC folates)		
Service #3 (#RBC folates)		
Service #4 (#RBC folates)		
Service #5 (#RBC folates)		

APPENDIX B: POST-INTERVENTION FOLLOW-UP DATA

Post-Intervention Follow-up Data	Value	Result
# Serum folate levels performed post-intervention	B1	
# RBC folate levels performed post-intervention	B2	
Post-intervention period (in months)	B3	
Ratio serum folate to RBC folate levels = **B1/B2 = B4**	B4	
# of RBC folate levels decreased per year **= A2 – (B2*(12/B3)) = B5**	B5	

QUESTIONS AND ANSWERS

QUESTION 1 OBJECTIVE

Define which testing is most useful to clinicians who are assessing the folate status of patients.

QUESTION 1

Which of the following tests should be used to identify nutritional folate deficiency?

A. Mean corpuscular volume
B. Plasma homocysteine
C. RBC folate
D. Serum folate
E. Serum methylmalonic acid

The correct answer is D. Serum folate is considered the preferred test for diagnosing nutritional folate deficiency.
A is incorrect. Although the mean corpuscular volume (MCV) may be elevated in folate deficiency, an increased MCV is not specific for folate deficiency.
B is incorrect. Plasma homocysteine levels do not reflect body folate levels.
C is incorrect. Serum folate levels are preferred to RBC folate levels to detect folate deficiency.
E is incorrect. Serum methylmalonic acid levels do not reflect body folate levels.

REFERENCES:

1. Farrell CJ, Kirsch SH, Herrmann M. Red cell or serum folate: what to do in clinical practice? *Clin Chem Lab Med*. 2013;51(3):555-569. doi:10.1515/cclm-2012-0639
2. Pillay TS, Oosthuizen NM. Why are we still measuring red cell folate instead of just serum folate? *J Clin Pathol*. 2014;67(4):289. doi:10.1136/jclinpath-2013-202086

QUESTION 2 OBJECTIVE

Understand the clinical and laboratory technical issues that impact tests used to detect nutritional folate deficiency.

QUESTION 2

Which of the following statements is true regarding clinical folate testing?

A. Folate deficiency is relatively common in the United States
B. It is not necessary to test B12 levels in patients with megaloblastic anemia
C. Folate deficiency is more common in the United States than vitamin B12 deficiency
D. Foods in the United States are supplemented with folate primarily to prevent anemia
E. Serum folate determinations are more precise than RBC folate measurements

The correct answer is E. Serum folate determinations are considered more precise than red cell folate measurements.
A is incorrect. Folate deficiency is uncommon in the United States, largely because many foods are supplemented with folic acid.
B is incorrect. Vitamin B12 levels should always be checked in patients with megaloblastic anemia because folic acid can correct anemia in B12 deficiency, however, it does not prevent neurologic complications.
C is incorrect. Since many foods are supplemented with folic acid in the United States, folate deficiency is relatively uncommon.
D is incorrect. Foods are supplemented with folic acid primarily to prevent neural tube defects.

REFERENCES:

Galloway M, Rushworth L. Red cell or serum folate? Results from the National Pathology Alliance benchmarking review. *J Clin Pathol*. 2003;56(12):924-926. doi:10.1136/jcp.56.12.924

QUESTION 3 OBJECTIVE

Recognize ways to facilitate the ordering of tests used to diagnose folate deficiency.

QUESTION 3

Which of the following interventions is most likely to reduce the use of RBC folate testing?

A. Adding a "reflex" test for RBC folate testing for patients with an elevated mean corpuscular volume
B. Making the RBC folate "not orderable" in the computer physician order entry system
C. Developing and performing the test in-house
D. Placing the RBC folate test on admission order sets

The correct answer is B. Most strategies that limit the ability to order a test will be effective in reducing the number of requests for that test.
A is incorrect. Adding a reflex in this case would actually increase the number of RBC folate tests performed.
C is incorrect. Bringing a test in-house often makes a test more visible and increases orders for that test.
D is incorrect. Placing the RBC folate test (or any test) on an order set would likely increase the utilization of that test.

REFERENCES:

1. Baron JM, Dighe AS. The role of informatics and decision support in utilization management. *Clinica Chimica Acta*. 2014;427:196-201. doi:10.1016/j.cca.2013.09.027
2. Ismail O, Chin-Yee I, Gob A, et al. Reducing red blood cell folate testing: a case study in utilisation management. *BMJ Open Quality*. 2019;8:e000531. doi:10.1136/bmjoq-2018-000531
3. MacMillan TE, Gudgeon P, Yip PM, Cavalcanti RB. Reduction in unnecessary red blood cell folate testing by restricting computerized physician order entry in the electronic health record. *Am J Med*. 2018; 131:939-944. doi: 10.1016/j.amjmed.2018.03.044

MODULE REFERENCES

1. Baron JM, Dighe AS. The role of informatics and decision support in utilization management. *Clinica Chimica Acta*. 2014;427:196-201. doi:10.1016/j.cca.2013.09.027
2. De Bruyn E, Gulbis B, Cotton F. Serum and red blood cell folate testing for folate deficiency: new features? *Eur J Haematol*. 2013; 92:354-359. doi:10.1111/ejh.12237
3. Devalia V, Hamilton MS, Molloy AM. Guidelines for the diagnosis and treatment of cobalamin and folate disorders. *Br J Haematol.* 2014;166(4):496-513.
4. Farrell CJ, Kirsch SH, Herrmann M. Red cell or serum folate: what to do in clinical practice? *Clin Chem Lab Med.* 2013; 51(3):555-569. doi:10.1515/cclm-2012-0639
5. Galloway M, Rushworth L. Red cell or serum folate? Results from the National Pathology Alliance benchmarking review. *J Clin Pathol.* 2003;56(12):924-926. doi:10.1136/jcp.56.12.924
6. Gudgeon P, Cavalcanti R. Folate testing in hospital inpatients. *Am J Med*. 2015; 128:56-59.
7. Ismail O, Chin-Yee I, Gob A, et al. Reducing red blood cell folate testing: a case study in utilisation management. *BMJ Open Quality*. 2019;8:e000531. doi:10.1136/bmjoq-2018-000531
8. Jaffe JP, Schilling RF. Erythrocyte folate levels: a clinical study. *Am J Hematol*.1991; 36(2):116-121. doi: 10.1002/ajh.2830360210
9. Joelson DW, Fiebig EW, Wu AHB. Diminished need for folate measurements among indigent populations in the post folic acid supplementation era. *Arch Pathol Lab Med.* 2007;131(3):477-480. doi:10.1043/1543-2165 (2007)131[477:DNFFMA]2.0.CO;2
10. MacMillan TE, Gudgeon P, Yip PM, Cavalcanti RB. Reduction in unnecessary red blood cell folate testing by restricting computerized physician order entry in the electronic health record. *Am J Med*. 2018; 131:939-944. doi: 10.1016/j.amjmed.2018.03.044
11. McDowell MA, Lacher DA, Pfeiffer CM, et al. Blood folate levels: the latest HNANES results. NCHS data brief number 6, May 2008. Center for Disease Control. Page reviewed November 6, 2015. Accessed May 2, 2023. https://www.cdc.gov/nchs/products/databriefs/db06.htm
12. Owen WE, Robarts WL. Comparison of five automated serum and whole blood folate assays. *Am J Clin Pathol. 2003*; 120(1):121-126. doi:10.1309/L2U6-HH5K-AYG4-8L40
13. Pillay TS, Oosthuizen NM. Why are we still measuring red cell folate instead of just serum folate? *J Clin Pathol.* 2014; 67(4):289. doi:10.1136/jclinpath-2013-202086

14. Robinson AR, Mladenovic J. Lack of clinical utility of folate levels in the evaluation of macrocytosis or anemia. *Am J Med.* 2001;110(2):88-90. doi:10.1016/s0002-9343(00)00670-7
15. Theisen-Toupal J, Horowitz G, Breu A. Low yield of outpatient serum folate testing: eleven years of experience. *JAMA Intern Med.* 2014; 174(10):1696-1697. doi:10.1001/jamainternmed.2014.3593