

Evidence-based Practice Center Systematic Review Protocol

Project Title: Laboratory biomarkers for iron deficiency anemia in late stage chronic kidney disease

I. Background and Objectives for the Systematic Review

Chronic kidney disease (CKD) is a serious condition with a worldwide impact. CKD patients are classified into five stages depending on the severity of the condition (CKD stage 1–5). When CKD progresses to its end stage, it would necessitate dialysis or kidney transplantation. Thus following kidney failure, patients are given three treatments options: hemodialysis, peritoneal dialysis, and kidney transplantation. A common complication of CKD is anemia, which develops early in the course of CKD and becomes increasingly severe as the disease progresses.¹ Anemia remains common among patients presenting for renal transplantation and persists in the post-transplant period.^{2,3} Anemia, with its associated fatigue, cognitive impairment, and diminished quality of life, is another significant problem for dialysis-dependent patients. According to the United States Renal Data System, 67.4 percent of patients initiating dialysis had hemoglobin (Hb) values below 11.0 g/dL.⁴ Despite its prevalence, anemia is generally treatable, and antianemic therapy is associated with reductions in mortality, morbidity, hospitalization, and medical costs in dialysis patients.⁵⁻¹¹ The most common cause of anemia in dialysis-dependent patients is inadequate erythropoietin production due to kidney damage, which is generally treated with erythropoiesis-stimulating agents (ESAs). Iron-deficiency anemia is the second most common cause of anemia in kidney dialysis-dependent patients and stems from inadequate diet and absorption, procedure-related iron losses from repeated laboratory testing, and blood retention in the dialyzer and tubing during dialysis.

The management of anemia in patients with CKD involves stimulating the generation of erythroblasts (erythropoiesis) and maintaining sufficient iron levels for optimum Hb production. Concentrations of Hb in nondialysis-dependent CKD patients who have anemia can be improved with oral iron supplementation (iron salts, e.g., ferrous sulfate). Among dialysis-dependent CKD patients (who may or may not be receiving ESA treatment), intravenous iron therapy is preferred over oral therapy because it decreases ESA dosages and significantly increases Hb concentrations.¹² ESAs are generally very effective in treating renal anemia in patients receiving hemodialysis and continuous ambulatory peritoneal dialysis but do not work well in patients with inadequate iron stores.¹³ Therefore, iron deficiency can prevent the effective treatment of anemia and may necessitate an increase in ESA dosages in an attempt to elicit an adequate response. The use of high doses is of concern not only because ESAs are very expensive, but also because higher dosing regimens of ESA are associated with adverse events, such as increased cardiovascular morbidity and mortality.^{12,14,15} For this reason, accurate assessment of iron status is essential to effective hemodialytic care.

Guidelines regarding the monitoring of iron deficiency and iron supplementation in patients on maintenance hemodialysis were first published by the National Kidney Foundation as part of their Kidney Disease Outcome Quality Initiative in 1997 and updated in 2000 and 2006.¹⁶ These guidelines suggest that Hb testing should be carried out annually in all patients with CKD and that such patients should be treated with ESAs when anemia is detected. Additionally, the guidelines stipulate that hemodialysis patients receiving erythropoietin should be monitored for iron deficiency monthly by measuring iron, total iron-binding capacity, and percent saturation of transferrin (iron/total iron-binding capacity * 100) and suggest that measurements of ferritin

concentrations be taken every 3 months. When treatment is required, the guidelines recommend the administration of sufficient iron to maintain a percent saturation of transferrin ≥ 20 percent and a serum ferritin level ≥ 100 ng/mL. The National Kidney Foundation guidelines have been widely adopted in dialysis centers across the United States. Similar best practice guidelines have been published for the management of anemia in patients with CKD in Europe.¹⁷

Unfortunately, traditional laboratory biomarkers of iron status in patients with CKD have several drawbacks: bone marrow biopsy involves risks of infection or bleeding at the biopsy site;¹⁸ serum ferritin and transferrin saturation are only useful when interpreted in the context of a patient's underlying erythropoietin responsiveness;¹⁹ the biological variability of serum iron, transferrin saturation, and ferritin is known to be large;¹⁹⁻²¹ and differences in successive measurements of 50 percent or more are not unusual for any of these markers. Additionally, there is an absence of an established reference method to serve as a gold standard for these assays, and considerable variability has been observed in comparisons of different assays, especially iron assays.^{22,23}

Another confounding factor is the effect of CKD-related inflammation on transferrin and ferritin. Transferrin and ferritin are both acute-phase reactants, and in the presence of an inflammatory condition, the transferrin concentration decreases and the ferritin concentration increases. When serum ferritin is used as a biomarker according to the thresholds defined by the National Kidney Foundation guidelines (< 100 ng/mL for the diagnosis of iron deficiency), the sensitivity is (approximately) only 50 percent.²⁴

Further complicating the matter, patients with CKD may suffer from various forms of iron deficiency, including absolute iron deficiency, functional iron deficiency, and an extreme case of functional iron deficiency known as reticuloendothelial blockage. The particular type of iron deficiency may affect the validity and reliability of laboratory test results for iron status and thus result in a dilemma regarding treatment decisions.²⁵

To find a more accurate and reliable test, newer biomarkers of iron status have been proposed. These include markers of iron deficiency, such as erythrocyte zinc protoporphyrin;²⁶ percentage of hypochromic erythrocytes;²⁷ reticulocyte Hb content;²⁸ and soluble transferrin receptor. Recent studies^{19,29,30} comparing the diagnostic values of these markers suggest that reticulocyte Hb content and percentage hypochromic erythrocytes could be valuable markers. In particular, reticulocyte Hb content has a lower biological variability ($\sim 3\%$) than traditional markers, such as transferrin saturation and ferritin (44% and 40%, respectively).¹⁹

Although a number of international guidelines have examined the use of both traditional and new serum iron biomarkers, their recommendations differ. Across guidelines, it is agreed that the optimal management of anemia in hemodialysis patients depends on diagnosis and management of iron-deficiency anemia. However, there is no consensus on a number of questions including:

- Which combination of iron biomarkers is required?
- Should the newer biomarkers be used as a replacement for older markers or as add-ons to older markers?
- How frequently should patients be tested for iron-deficiency anemia?
- What targets of iron therapy should be achieved?

Accurate management of iron status is expected to gain new attention after the recent adoption by the Centers for Medicare and Medicaid Services of a bundled reimbursement system for dialysis, where payment is made for groups of services—such as dialysis treatment, medical

treatments, and laboratory tests—rather than by individual service.³¹ In view of considerable clinical uncertainty, and the difficulties associated with known assays, the high biological variability associated with laboratory biomarkers, and the need for frequent assessment to guide treatment with ESAs, a systematic review is of priority. The focus of our proposed review would be to evaluate the strength of evidence for using these newly suggested markers, either as replacements or add-ons to currently used markers, in managing iron-replacement therapy in patients with CKD.

II. The Key Questions

Based on the public comments, we have revised the Key Questions (KQs) and study eligibility criteria to clarify the focus of the current comparative effectiveness review by using more precise definitions for patient populations and interventions of interest. Specifically, we clarified that different forms of iron deficiency exist in cases of anemia in patients with CKD, including absolute iron deficiency, functional iron deficiency, and an extreme case of functional iron deficiency known as reticuloendothelial blockage. Different forms of iron-deficiency anemia could affect the diagnostic test performance of iron-status markers and the effectiveness of treatments for iron-deficiency anemia. Thus, we are only interested in studies that report the iron status of study populations before they receive treatments to correct iron or anemia status. Along the same line of reasoning, we clarified that we are interested in comparing the use of newer laboratory biomarkers with the use of older laboratory biomarkers of iron status as part of the management strategies for iron-deficiency anemia.

Four KQs will be addressed in our systematic review:

Question 1 (Overarching Question)

What is the impact on patient-centered outcomes of using the newer laboratory biomarkers as a replacement for or an add-on to the older laboratory biomarkers of iron status for the diagnosis and management of iron deficiency anemia in stages 3–5 nondialysis and dialysis patients with CKD, and in patients with a kidney transplant?

Since test results have little direct impact on patient-relevant outcomes, the utility of medical tests is usually determined by their indirect effects on outcomes, that is, through their influence on therapeutic decisionmaking. Although overarching studies that assess the overall impact of tests on the clinical management process provide most of the direct evidence for answering this KQ, they are often challenging or infeasible to conduct. *If direct evidence is not sufficient to address this question, the overarching question can be indirectly answered by the following three interlinked questions:*

Key Question 2

What is the diagnostic test accuracy of newer markers of iron status (content of Hb in reticulocytes, percentage of hypochromic red blood cells, erythrocyte zinc protoporphyrin, soluble transferrin receptor, hepcidin, and superconducting quantum interference devices) as a replacement for or an add-on to the older markers (bone marrow iron stores, serum iron,

transferrin saturation, iron-binding capacity, and ferritin) in stages 3–5 nondialysis and dialysis patients with CKD and in patients with a kidney transplant?

- a. What reference standards are used for the diagnosis of iron status in studies evaluating test accuracy?
- b. What are the adverse effects or harms associated with testing using newer and/or older markers of iron status?

Question 3

In stages 3–5 nondialysis and dialysis CKD patients with iron-deficiency anemia, what is the impact of managing iron status based on newer laboratory biomarkers either alone or in addition to older laboratory biomarkers on intermediate outcomes (e.g., improvement in Hb levels, dose of ESAs, time in target Hb range), compared with managing iron status based on older laboratory biomarkers alone?

- a. What are the adverse effects or harms associated with the treatments guided by tests of iron status?

Question 4

What factors affect the test performance and clinical utility of newer markers of iron status in stages 3–5 nondialysis and dialysis CKD patients with iron-deficiency anemia? For example:

- Biological variation in diagnostic indices
- Use of different diagnostic reference standards
- Type of dialysis (i.e., peritoneal or hemodialysis)
- Patient subgroups (i.e., age, sex, comorbid conditions, ESA resistance, protein energy malnutrition secondary to an inflammatory state, hemoglobinopathies [e.g., thalassemia and sickle cell anemia])
- Route of iron administration (i.e., oral or intravenous)
- Treatment regimen (i.e., repletion or continuous treatment)
- Interactions between treatments (i.e., patients treated with vs. without ESAs, patients treated with vs. without iron-replacement therapy)
- Other factors

PICOTS Framework

Eligibility criteria for KQ1 (overarching question)

We will include comparative studies of different test-oriented treatments, defined as treatments for correcting iron status, as determined by measurements of older laboratory biomarkers in nondialysis-dependent and dialysis-dependent patients with stage 3, 4, or 5 CKD.

- **Populations:**

- Pediatric and adult nondialysis-dependent patients with stage 3, 4, or 5 CKD
- Patients with CKD undergoing dialysis (hemodialysis or peritoneal dialysis)
- Patients with a kidney transplant

- **Interventions:**
 - Newer laboratory biomarkers (i.e., content of Hb in reticulocytes, percentage of hypochromic red blood cells, erythrocyte zinc protoporphyrin, soluble transferrin receptor, hepcidin, and superconducting quantum interference devices) to diagnose and manage iron-deficiency anemia either as a replacement for or in addition to older laboratory biomarkers

- **Comparators:**
 - Older laboratory biomarkers of iron status (i.e., bone marrow iron stores, serum iron, transferrin saturation, iron-binding capacity, and ferritin) to diagnose and manage iron-deficiency anemia

- **Outcomes:**
 - Mortality
 - Morbidity (e.g. cardiac or liver toxicity, infection)
 - Quality of life, measured with standardized scales Kidney Disease Quality of Life, Health Related Quality of Life, Medical Outcomes Study Short Form-36 [SF-36], Pediatric Quality of Life Inventory, etc.)
 - Adverse effects or harms associated with testing and associated treatments (e.g., test-related anxiety, adverse events secondary to venipuncture, effects of iron overload with iron treatments, cardiovascular complications from use of erythropoietin at higher Hb levels, etc.)

- **Study designs:**
 - Randomized controlled trials
 - Nonrandomized controlled trials
 - Observational studies with concurrent comparison groups

Eligibility criteria for KQs 2–4

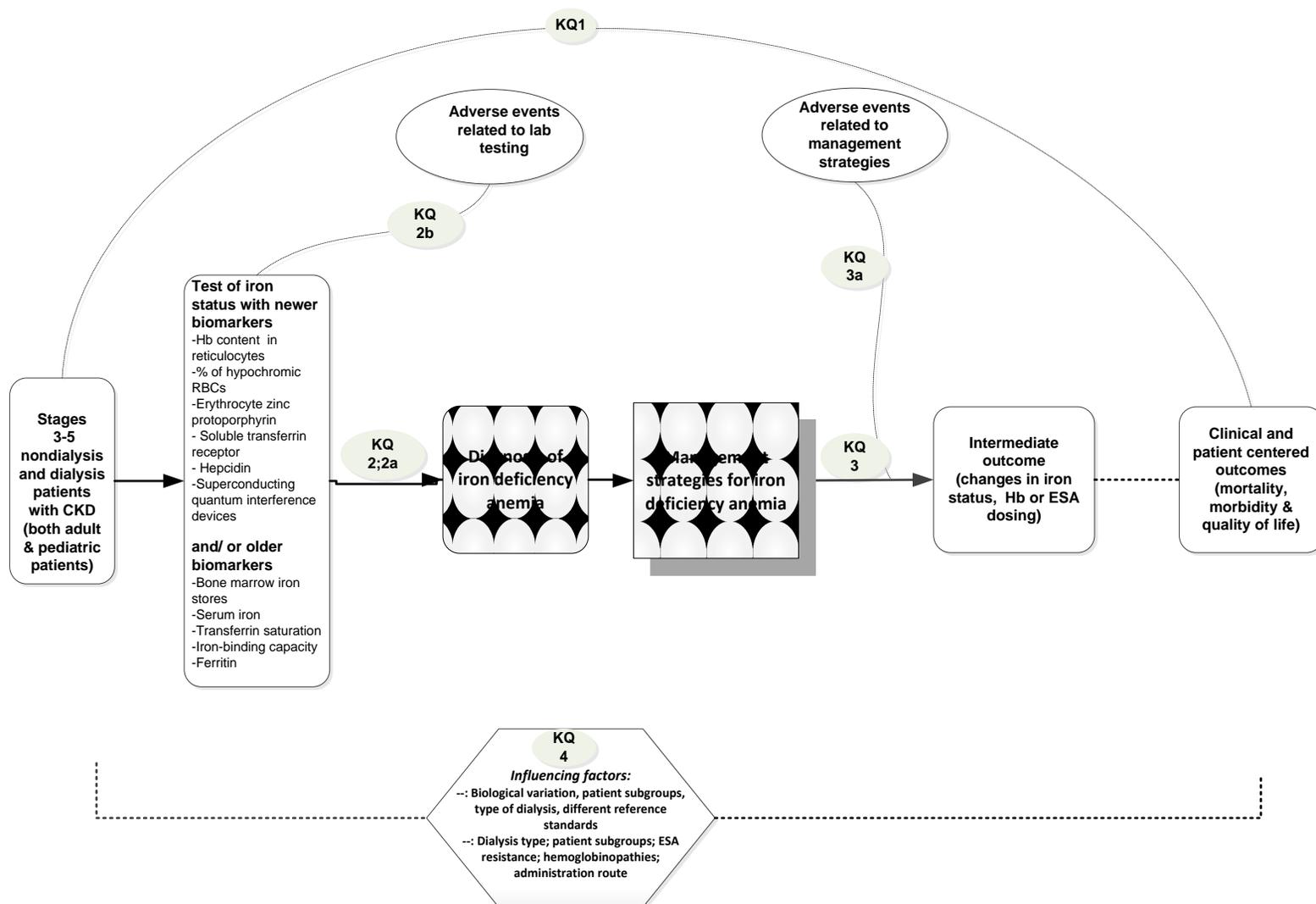
- **Populations:**
 - Pediatric and adult nondialysis-dependent patients with stage 3, 4, or 5 CKD
 - Patients with CKD undergoing dialysis (hemodialysis or peritoneal dialysis)
 - Patients with a kidney transplant

- **Interventions:**

- Newer laboratory biomarker alone or in combination with older laboratory biomarkers of iron status
- Newer laboratory biomarker include content of Hb in reticulocytes and percentage of hypochromic red blood cells, as well as other novel markers, such as erythrocyte zinc protoporphyrin, soluble transferrin receptor, hepcidin, and superconducting quantum interference devices
- **Comparators:**
 - Older laboratory biomarkers of iron status, which include bone marrow iron stores, serum iron, transferrin saturation, iron-binding capacity, and ferritin
- **Outcomes:**
 - **KQs 2 and 4:**
 - Measures of diagnostic test performance (e.g., sensitivity, specificity, predictive values, area under the receiver operating curve) comparing newer markers with older markers of iron status, including any reference standard used to analyze sensitivity and specificity in the original study, such as functional iron deficiency anemia as defined by response or no response to treatment
 - Adverse effects or harms associated with laboratory testing
 - **KQs 3 and 4:**
 - Intermediate outcomes
 - Increase in Hb or hematocrit, or more consistent maintenance of Hb or hematocrit within the desired range
 - Use of erythropoiesis-stimulating agents: Use of erythropoiesis-stimulating agents for maintenance of Hb within the desired range (stable dose in contrast to escalating dose resulting in net decreased ESA dose in hyporesponsive patients or actual decreased ESA dose in relatively responsive patients)
 - Adverse effects or harms associated with different management strategies
- **Setting**
 - Any setting: primary or specialty care, in a facility or at home, and inpatient or outpatient

III. Analytic Framework

Please see the KQs for detailed study eligibility criteria.



CKD = chronic kidney disease; ESA = erythropoiesis-stimulating agents; Hb = hemoglobin

Source: www.effectivehealthcare.ahrq.gov

Published Online: August 29, 2011

IV. Methods

A. Criteria for Inclusion/Exclusion of Studies in the Review

We will use the eligibility criteria for populations, interventions, comparators, outcomes, and study designs or setting (PICOS) as enumerated in Section II pertaining to the biomarkers of interest as listed in the KQs above. Inclusion/exclusion criteria for study design are detailed below.

- **Study design**

- **KQ 2:**

- Randomized controlled trials
- Nonrandomized comparative studies
- Prospective or retrospective cohort studies
- Cross sectional studies
- Case control studies
- N > 10 subjects (per arm, if there are ≥2 arms)

- **KQs 3 and 4:**

- Randomized controlled trials
- Nonrandomized comparative studies
- Observational studies with concurrent comparison groups
- N > 10 subjects (per arm, if there are ≥2 arms)

- **Other criteria:**

- We will exclude studies that only enrolled patients with anemia unrelated to iron deficiency or CKD.
- We will exclude studies that enrolled patients with anemia related to chronic disease but who have no underlying kidney disease.
- We will exclude studies that reported only data on analytical validity, such as correlations, mean change with treatment, or intrasubject and intersubject variation.

B. Searching for the Evidence: Literature Search Strategies for Identification of Relevant Studies To Answer the Key Questions

Appendix 1 describes our proposed literature search strategy. This search will be conducted in MEDLINE[®] and in the Cochrane Central Register of Controlled Trials. We will screen all abstracts available in English. Abstracts will be manually screened based on the eligibility criteria and exclusions cross-checked by a second member of the team. Any studies that are accepted based on their abstracts will then be reviewed in full. For those articles not available in English, we will identify and review these articles, provided native language speakers can be

identified. A list of articles excluded because of language will be included in the final report. Full-text articles will be screened independently by two investigators for eligibility. Disagreement about any article's eligibility will be resolved by consensus. The reasons for excluding these articles will be tabulated. We will ask our technical experts for potentially missing articles. All suggested articles will be screened for eligibility by using the same criteria as for the original articles. If necessary, we will revise the literature search to find articles similar to those missed in the original search. Additional studies will be identified through existing guidelines, narrative and systematic reviews, review of relevant conference proceedings, Scientific Information Packages from manufacturers, and a search of U.S. Food and Drug Administration databases. Following submission of the draft report, an updated literature search (using the same search strategy) will be conducted. Any additional studies that meet the eligibility criteria will be added to the final report.

C. Data Abstraction and Data Management

Each study will be extracted by one experienced methodologist. The extraction will be reviewed and confirmed by at least one other methodologist. Any disagreements will be resolved by discussion among the team. Data will be extracted into standard forms. The basic elements and design of these forms will be the similar to those we have used for other comparative effectiveness reviews and will include elements that address population characteristics, sample size, study design, descriptions of the test and reference standard, analytic details, and outcomes. Prior to extraction, the form will be customized to capture all elements relevant to the KQs. We will use separate forms for questions related to diagnostic test performance (KQ 2) and factors affecting the diagnostic test performance and the effectiveness of test-oriented treatments (KQ 4). We will test the forms on several studies and revise as necessary before full data extraction.

D. Assessment of Risk of Bias of Individual Studies

We will assess the methodological quality, or risk of biases, for each individual study by using the assessment instrument detailed by the Agency for Healthcare Research and Quality in its *Methods Guide for Effectiveness and Comparative Effectiveness Review*, hereafter referred to as the *AHRQ Methods Guide*.³² Briefly, we will rate each study as being of high, medium, or low risk of bias based on their adherence to well-accepted standard methodologies (i.e., QUADAS³³ for studies of diagnostic accuracy and the Cochrane risk of bias tool for intervention studies³⁴). We will assess and report each methodological quality item (Yes, No, or Unclear/Not reported) for all qualifying studies. In addition, we will consider the clarity and consistency in reporting as part of the overall judgment of risk of bias. When possible, we will perform sensitivity analyses to assess the impact of each quality item on the meta-analytic results. The grading will be outcome-specific, such that a given study that reports its primary outcome well but did an incomplete analysis of a secondary outcome would be graded of different quality for the two outcomes. Studies of different designs will be graded within the context of their study design. Thus, randomized controlled trials will be graded as having high, medium, or low risk of bias, and observational studies will be separately graded as having high, medium, or low risk of bias.

E. Data Synthesis

All included studies will be summarized in narrative form and in summary tables that tabulate the important features of the study populations, design, intervention, outcomes, and results. For example, population characteristics include age, sex, and race; design characteristics include recruitment and sampling; intervention characteristics include cutoffs used in index and reference tests; outcomes include mortality, morbidity, and quality of life; and results include sensitivity, specificity, and hazard ratio, among others. A meta-analysis of diagnostic tests, such as summary receiver operating curve, will be undertaken when there are more than three unique studies that used the same reference standard. For KQ 3, which evaluates treatments guided by tests of iron status on intermediate and clinical outcomes, we plan to perform meta-analyses where there are at least three unique studies that are deemed to be sufficiently similar in population and have the same comparison of interventions and the same outcomes. We will solicit input from our Technical Expert Panel (TEP) on our assessment of whether the included studies are clinically heterogeneous enough to be excluded from a meta-analysis or not. We plan to use the random-effects model for all meta-analyses.

F. Grading the Evidence for Each Key Question

We will follow the *AHRQ Methods Guide*³² to evaluate the strength of the body of evidence for each KQ with respect to four domains: risk of bias, consistency, directness, and precision. Briefly, we will define the risk of bias (low, medium, or high) based on the study design and the methodological quality of the studies.

We will rate the consistency of the data as: *no inconsistency*, *inconsistency present*, or *not applicable* if there is only one study available. We do not plan to use rigid counts of studies as standards of evaluation (e.g., 4 of 5 agree, therefore consistent) but instead will assess the direction, magnitude, and statistical significance of all studies and make a determination. We will describe our logic where the study findings are not unanimous.

We will assess the precision (precise or imprecise) of the evidence based on the degree of certainty surrounding an effect estimate. A precise estimate is an estimate that would allow a clinically useful conclusion. An imprecise estimate is one for which the confidence interval is wide enough to include clinically distinct conclusions (e.g., both clinically important superiority and inferiority—a situation in which the direction of effect is unknown), a circumstance that would preclude a conclusion.

Finally, we will rate the body of evidence based on four strength of evidence levels: high, moderate, low, and insufficient.³² These ratings will be based on our level of confidence that the evidence reflects the true effect for the major comparisons of interest.

G. Assessing Applicability

We will follow the *AHRQ Methods Guide*³² to evaluate the applicability of included studies to each patient population of interest, that is, nondialysis-dependent patients with stage 3, 4, or 5 CKD, patients with CKD who are undergoing hemodialysis or peritoneal dialysis, and patients who have had a kidney transplant. We will evaluate studies of pediatric, adult, or elderly adults separately if data are available.

VI. Definition of Terms

The following definitions were adapted from published articles or from the National Kidney Foundation's Kidney Disease Outcome Quality Initiative guidelines.^{1,35}

- *Absolute iron deficiency anemia*: Individuals with a transferrin saturation (TSAT) level <20 percent and a ferritin concentration <100 µg/L.
- *Functional iron deficiency anemia*: Individuals with a TSAT level <20 percent and a ferritin level of 100–700 µg/L. Functional iron deficiency is associated with adequate iron stores but insufficient release of iron to meet the demands of erythropoiesis. It is commonly seen among ESA-treated patients due to the inability of transferrin-bound iron to supply an adequate amount of Hb substrate for an increasing number of red blood cells in the bone marrow. In functional iron deficiency, TSAT levels may dip below 20 percent, whereas serum ferritin levels often remain normal or elevated.
- *Reticuloendothelial block*: Individuals with a TSAT <20 percent and a serum ferritin level of 100–800+ µg/L. Reticuloendothelial blockade is associated with normal or increased ferritin levels, increased levels of C-reactive protein and ESR, and low levels of TSAT.²⁵
- *Iron-replete status*: Individuals with a TSAT concentration ≥20 percent and a ferritin level ≥100 µg/L.
- *Definitions of CKD stages 1–5*:
 - CKD stage 1: a glomerular filtration rate (GFR) >90 ml/min, with some sign of kidney damage on other tests (if all the other kidney tests are normal, there is no CKD)
 - CKD stage 2: a GFR between 60 and 90 ml/min with some sign of kidney damage (if all the kidney tests are normal, there is no CKD)
 - CKD stage 3: a GFR between 30 and 59 ml/min and a moderate reduction in kidney function
 - CKD stage 4: a GFR between 15 and 29 ml/min and a severe reduction in kidney function
 - CKD stage 5: a GFR <15 ml/min and established kidney failure to the degree when dialysis or a kidney transplant may be needed.

V. References

1. National Kidney Foundation. KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Anemia in Chronic Kidney Disease. *Am J Kidney Dis* 2006 May;47(5 Suppl 3):S1-145. PMID: 16678659. Erratum in: *Am J Kidney Dis* 2006 Sep;48(3):518.
2. Mix TC, Kazmi W, Khan S, et al. Anemia: a continuing problem following kidney transplantation. *Am J Transplant* 2003 Nov;3(11):1426-33. PMID: 14525605.
3. Vanrenterghem Y, Ponticelli C, Morales JM, et al. Prevalence and management of anemia in renal transplant recipients: a European survey. *Am J Transplant* 2003 Jul;3(7):835-45. PMID: 12814475.
4. United States Renal Data System. 2010 USRDS Annual Data Report: Vol. 2 Atlas of End-Stage Renal Disease in the United States. Bethesda, MD, National Institute of Diabetes and Digestive and Kidney Diseases; 2010. Available at: <http://www.usrds.org/atlas.htm>. Accessed August 25, 2011.
5. Robertson HT, Haley NR, Guthrie M, et al. Recombinant erythropoietin improves exercise capacity in anemic hemodialysis patients. *Am J Kidney Dis* 1990 Apr;15(4):325-32. PMID: 2321645.
6. Pickett JL, Theberge DC, Brown WS, et al. Normalizing hematocrit in dialysis patients improves brain function. *Am J Kidney Dis* 1999;33:1122-1130. PMID: 10352201.
7. Foley RN, Parfrey PS, Harnett JD, et al. The impact of anemia on cardiomyopathy, morbidity, and mortality in end-stage renal disease. *Am J Kidney Dis* 1996 Jul;28(1):53-61. PMID: 8712222.
8. Harris DC, Chapman JR, Stewart JH, et al. Low dose erythropoietin in maintenance haemodialysis: improvement in quality of life and reduction in true cost of haemodialysis. *Aust N Z J Med* 1991 Oct;21(5):693-700. PMID: 1759917.
9. Evans RW, Rader B, Manninen DL. The quality of life of hemodialysis recipients treated with recombinant human erythropoietin. Cooperative Multicenter EPO Clinical Trial Group. *JAMA* 1990 Feb 9;263(6):825-30. PMID: 2404150.
10. Stevens ME, Summerfield GP, Hall AA, et al. Cost benefits of low dose subcutaneous erythropoietin in patients with anaemia of end stage renal disease. *BMJ* 1992 Feb 22;304(6825):474-7. PMID: 1547417.
11. Collins AJ, Li S, Ebben J, et al. Hematocrit levels and associated Medicare expenditures. *Am J Kidney Dis* 2000 Aug;36(2):282-93. PMID: 10922306.
12. Besarab A, Coyne DW. Iron supplementation to treat anemia in patients with chronic kidney disease. *Nat Rev Nephrol* 2010 Dec;6(12):699-710. PMID: 20956992.
13. Macdougall IC, Hutton RD, Cavill I, et al. Poor response to treatment of renal anaemia with erythropoietin corrected by iron given intravenously. *BMJ* 1989 Jul 15;299(6692):157-8. PMID: 2504356.
14. Singh AK, Szczech L, Tang KL, et al, for the CHOIR Investigators. Correction of anemia with epoetin alfa in chronic kidney disease. *N Engl J Med* 2006 Nov 16;355(20):2085-98. PMID: 17108343.
15. Singh AK, Fishbane S. The optimal hemoglobin in dialysis patients—a critical review. *Semin Dial* 2008 Jan-Feb;21(1):1-6. PMID: 18251947.
16. IV. NKF-K/DOQI Clinical Practice Guidelines for Anemia of Chronic Kidney Disease: update 2000. *Am J Kidney Dis* 2001Jan;37(1 Suppl 1):S182-238. PMID: 11229970. Erratum in: *Am J Kidney Dis* 2001 Aug;38(2):442.
17. Locatelli F, Aljama P, Barany P, et al, for the European Best Practice Guidelines Working Group. Revised European best practice guidelines for the management of anaemia in patients with chronic renal failure. *Nephrol Dial Transplant* 2004 May;19 Suppl 2:ii1-47. PMID: 15206425.
18. Bain BJ. Bone marrow biopsy morbidity: review of 2003. *J Clin Pathol* 2005 Apr;58(4):406-8. PMID: 15790706.
19. Fishbane S, Shapiro W, Dutka P, et al. A randomized trial of iron deficiency testing strategies in hemodialysis patients. *Kidney Int* 2001Dec;60(6):2406-11. PMID: 11737617.

20. Winkel P, Statland BE, Bokelund H. Factors contributing to intra-individual variation of serum constituents: 5. Short-term day-to-day and within-hour variation of serum constituents in healthy subjects. *Clin Chem* 1974 Dec;20(12):1520-7. PMID: 4430129.
21. Winkel P, Statland BE, Bokelund H. The effects of time of venipuncture on variation of serum constituents. Consideration of within-day and day-to-day changes in a group of healthy young men. *Am J Clin Pathol* 1975 Oct;64(4):433-47. PMID: 1239188.
22. Tietz NW, Rinker AD, Morrison SR. When is a serum iron really a serum iron? The status of serum iron measurements. *Clin Chem* 1994 Apr;40(4):546-51. PMID: 8149608.
23. Eckfeldt JH, Witte DL. Serum iron: would analytical improvement enhance patient outcome? *Clin Chem* 1994 Apr;40(4):505-7. PMID: 8149600. Comment on: *Clin Chem* 1994 Apr;40(4):546-51. PMID: 8149600.
24. Fishbane S, Kalantar-Zadeh K, Nissenson AR. Serum ferritin in chronic kidney disease: reconsidering the upper limit for iron treatment. *Semin Dial* 2004 Sep-Oct;17(5):336-41. PMID: 15461737.
25. Wish JB. Assessing iron status: beyond serum ferritin and transferrin saturation. *Clin J Am Soc Nephrol* 2006 Sep;1 Suppl 1:S4-8. PMID: 17699374.
26. Fishbane S, Lynn RI. The utility of zinc protoporphyrin for predicting the need for intravenous iron therapy in hemodialysis patients. *Am J Kidney Dis* 1995 Mar;25(3):426-32. PMID: 7872320.
27. Macdougall IC, Cavill I, Hulme B, et al. Detection of functional iron deficiency during erythropoietin treatment: a new approach. *BMJ* 1992 Jan 25;304(6821):225-6. PMID: 1739797.
28. Braun J, Lindner K, Schreiber M, et al. Percentage of hypochromic red blood cells as predictor of erythropoietic and iron response after i.v. iron supplementation in maintenance haemodialysis patients. *Nephrol Dial Transplant* 1997 Jun;12(6):1173-81. PMID: 9198047.
29. Brugnara C, Schiller B, Moran J. Reticulocyte hemoglobin equivalent (Ret He) and assessment of iron-deficient states. *Clin Lab Haematol* 2006 Oct;28(5):303-8. PMID: 16999719.
30. Tessitore N, Solero GP, Lippi G, et al. The role of iron status markers in predicting response to intravenous iron in haemodialysis patients on maintenance erythropoietin. *Nephrol Dial Transplant* 2001 Jul;16(7):1416-23. PMID: 11427634.
31. Centers for Medicare and Medicaid Services. CMS proposes new prospective payment system for renal dialysis facilities [press release]. September 15, 2009. Available at: http://www.asn-online.org/policy_and_public_affairs/docs/CMS%20Releases%20Proposal%20for%20New%20ESRD%20Bundled%20Payment%20System.pdf. Accessed: August 25, 2011.
32. Methods Guide for Effectiveness and Comparative Effectiveness Reviews. AHRQ Publication No. 10(11)-EHC063-EF. Rockville, MD: Agency for Healthcare Research and Quality; August 2011. Chapters available at: www.effectivehealthcare.ahrq.gov.
33. Whiting P, Rutjes A, Reitsma J, et al. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. *BMC Med Res Methodol* 2003 Nov 10;3:25. PMID: 14606960.
34. Higgins JPT, Green S, eds. *Cochrane handbook for systematic reviews of interventions*. Version 5.1.0 [updated March 2011]. London: The Cochrane Collaboration; 2011.
35. Horl WH. Clinical aspects of iron use in the anemia of kidney disease. *J Am Soc Nephrol* 2007 Feb;18(2):382-93. PMID: 17229908.

VII. Summary of Protocol Amendments

In the event of protocol amendments, the date of each amendment will be accompanied by a description of the change and the rationale.

VIII. Review of Key Questions

For all EPC reviews, key questions were reviewed and refined as needed by the EPC with input from Key Informants and the Technical Expert Panel (TEP) to assure that the questions are specific and explicit about what information is being reviewed. In addition, for Comparative Effectiveness reviews, the key questions were posted for public comment and finalized by the EPC after review of the comments.

IX. Key Informants

Key Informants are the end users of research, including patients and caregivers, practicing clinicians, relevant professional and consumer organizations, purchasers of health care, and others with experience in making health care decisions. Within the EPC program, the Key Informant role is to provide input into identifying the Key Questions for research that will inform healthcare decisions. The EPC solicits input from Key Informants when developing questions for systematic review or when identifying high priority research gaps and needed new research. Key Informants are not involved in analyzing the evidence or writing the report and have not reviewed the report, except as given the opportunity to do so through the public review mechanism.

Key Informants must disclose any financial conflicts of interest greater than \$10,000 and any other relevant business or professional conflicts of interest. Because of their role as end-users, individuals are invited to serve as Key Informants and those who present with potential conflicts may be retained. The TOO and the EPC work to balance, manage, or mitigate any potential conflicts of interest identified.

X. Technical Experts

Technical Experts comprise a multi-disciplinary group of clinical, content, and methodological experts who provide input in defining populations, interventions, comparisons, or outcomes as well as identifying particular studies or databases to search. They are selected to provide broad expertise and perspectives specific to the topic under development. Divergent and conflicted opinions are common and perceived as health scientific discourse that results in a thoughtful, relevant systematic review. Therefore study questions, design and/or methodological approaches do not necessarily represent the views of individual technical and content experts. Technical Experts provide information to the EPC to identify literature search strategies and recommend approaches to specific issues as requested by the EPC. Technical Experts do not do analysis of any kind nor contribute to the writing of the report and do not have an opportunity to review the report until the public review period.

Technical Experts must disclose any financial conflicts of interest greater than \$10,000 and any other relevant business or professional conflicts of interest. Because of their unique clinical or content expertise, individuals are invited to serve as Technical Experts and those who present with potential conflicts may be retained. The TOO and the EPC work to balance, manage, or mitigate any potential conflicts of interest identified.

XI. Peer Reviewers

Source: www.effectivehealthcare.ahrq.gov

Published Online: August 29, 2011

Approximately five experts in the field will be asked to peer review the draft report and provide comments. Peer reviewers are invited to provide written comments on the draft report based on their clinical, content, or methodological expertise. The peer reviewer may represent stakeholder groups such as professional or advocacy organizations with knowledge of the topic. On some specific reports such as reports requested by the Office of Medical Applications of Research, National Institutes of Health there may be other rules that apply regarding participation in the peer review process. Peer review comments on the preliminary draft of the report are considered by the EPC in preparation of the final draft of the report. Peer reviewers do not participate in writing or editing of the final report or other products. The synthesis of the scientific literature presented in the final report does not necessarily represent the views of individual reviewers. The dispositions of the peer review comments are documented and will, for CERs and Technical briefs, be published three months after the publication of the Evidence report.

Potential Reviewers must disclose any financial conflicts of interest greater than \$10,000 and any other relevant business or professional conflicts of interest. Invited Peer Reviewers may not have any financial conflict of interest greater than \$10,000. Peer reviewers who disclose potential business or professional conflicts of interest may submit comments on draft reports through the public comment mechanism.

It is our policy not to release the names of the Peer reviewers or TEP panel members until the report is published so that they can maintain their objectivity during the review process.

Appendix 1. Preliminary Search Strategy

Database(s): Ovid MEDLINE(R) 1948 to March Week 2 2011, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations March 18, 2011

Search Strategy:

#	Searches	Results
1	percentage of hypochromic erythrocytes.mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier]	15
2	(percentage adj2 hypochromic erythrocytes).tw.	15
3	*Reticulocytes/pa [Pathology]	75
4	(%HYPO and CHR).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier]	10
5	*Erythrocyte Indices/	768
6	Erythrocyte Count.mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier]	9,779
7	*Erythrocytes/an, du, me [Analysis, Diagnostic Use, Metabolism]	18,611
8	*Erythrocytes/pa	973
9	Erythropoiesis/ph	1,203
10	*Reticulocytes/ch	64
11	*Reticulocyte Count/	154
12	Ferritins/bl	6,714
13	*Hemoglobins/an	4,903
14	*Erythrocyte Indices/	768
15	Reticulocytes/me	4,553
16	Transferrin/an	3,747
17	TSAT.tw.	177
18	exp Anemia, Hypochromic/ or exp Anemia, Iron-Deficiency/	14,138
19	hypochromic an?emia.mp.	765
20	hypochromic erythrocytes.mp.	37
21	Anemia, Iron-Deficiency/dt	1,151
22	(transferrin adj saturation).af.	2,323
23	exp transferrin/	14,302
24	hepcidin.af.	1,261
25	(zinc adj protoporphyrin).af.	5
26	erythrocyte zinc protoporphyrin.mp.	1
27	superconducting quantum interference device.mp.	322
28	*Biological Markers/an, bl, me [Analysis, Blood, Metabolism]	9,955
29	*ferritins/ or *apoferritins/	6,686
30	acute-phase proteins/ or exp transferrin/	18,409
31	transferrin.mp.	26,930
32	Transferrin/ad, an, bl, du, de, me, pk, tu [Administration & Dosage, Analysis, Blood, Diagnostic Use, Drug Effects, Metabolism, Pharmacokinetics, Therapeutic Use]	10,236
33	or/1-32	99,426
34	exp "sensitivity and specificity"/	325,176

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35	exp Predictive Value of Tests/	107,715
36	exp ROC CURVE/	18,819
37	exp Mass Screening/	84,398
38	exp diagnosis/	5,287,805
39	exp REPRODUCIBILITY OF RESULTS/	208,459
40	exp false negative reactions/ or false positive reactions/	30,435
41	predictive value.tw.	43,735
42	(sensitivity or specificity).tw.	581,666
43	accuracy.tw.	167,564
44	screen\$.tw.	353,034
45	diagno\$.tw.	1,330,720
46	roc.tw.	13,704
47	reproducib\$.tw.	89,883
48	(false positive or false negative).tw.	39,644
49	likelihood ratio.tw.	4,561
50	accuracy.tw.	167,564
51	di.fs.	1,690,735
52	biological variability.mp.	655
53	reference values.tw.	7,907
54	reference standard\$.tw.	6,224
55	or/34-54	6,987,404
56	(NeoRecormon or Aranesp or Methoxy Polyethylene Glycol Epoetin Beta or MIRCERA or Epoetin or Dynepo or PDpoetin).af. or (NeoRecormon or Aranesp or Methoxy Polyethylene Glycol Epoetin Beta or MIRCERA or Epoetin or Dynepo or PDpoetin).tw.	2,115
57	(epogen or epotin or betapoietin or relpoietin or epokine or procrit or eprex or darbopoietin).af. or (epogen or epotin or betapoietin or relpoietin or epokine or procrit or eprex or darbopoietin).tw.	213
58	exp recombinant erythropoietin/ or recombinant erythropoietin.mp.	5,167
59	or/56-58	5,797
60	exp Renal Replacement Therapy/ or exp Renal Dialysis/ or exp Kidney Transplantation/ or exp Kidney Function Tests/ or renal.mp. or nephro\$.mp. or kidney.mp. or ur?emia.tw. or h?emodialysis.tw.	780,133
61	hemodialysis.af.	41,409
62	peritoneal dialysis.mp. or exp Peritoneal Dialysis/	23,533
63	exp Kidney Diseases/ or exp Kidney Failure, Chronic/ or chronic kidney disease.mp. or exp Chronic Disease/ or exp Kidney Glomerulus/	556,761
64	or/60-63	999,951
65	33 and 55 and 64	4,992
66	33 and 59 and 64	563
67	65 and 66	361
68	65 not 67	4,631
69	68 or 66	5,194
70	remove duplicates from 69	5,168

