Journal of Clinical Laboratory Analysis 14:271–273 (2000)

New Automated Chemiluminescent Assay for Erythropoietin

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Erythropoietin (EPO) is a polypeptide hormone produced by the kidney that regulates erythropoiesis by controlling the proliferation and differentiation of erythroid progenitors in bone marrow. Assays for EPO are used to monitor dosage and response to human recombinant erythropoietin also may have diagnostic utility in the differential diagnosis of anemia and polycythemia. We evaluated an automated, chemiluminescent immunoassay for EPO (DPC Immulite) in terms of precision, linearity, interference, and correlation with reference assays. The Immulite assay demonstrated acceptable correlation with the reference immunochemiluminometric method (slope = 1.087, *y* intercept = 0.567, *R* value = 0.990). Within-run CVs ranged from 2.3% to 5.0%, while between-run CVs ranged from 4.1% to 9.5%. Linearity extended beyond the manufacturer's stated claims, and recovery ranged from 96.8% to 100.9% across the concentrations tested. No significant interference was noted with hemoglobin, bilirubin, or triglyceride. Overall, this method compares favorably with the existing immunochemiluminometric reference method and offers clinical laboratories an alternative for the analysis of erythropoietin. J. Clin. Lab. Anal. 14:271–273, 2000. © 2000 Wiley-Liss, Inc.

Key words: immunoassay; anemia; polycythemia; kidney; hormone

Erythropoietin (EPO) is produced by proximal renal tubular cells in response to decreased tissue oxygen tension in the kidney (1). Erythropoietin regulates erythropoiesis by stimulating the formation and maturation of erythroid progenitors, resulting in increased red cell mass and elevated tissue oxygen levels.

The availability of recombinant human erythropoietin since the mid-1980s has made possible the development of sensitive and specific immunoassay methods for measuring EPO levels (2–4). Erythropoietin assays are useful diagnostic tools for monitoring the dosage and response to recombinant human erythropoietin therapy used for some types of anemias. Serum EPO levels are usually inappropriately low in patients with end-stage renal failure due to underproduction of EPO by the kidney. Treatment with recombinant human erythropoietin can correct the anemia in these patients, but due to the expense of the drug and the potential for hypertension and/or thrombosis, close monitoring of serum EPO levels is indicated (5).

Serum EPO levels are also useful in determining the cause of polycythemia (6). Polycythemia, an overproduction of erythrocytes, may result from increased EPO secretion in response to uncompensated hypoxia, or it may be autonomous and independent of EPO levels, as is the case with polycythemia vera (7). Distinguishing between the causes of erythrocytosis is complicated because both primary and secondary polycythemias can have EPO levels that overlap with the normal reference range. In general, however, patients with secondary polycythemia have a relatively higher concentration of EPO than patients with polycythemia vera (8,9).

The Diagnostics Products Corporation (DPC, Los Angeles, CA) Immulite fully automated chemiluminescent enzyme-labeled immunometric assay for EPO measurement is a sensitive and specific in-house method for the quantitation of this hormone. In this study, we assessed the performance of the DPC Immulite EPO assay in terms of precision, linearity, interference, and correlation with reference methods.

MATERIALS AND METHODS

The DPC assay consists of a ligand-labeled monoclonal anti-EPO capture antibody, an alkaline phosphatase-labeled polyclonal conjugate antibody, and solid-phase anti-ligand-coated polystyrene beads. Patient sample and ligand-labeled anti-EPO antibody are incubated with the solid phase, and EPO in the patient sample binds to the monoclonal antibody and is immobilized onto the solid phase. Alkaline phosphatase-labeled polyclonal anti-EPO antibody is then introduced, which binds to the EPO attached to the solid phase, and un-

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Received 10 January 2000; Accepted 12 June 2000

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bound enzyme conjugate is removed by a wash step. A chemiluminescent substrate is added, and the photon output, which is proportional to the EPO concentration in the sample, is measured by a luminometer.

Comparison versus an immunochemiluminometric (ICMA) method was performed by analysis of 20 patient samples obtained from the Mayo Clinic with EPO concentrations ranging from 1.5 to 136 mU/ml. The samples were assayed by the DPC method under investigation, and results were correlated with those from results from the Mayo Reference Laboratory immunochemiluminometric assay.

Comparison versus a radioimmunoassay (RIA) method was performed by analysis of 50 samples obtained at the University of Alabama at Birmingham from normal individuals as well as from patients with anemia and renal disease. Patient samples were selected to encompass a serum ferritin range of 8–1,054 ng/ml, with EPO concentrations ranging from 3.0 to 156.0 mU/ml. The samples were assayed by the DPC method under investigation, and results were correlated with those from a commercial RIA (SmithKline Beecham Clinical Laboratories, Quest Diagnostics, Rockville, MD), with a lower limit of detection of 5 mU/ml. For graphical purposes, samples with an RIA EPO level of less than 5 mU/ml were plotted as 5 mU/ml.

Linearity studies were performed using non-human serum matrix calibrators from DPC with target concentrations ranging from 0 to 193 mU/ml. The limit of detection was established by analyzing the zero calibrator five times. Within-run precision was performed on 20 consecutive replicates, while between-run precision was calculated using data from 10 separate runs, performed in duplicate.

Interference studies were performed using three serum pools spiked with increasing amounts of bilirubin, hemoglobin, or triglyceride. Bilirubin was obtained from Sigma Chemical Co. (St. Louis, MO). Hemoglobin was obtained from osmotically shocked heparinized red blood cells. For triglyceride interference studies, Liposyn, a commercial fat emulsion produced by Abbot (Abbot Park, IL), was used. Erythropoietin levels were compared to the original serum pool plus vehicle, and the percentage change was calculated for each concentration. Five to seven concentrations each of bilirubin, hemoglobin, or triglyceride were tested at concentrations ranging from 1.5 to 25.3 mg/L of bilirubin, 0.01 to 0.5 g/dl of hemoglobin, and 150 to 2,401 mg/dl of lipid, respectively.

RESULTS

Acceptable correlation was obtained between the Immulite® EPO assay and the reference immunochemiluminometric method (Fig. 1). The *R* value was 0.990 with a *y* intercept of 0.567, a slope of 1.087, and $S_{y,x}$ of 6.3. Correlation between the Immulite EPO assay and the radioimmunoassay method was not as good, with an *R* value of 0.936, a slope of 1.653, a *y* intercept of 4.477, and $S_{y,x}$ of 13.4 (Fig. 2). The positive bias of the Immulite EPO assay compared to the RIA may be partly (but not entirely) accounted for by the fact that the normal EPO range for the RIA is <25 mU/ml, compared to a normal range of 2.6–34.0 mU/ml for the Immulite EPO assay.

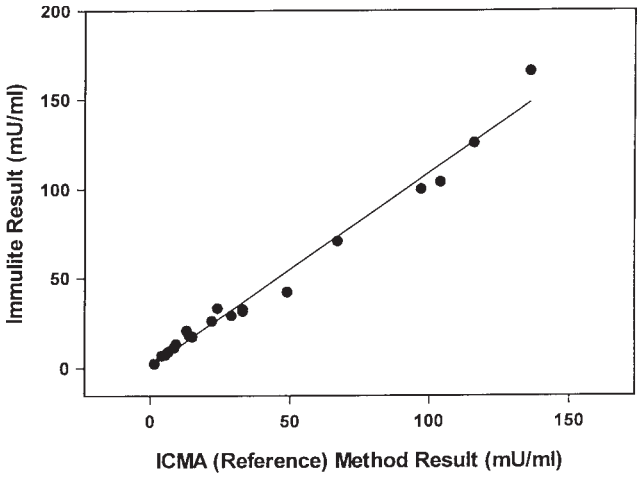

Fig. 1. Comparison of DPC Immulite erythropoietin results from 20 patients versus results obtained using a reference immunochemiluminometric (ICMA) method. Levels of circulating erythropoietin are determined by the DPC Immulite chemiluminescent erythropoietin assay and plotted against those obtained with a reference immunochemiluminometric (ICMA) method. The slope is 1.087, the *y* intercept is 0.567, the *R* value is 0.990, and the $S_{y,x}$ is 6.3.

The Immulite EPO assay demonstrated good linearity over the manufacturer's range of 0.24–200 mU/ml (Fig. 3). In serial dilution studies, recoveries varied from 97.3% to 100.9% throughout the range of concentrations tested. The lower limit of detection for the Immulite assay was determined to be 0.16

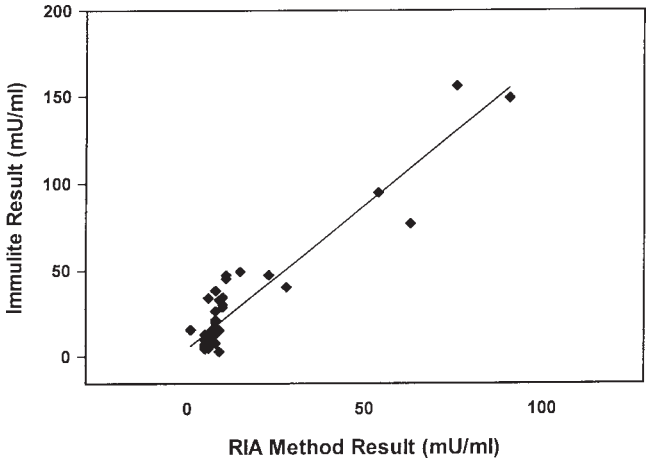

Fig. 2. Comparison of DPC Immulite erythropoietin results from 50 patients versus results obtained using a reference radioimmunoassay (RIA) method. Levels of circulating erythropoietin are determined by the DPC Immulite chemiluminescent erythropoietin assay and plotted against those obtained with a reference radioimmunoassay (RIA) method. The slope is 1.653, the *y* intercept is 4.477, the *R* value is 0.936, and the $S_{y,x}$ is 13.4.

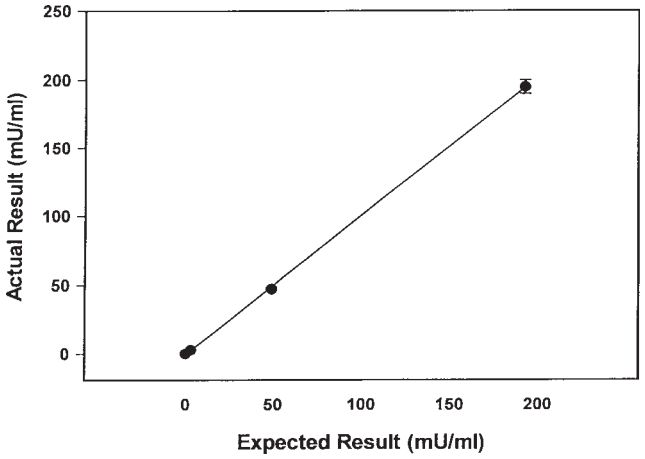

Fig. 3. Linearity of the DPC Immulite erythropoietin assay. Linearity of the assay was confirmed by running four DPC-supplied calibrators with expected EPO concentrations of 0, 3.0, 48.9, and 193.0 mU/ml in quintuplicate. Data are shown as the mean ± SD. The slope is 1.010 and the *y* intercept is –0.584.

mU/ml, which is better than the manufacturer's recommended lower limit of detection of 0.24 mU/ml.

Within-run precision was assessed at three levels of controls containing 15.3, 30.1, and 60.0 mU/ml of EPO, respectively. Between-run precision was measured using the same controls. The low control had a within-run mean of 14.72 mU/ml, a within-run CV of 2.6%, a between-run mean of 13.85 mU/ml, and a between-run CV of 9.5%. The middle control had a within-run mean of 29.79 mU/ml, a within-run CV of 5.0%, a between-run mean of 28.33 mU/ml, and a between-run CV of 4.5%. The high control had a within-run mean of 59.72 mU/ml, a within-run CV of 2.3%, a between-run mean of 56.77 mU/ml, and a between-run CV of 4.1%.

Interference studies demonstrated less than 10% interference for the concentrations of bilirubin, hemoglobin, and lipid tested. The single greatest interference was –8.3%, which was observed at a bilirubin concentration of 25.3 mg/dl.

DISCUSSION

Erythropoietin measurements are useful for monitoring dosage and response to human recombinant erythropoietin, and they also may have utility in the differential diagnosis of anemia and polycythemia. In secondary polycythemia, the EPO level is generally elevated, while in polycythemia vera and anemia associated with chronic renal failure, the EPO level tends to be low. In order to follow EPO levels in patients on recombinant human erythropoietin, an accurate and precise assay is required in order to implement proper dosing while avoiding side effects. In order to provide fast turnaround times to clinicians administering therapy, it is ideal to have an assay available in-house.

Before a laboratory can implement an automated EPO method, however, it is essential for the assay to correlate with an existing reference method, which the DPC EPO assay does. Within-run CVs of 2.3–5.0% and between-run CVs of 4.1–9.5% for the DPC EPO assay are acceptable. Furthermore, the DPC Immulite® assay sensitivity is well below the normal EPO reference range (2.6–34.0 mU/ml), thus making it suitable both for monitoring recombinant human erythropoietin levels and for assisting in the diagnosis of polycythemia vera. The DPC Immulite EPO assay offers the additional significant advantage of the improved turnaround time that comes with an in-house assay. The Immulite method is fully automated, reducing the amount of technologist time required to perform the test. We conclude that the DPC Immulite chemiluminescent erythropoietin assay is an acceptable alternative to the immunochemiluminometric reference assay and, as a result, have implemented this assay in our own laboratory.

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