

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

K052223

B. Purpose for Submission:

New Device

C. Measurand:

Erythropoietin (EPO)

D. Type of Test:

Quantitative, Immunoenzymatic

E. Applicant:

Beckman Coulter, Inc.

F. Proprietary and Established Names:

Access Erythropoietin (EPO) Assay and Calibrator

G. Regulatory Information:

1. Regulation section:

21 CFR 864.7250

21 CFR 862.1150

2. Classification:

Class II

3. Product code:

GGT

JIT

4. Panel:

81 (Hematology)

H. Intended Use:

1. Intended use(s):

The Access EPO assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of erythropoietin levels in human serum and plasma (heparin) using the Access Immunoassay Systems. This assay is intended as an aid in the diagnosis of anemias and polycythemias. With the advent of the administration of recombinant erythropoietin as a biologic therapy to increase red blood cell mass, an erythropoietin assay may be used also to aid in the prediction and monitoring of response to recombinant erythropoietin treatment in persons with anemias.

The Access EPO calibrators are intended to calibrate the Access EPO assay for the quantitative determination of EPO levels in human serum and plasma (heparin) using the Access Immunoassay Systems.

2. Indication(s) for use:

Same as above.

3. Special conditions for use statement(s):

Not applicable.

4. Special instrument requirements:

For use with the Access family of Immunoassay analyzers (Access, Access 2, Synchron LX®i 725, UniCel DxI 800, UniCel DxC 600i)

I. Device Description:

The Access EPO assay consists of the reagent pack and calibrators. The reagent pack consist of three specific reagents; (1) Paramagnetic particles coated with goat anti-mouse IgG: mouse anti-recombinant human EPO monoclonal antibody, BSA, sodium azide and ProClin 300, (2) Chicken anti-recumbent mouse alkaline phosphatase (bovine) conjugate, BSA, 0.1% sodium aide and 0.7% ProClin 300, (3) TRIS saline buffer containing BSA, proteins (chicken, bovine, mouse), <0.1% sodium azide and 0.17% ProClin 300. Two reagent packs containing 50 tests per pack are provide for a total of 100 assay determinations. The calibrator kit provides calibrators at 6 levels: zero (S0) and approximately 5, 25, 125, 375, 750ml mIU/ml (S1 – S5) and one Calibration Card. The S0 calibrator vial contains 10 ml and each S1 – S5 vial contains

2.5mL. Other items needed to perform the assay include the Access substrate and wash buffers for use with the Access family of Immunoassay Analyzers in the clinical laboratory.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Quantikine IVD Erythropoietin ELISA Kit - R & D Systems, Inc.
 IMMULITE EPO - Diagnostic Products Corp.

Nichols Advantage Chemiluminescent Erythropoietin Immunoassay - Nichols Institute Diagnostics

EPO [Erythropoietin] ELISA Assay - Sangui Bio Tech, Inc.

2. Predicate 510(k) number(s):

K936016, K983203, K980737, K992799

3. Comparison with predicate:

Similarities					
<i>Characteristic</i>	<i>BCI Access EPO</i>	<i>RDS Quantikine</i>	<i>DPC Immulite</i>	<i>Nichols Advantage</i>	<i>Sangui Bio EPO ELISA Kit</i>
Indications for use	For the quantitative determination of erythropoietin levels in human serum and plasma (heparin) using the Access Immunoassay Systems. This assay is intended as an aid in the diagnosis of anemias and polycythemias. With the advent of the administration of recombinant erythropoietin as a biologic therapy to increase red blood cell mass, an erythropoietin assay may be used also to aid in the prediction and monitoring of response to recombinant erythropoietin treatment in persons with anemias.	For the quantitative measurement of EPO levels in serum or plasma by enzyme linked immunosorbent assay (ELISA). The quantitative determination of EPO aids in the diagnosis of anemias and polycythemias.	For the quantitative measurement of EPO in serum or heparinized plasma. It is intended strictly for the in vitro diagnostic use as an aid in the diagnosis of anemias and polycythemias.	For the quantitative determination of EPO concentration in human serum as an adjunct in the diagnosis of anemia and polycythemias.	For the quantitative determination of erythropoietin levels in human serum. This assay is intended as an aid in the diagnosis of anemias and polycythemias. With the advent of the administration of recombinant erythropoietin as a biologic therapy to increase red blood cell mass, an erythropoietin assay may be used also to aid in the prediction and monitoring of response to recombinant erythropoietin treatment in persons with anemias.

Similarities					
<i>Characteristic</i>	<i>BCI Access EPO</i>	<i>RDS Quantikine</i>	<i>DPC Immulite</i>	<i>Nichols Advantage</i>	<i>Sangui Bio EPO ELISA Kit</i>
Assay type/format	2-site simultaneous immunometric (sandwich) chemiluminescent	ELISA	2-site sequential immunometric (sandwich) chemiluminescent	Same	2-site simultaneous immunometric (sandwich) chemiluminescent (ELISA)
Composition	<p>Paramagnetic particles coated with goat anti-mouse IgG: mouse anti-recombinant human EPO monoclonal antibody, BSA, sodium azide and ProClin 300.</p> <p>Chicken anti-recumbent mouse alkaline phosphatase (bovine) conjugate, BSA, 0.1% sodium azide and 0.7% ProClin 300,</p> <p>TRIS saline buffer containing BSA, proteins (chicken, bovine, mouse), <0.1% sodium azide and 0.17% ProClin 300</p>	<p>Microplate - polystyrene microplate coated with mouse monoclonal antibody against recombinant human EPO.</p> <p>Conjugate - anti EPO polyclonal (rabbit) antibody: horseradish peroxidase conjugate w/ thimerosal as a preservative</p>	<p>Solid phase - Polystyrene bead coated with an anti-ligand derived from streptavidin.</p> <p>Reagent – ligand labeled murine monoclonal anti-EPO antibody with preservative. Alkaline phosphatase conjugated to goat polyclonal anti-EPO antibody in buffer, with preservatives.</p>	<p>Streptavidin coated magnetic particles in a buffer containing goat, rabbit and mouse gamma globulin with sodium azide and ProClin 300.</p> <p>Acridinium ester-labeled mouse monoclonal antibody to human EPO in a buffered protein solution with antimicrobial agents. Biotin labeled mouse monoclonal antibody to human EPO in a buffered protein solution, with sodium azide and ProClin 300.</p>	<p>Carboxyl-terminal mouse monoclonal antibody & an affinity purified region-restricted amino-terminal sheep antibody. Solid phase coated with avidin. Capture antibodies are coupled with biotin. Horseradish peroxidase labeled Tag antibody.</p>
Measuring/ Reportable Range	0.6 - 750 mIU/mL			5-700 mIU/mL	Unknown
Expected Values	2.59-18.50 mIU/mL	3.3 – 16.6 mIU/mL	3.7 – 29.5 mIU/mL	5.0 – 25.1 mIU/ml	Unknown
Analytical Sensitivity	≤ 0.6 mIU/mL	~ <0.6 mIU/mL			Unknown
Standardization	WHO 2 nd IRP 67/343	WHO 2 nd IRP 67/343	WHO 2 nd IRP 67/343	WHO 1 st IS 87/684	Unknown
Sample Types	Serum, heparinized plasma	Serum & EDTA plasma	Serum, heparinized plasma	Serum	Serum
Sample Size	85µl		100 µl		Unknown

Differences				
<i>Characteristic</i>	<i>BCI Access EPO</i>	<i>RDS Quantikine</i>	<i>DPC Immulite</i>	<i>Nichols Advantage</i>
Calibrator Stability	28 days	Each run	2 weeks	1 week
Sample Size	85µl	≥ 0.25 mL		200 µl
Analytical Sensitivity	≤ 0.6 mIU/mL		0.24 mIU/mL	1.2 mIU/mL
Measuring/ Reportable Range	0.6 - 750 mIU/mL	0 – 200 mIU/mL	0 – 200 mIU/mL	

K. Standard/Guidance Document Referenced (if applicable):

Document for Special Controls for Erythropoietin Assay Premarket Notification [510(k)s] – April 1999

Abbreviated 510(k) Submission for In Vito Diagnostic Calibrators – February 1999

EP5A Evaluation of Precision Performance of Clinical Chemistry Device Approved Guideline, CLSI/NCCLS

EP7-P Interference Testing in Clinical Chemistry, CLSI/NCCLS

EP9-A2 Method Comparison and Bias Estimation Using Patient Samples, Approved Standard-Second Edition, CLSI/NCCLS

EP14A2 Evaluation of Matrix Effects, CLSI/NCCLS

C28-A2 How to Define and Determine Reference Intervals in the Clinical Laboratory, CLSI/CLSI/NCCLS

L. Test Principle:

The Access EPO assay is a two-site immunoenzymatic (“sandwich”) assay. A sample is added to a reaction vessel along with the paramagnetic particles coated with mouse monoclonal anti-EPO blocking reagent and the alkaline phosphatase conjugate. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportion to the concentration of EPO in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Precision testing was performed using patient samples and three levels of controls (0-10, 10-100, 100-500 mIU/mL), in duplicate, for 20 runs, completing 2 runs per day, for at least 10 day. The assay exhibits total precision of ≤ 10% CV at EPO concentrations greater than 3 mIU/mL. Assay precision was tested at concentrations from approximately 9 to 475 mIU/mL. The within-run imprecision ranged from 1.8% CV to 8.7% CV. Total imprecision ranged from 2.6% CV to 8.7% CV.

b. *Linearity/assay reportable range:*

Dilution recovery studies were performed by diluting multiple human serum and plasma (heparin) samples at various levels with Access EPO Calibrator S0. Sample mean recovery values for all serum and plasma samples were within the range of $100 \pm 15\%$.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

EPO reagents are stable for 28 days after opening and calibrators are stable for 90 days after opening. The calibration curve is stable for 28 days.

The measurand in the Access EPO Calibrators is traceable to the WHO Second IRP (67/343) a urine-derived form of human erythropoietin. Tractability process is based on EN ISO 17511.

d. *Detection limit:*

The detection limit was determined by processing a six point calibration curve, controls, and ten replicates of zero calibrator in multiple assays. The lowest detectable level of EPO distinguishable from zero (Access EPO Calibrator S0) with 95% confidence is ≤ 0.6 mIU/mL.

e. *Analytical specificity:*

The Access EPO assay was tested using spiked samples for interference of normal human blood constituents [hemoglobin, triglycerides (triolein), bilirubin and human serum albumin], heparin, and commonly encountered medications (acetaminophen, acetylsalicylic acid, ibuprofen, multi vitamins). A cross-reactivity study was also performed of compounds with similar molecular structure. The assay exhibited no significant interference or cross reactivity with these substances or compounds.

f. *Assay cut-off:*

Not applicable.

2. Comparison studies:

a. *Method comparison with predicate device:*

An internal and external study was performed with the Access EPO and RDS Quantikine ELISA assays. The internal study included apparently healthy adults and adults with elevated EPO concentration levels. The external study included normal subjects, subjects diagnosed with anemia or polycythemia, subjects on erythropoietin therapy, and procured or spiked samples. Samples

for both tests were selected to cover the predicate assay range (2.5 – 200 mIU/mL). The results are as follows:

Internal Site Study: Slope of 1.0511, intercept of –1.3595, and correlation coefficient (r) of 0.988. For this study N=103 with an EPO concentration range of approximately 3 to 182 mIU/mL.

External Site Study: Slope of 1.1216, intercept of –2.4168, and correlation coefficient (r) of 0.995. For this study N=113 with an EPO concentration range of approximately 3 to 193 mIU/mL.

b. *Matrix comparison:*

A comparison study was performed using matched serum and plasma (heparin) samples and three matched serum and plasma samples, each spiked with four different concentrations of erythropoietin to obtain samples with concentrations throughout the analytical range of the assay. The results as follows met the design specification.

n	Slope (95% Confidence Interval)	Intercept (mIU/mL) (95% Confidence Interval)	Correlation Coefficient (r)
80	1.0153	0.1827	0.998

3. Clinical studies:

a. *Clinical Sensitivity:*

Not applicable.

b. *Clinical specificity:*

Not applicable.

c. Other clinical supportive data (when a. and b. are not applicable):

4. Clinical cut-off:

No Applicable.

5. Expected values/Reference range:

Serum and plasma (heparin) samples were obtained from 122 apparently healthy adults in the Minneapolis/St. Paul metropolitan area in Minnesota and analyzed. The result below was based on 95% non parametric analysis of normal samples:

n	Range of Observation (mIU/mL)	Expected Range (mIU/mL)
122	1.48 – 31.88	2.59 – 18.5

It is noted in the package insert that “the EPO concentration is in normal individuals can be affected by altitude, pregnancy and other factors” and recommended that each laboratory establish its own reference ranges to assure proper representation of specific populations.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.