510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K183088

B. Purpose for Submission:

Clearance of a new device

C. Measurand:

Erythropoietin (EPO)

D. Type of Test:

Quantitative

E. Applicant:

Axis-Shield Diagnostics

F. Proprietary and Established Names:

ADVIA Centaur Erythropoietin (EPO) Assay

G. Regulatory Information:

1. Regulation section:

   21 CFR 864.7250, Erythropoietin assay

2. Classification:

   Class II

3. Product code:

   GGT, Assay, Erythropoietin

4. Panel:

   Hematology (81)

H. Intended Use:

1. Intended use(s):

   The ADVIA Centaur® Erythropoietin (EPO) assay is for in vitro diagnostic use in the quantitative measurement of erythropoietin in pediatric and adult human serum or plasma (K2-EDTA, lithium heparin, sodium heparin) using the ADVIA Centaur XP system. Measurement of erythropoietin is used as an aid in the diagnosis of anemias and polycythemias.

2. Indication(s) for use:

   Same as Intended Use.

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   ADVIA Centaur XP

I. Device Description:

The device is a fully automated, one-step sandwich immunoassay which uses chemiluminescent technology. The assay utilizes an acridinium-ester-labeled monoclonal mouse anti-EPO antibody in the Lite Reagent. The Solid Phase consists of anti-EPO monoclonal antibody-coated paramagnetic microparticles. The assay consists of the following components:

- ADVIA Centaur EPO ReadyPack primary reagent pack; Lite Reagent which is a 10 mL reagent pack that contains monoclonal mouse anti-EPO antibody labeled with DMAE acridinium conjugate reagent in buffer with bovine serum albumin (BSA), surfactant, and sodium azide
- ADVIA Centaur EPO ReadyPack primary reagent pack; Solid Phase Reagent which is a 24 mL reagent pack that contains anti-EPO mouse monoclonal antibody coated streptavidin microparticles in buffer with BSA, surfactant, and preservatives
- ADVIA Centaur EPO Calibrator which is a 2.0 mL vial that contains recombinant human EPO, calf serum, sodium azide and preservative

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Beckman Coulter Access EPO Assay

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

   K052223

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	The ADVIA Centaur Erythropoietin (EPO) assay is for in vitro diagnostic use in the quantitative measurement of erythropoietin in pediatric and adult human serum or plasma using the ADVIA Centaur XP system. Measurement of erythropoietin is used as an aid in the diagnosis of anemias and polycythemias.	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 of anemias.
Assay technology	Chemiluminescent microparticle immunoassay (CMIA)	Chemiluminescent microparticle immunoassay (CMIA)
Measurement	Quantitative (mIU/mL)	Quantitative (mIU/mL)

Differences		
Item	Device	Predicate
Substrate/signal generation	Acridinium tracer	Alkaline phosphatase
Conjugate antibody	Monoclonal mouse anti-EPO antibody	Polyclonal chicken anti-recombinant mouse EPO antibody
Calibration	2-point calibration using two levels	6-level calibration
Calibration frequency	14 days	28 days
Specimen type	Human serum and plasma (K_2EDTA, lithium heparin, sodium heparin)	Human serum and plasma (heparin)
Linear range	0.83–750 mIU/mL	3.2–557.2 mIU/mL

Differences		
Item	Device	Predicate
Measurement range	0.83–750 mIU/mL	0.6–750 mIU/mL
Calibration	2-point calibration using two levels	6-level calibration

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

CLSI EP05-A3; Evaluation of Precision Performance of Qualitative Measurement Methods; Approved Guideline - Third Edition.

CLSI EP06-A; Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline.

CLSI EP07-A2; Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition.

CLSI EP17-A2; Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline - Second Edition.

CLSI EP25-A; Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline.

CLSI EP28-A3c; Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition.

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 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional 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. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

   Precision performance was evaluated in a 20-day single site study and a 5-day multisite study conducted at three sites. Seven serum samples with different EPO

concentrations spanning 1.55–474.53 mIU/mL were tested in duplicate for two runs per day in the 20-day single site study and in triplicate in two runs per day in the 5-day multisite study.

The results of the 20-day single site precision study were found to be acceptable and are described in the table below:

Sample	N	Mean	Within-Run		Between-Run		Between-Day		Between-Lot		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	240	1.81	0.10	5.49%	0.09	5.09%	0.08	4.23%	0.11	6.34%	0.19	10.69%
2	240	4.74	0.16	3.45%	0.12	2.58%	0.10	2.19%	0.20	4.25%	0.31	6.44%
3	240	9.71	0.24	2.48%	0.23	2.36%	0.11	1.10%	0.36	3.69%	0.50	5.15%
4	240	26.23	0.62	2.37%	0.67	2.55%	0.60	2.30%	0.95	3.61%	1.45	5.52%
5	240	97.48	1.75	1.80%	2.01	2.06%	1.96	2.01%	2.76	2.84%	4.31	4.42%
6	240	225.95	4.02	1.78%	5.30	2.35%	2.69	1.19%	4.97	2.20%	8.73	3.86%
7	240	586.83	10.69	1.82%	8.51	1.45%	6.10	1.04%	7.77	1.32%	16.86	2.87%

The results of the 5-day multisite precision study were found to be acceptable and are described in the table below:

Sample	N	Mean	Within Run		Between Run		Between Day		Between Site		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	90	1.60	0.10	6.52%	0.00	0.00%	0.07	4.27%	0.03	1.72%	0.13	7.98%
2	90	4.24	0.13	3.16%	0.03	0.82%	0.10	2.36%	0.00	0.00%	0.17	4.03%
3	90	10.21	0.23	2.30%	0.15	1.44%	0.17	1.63%	0.09	0.90%	0.34	3.29%
4	90	27.84	0.50	1.79%	0.40	1.44%	0.46	1.66%	0.43	1.56%	0.90	3.23%
5	90	99.63	1.78	1.79%	0.95	0.95%	1.14	1.15%	1.47	1.47%	2.74	2.75%
6	90	266.91	3.89	1.46%	4.90	1.83%	0.44	0.16%	5.22	1.95%	8.16	3.06%
7	90	481.47	7.08	1.47%	5.72	1.19%	4.80	1.00%	8.43	1.75%	13.30	2.76%

In addition to the two studies described above, an additional precision study was conducted with four samples spiked with rhEPO and four native EPO samples at four different concentrations across the measurement range as well as three fresh samples at three different concentrations. Each sample was tested in 48 replicates over three days with two runs per day and eight replicates per run. The results of the study were found to be acceptable.

b. Linearity/assay reportable range:

Three samples with elevated EPO concentrations (>600 mIU/mL) were diluted with a low concentration sample pool to create a nine-level dilution series per sample. Each sample was tested in five replicates, using three reagent lots on one ADVIA Centaur XP (analyzer). The linear range for the ADVIA Centaur Erythropoietin (EPO) assay is 0.83–750 mIU/mL.

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

Traceability

The ADVIA Centaur EPO assay is traceable to the World Health Organization (WHO) 2nd International Reference Preparation for Erythropoietin (Human, urinary derived); NIBSC code: 67/343. The ADVIA Centaur EPO assay is also traceable to the 3rd World Health Organization (WHO) International standard for Erythropoietin, recombinant, for bioassay; NIBSC code: 11/170.

Sample Stability

A sample stability study was conducted with pooled serum and plasma samples as well as neat native samples to demonstrate stability under room temperature conditions for 8 hours. The pooled samples were tested at T0, 1, 2, 4, 18 and 20 hours. The native samples were tested at T0, 4, 8, 16, 24, and 25 hours. All subsequent time points were within acceptable limits of timepoint zero. Room temperature serum and plasma sample stability was established at 20 hours.

A similar study was conducted with pooled serum and plasma samples as well as neat native samples at multiple concentrations across the measurement range to demonstrate stability under refrigerated storage conditions. All timepoints were within acceptable limits of timepoint zero. Sample stability for serum and plasma was established at 7 days under refrigerated storage conditions (2−8ºC).

Sample stability studies were also conducted to substantiate sample storage claims at -20ºC with pooled samples at multiple concentrations across the measurement range to demonstrate stability under frozen storage conditions for 7 days. Each sample was tested at multiple timepoints and the results of the study were within acceptable limits. Sample stability was established for serum and plasma samples at -20ºC for 7 days.

Lastly, a freeze-thaw study was performed with multiple pooled serum and plasma samples at concentrations across the measurement range. The results of the study support the claimed maximum number of three freeze-thaw cycles for both serum and plasma samples.

d. Detection limit:

The limit of blank (LoB) was determined by testing four blank samples (calf serum) on one analyzer with three reagent lots, over three days. Each sample was analyzed in one run per day and five replicates per run, providing a total of 180 replicates. LoB was calculated by rank ordering the 72 samples per microcuvette lot from low to high and averaging the 68th and 69th results (being the 95th percentile). LoB was determined to be 0.46 mIU/mL.

The limit of detection (LoD) was determined by testing human serum and plasma samples with low EPO concentrations. The study was conducted with 10 samples (eight serum and two plasma) by one analyzer and three reagent lots, over 5–6 days per reagent lot. Each sample was analyzed in one run per day and 3–6 replicates per run, providing 259–323 replicates per reagent lot. LoD was calculated by

nonparametric analysis and was determined to be 0.75 mIU/mL.

The limit of quantitation (LoQ) was determined by testing human serum and plasma samples with low EPO concentrations. The study was conducted with 10 samples (eight serum and two plasma) by one analyzer and three reagent lots, over 5–6 days per reagent lot. Each sample was analyzed in one run per day and 3–6 replicates per run, providing 259–323 replicates per reagent lot. LoQ was determined to be 0.83 mIU/mL.

e. Analytical specificity:

Interference studies were performed by spiking pooled human serum with rhEPO to achieve two levels (4–6 mIU/mL and 25–35 mIU/mL). Each of these samples were divided into a test pool with the potential interferent added and a control sample with no added interferent. For substances identified as interferents at the highest concentration tested, a concentration-response curve evaluating multiple concentrations of the interfering substance was generated to determine the non-interfering concentration. A total of 11 potential endogenous interferents and six potential exogenous interferents were evaluated. Each sample was tested in five replicates on one ADVIA Centaur XP. The highest tested concentrations at which no significant interference was observed (defined by the sponsor as ≤ 10% difference) are presented in the following table:

Endogenous Substances	**Highest concentration tested with no significant interference**
Hemoglobin	500 mg/dL
Conjugated bilirubin	40 mg/dL
Unconjugated bilirubin	60 mg/dL
Intralipid	3000 mg/dL
Albumin	6 g/dL
Cholesterol	500 mg/dL
EPO Soluble Receptor	15 ng/mL
Human gamma globulins (IgG)	4.9 g/dL
Rheumatoid Factor	200 IU/mL
Total protein	12 g/dL
Triglycerides	1000 mg/dL
Exogenous Substances	**Highest concentration tested with no significant interference**
Acetaminophen	14 mg/dL
Acetylsalicylic acid	50 mg/dL
Biotin	100 mg/dL
Heparin	8000 U/dL
Ibuprofen	40 mg/dL
Silwet L720	0.2 mg/dL

Cross Reactivity:

To determine the cross reactivity of potential cross reactants with the ADVIA Centaur EPO assay, two serum samples were examined. One sample was pooled normal serum at 4–6 mIU/mL and the other was pooled normal serum with spiked rhEPO to achieve an EPO concentration of 25–35 mIU/mL. Either one or four concentrations of the cross reactant was added to these two serum samples for the calculation of % cross-reactivity. No cross-reactivity was observed. The results of the study are present in the following table:

Cross-Reactant	Concentration	% Cross reactivity
α-2-Macroglobulin	400 mg/dL	-0.01
Transferrin (iron saturated)	200 mg/dL	0.00
Transferrin (non-saturated)	200 mg/dL	0.00
rh Thrombopoietin	10,000 ng/mL	-0.01
α-1-Acid Glycoprotein	80 mg/dL	0.01
α-1-Antitrypsin	200 mg/dL	0.00
α-and β-Globulins	5 g/dL	0.00
Gamma Globulins	6 g/dL	0.00

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

Four different method comparison studies were performed: single site (in-house) with frozen samples, multisite study with frozen samples, and two single site studies with native samples.

For the single site using frozen samples, a total of 222 frozen serum samples were thawed and measured in singlicate. Among these samples were: 22 infants (1 month to 2 years), 12 children (2 to 12 years), 20 adolescents (12 to 21 years), and 20 collected from EPO therapy donors. Additionally, five serum samples were collected from patients with the following conditions: chronic kidney disease, lymphoma, myeloma, non-renal anemia, polycythemia, and renal anemia. Ninety-two (41%) samples were from the U.S., 21 (10%) contrived, and 6 samples were excluded due to "device errors or a result not within the measurement interval of the predicate device or new device were excluded". The study was performed with three reagent lots and testing was performed on the ADVIA Centaur XP for the subject assay and the Beckman Coulter Access Immunoassay system for the predicate assay. A total of 216 samples in the range of 3.29–691.60 mIU/mL were evaluated in the Passing-Bablok regression analysis. The results of the study are as follows: $y= 0.99x + 0.81$ mIU/mL, and $r=0.99$.

For the multisite study using frozen samples, testing using the candidate device ADVIA Centaur EPO assay was performed at three sites and testing using the predicate device, Beckman Access EPO, was performed at a single site. A total of 341 frozen serum samples were thawed and measured. Among these samples, 28 were adolescents (12 to 21 years) and 20 were collected from EPO therapy donors. Additionally, serum samples collected from patients with the following conditions were tested: chronic kidney disease, lymphoma, myeloma, non-renal anemia, and polycythemia. Fourteen samples were removed as nine samples generated no result by the instrument and five were outside the measurement range.

For the first single site study using native samples with endogenous EPO stored frozen, 140 samples were tested across the measurement range on both the candidate and predicate devices. This study included more samples at the lower end of the measuring range to cover medical decision limits around 1.5–2.5 mIU/mL and <1.4 mIU/mL. The results of the study are as follows: y= 1.00x + 0.23 mIU/mL.

For the second single site study using fresh native samples with endogenous EPO stored at room temperature, 100 samples were tested across the measurement range on both the candidate and predicate devices. This study included samples in the following range: 4.00–445.68 mIU/mL. The results of the study were evaluated with Passing-Bablok regression analysis. The results of the study are as follows: y= 1.07x – 0.00 mIU/mL, and r=1.00. The 95% CI were reported for the slope (1.04, 1.10), intercept (-0.42, 0.41) and r value (1.00, 1.00).

b. Matrix comparison:

A study was performed to compare serum to the other five sample types including: K_2EDTA, LiHep, NaHep, PST (plasma separator tube), and SST (serum separator tube), using the ADVIA Centaur EPO assay. Each sample was tested in singlicate and the study was performed with one reagent lot. Samples were collected from 23 donors for each of the six sample types and each sample with each collection tube type was split into three aliquots: one was tested as the neat sample that ranged 4.39–33.32 mIU/mL, while the second and third aliquots were spiked with rhEPO to achieve about 300 and 600 mIU/mL to cover the medium and high end of the AMR, respectively. The results of the study were found to be acceptable.

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):

Not applicable

4. Clinical cut-off**:**

   Not applicable

5. Expected values/Reference range:

   A reference range study was performed to determine the expected EPO concentration for the adult population. A total of 251 serum samples collected from 128 apparently healthy adult males and 123 apparently healthy adult females in the U.S. Using non-parametric data analysis, the 95% central reference interval was calculated. The reference range for adult males and females are 5.41–36.90 mIU/mL and 2.92–35.17 mIU/mL, respectively.

   In addition, a reference range study was performed for the pediatric population with a total of 266 serum samples collected from 140 apparently healthy males and 140 apparently healthy females in the U.S. A total of 14 samples in the infant group (> 1 month – 2 years), 132 samples in the child group (> 2–12 years), and 120 samples in the adolescent group (>12–21 years) were included in the data analysis.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable.

O. Conclusion:

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