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

A. 510(k) Number:

k130020

B. Purpose for Submission:

New device

C. Measurand:

Total beta human chorionic gonadotropin (βhCG) in human serum and plasma

D. Type of Test:

Quantitative chemiluminescent immunoassay

E. Applicant:

Beckman Coulter, Inc.

F. Proprietary and Established Names:

Access Total βhCG (5th IS) Assay

Access Total βhCG (5th IS) Calibrators

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
DHA	II	21 CFR 862.1155, Human chorionic gonadotropin (HCG) test system	Chemistry (75)
JIX	II	21 CFR 862.1150, Calibrator	Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See Indications for Use below

2. Indication(s) for use:

   The Access Total βhCG (5^{th} IS) assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of total βhCG levels in human serum and plasma using the Access Immunoassay Systems. The assay is intended for use as an aid in the early detection of pregnancy.

   The Access Total βhCG (5^{th} IS) calibrators are intended to calibrate the Access Total βhCG (5^{th} IS) assay for the quantitative determination of total βhCG levels in human serum and plasma using the Access Immunoassay Systems.

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   Beckman UniCel DxI 800.

I. Device Description:

The Access Total βhCG (5^{th} IS) assay consists of the reagent pack and calibrators. Other items needed to run the assay include substrate and wash buffers. The Access Total βhCG (5^{th} IS) assay reagent pack, Access Total βhCG (5^{th} IS) Calibrators, along with the Access wash buffer and substrate, are designed for use with the UniCel DxI 800 Analyzer in a clinical laboratory setting.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Siemens ADVIA Centaur Total hCG Assay

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

   k925277

3. Comparison with predicate:

Similarities		
Item	Access Total βhCG (5th IS) Assay and Access Total βhCG (5th IS) Calibrators	Predicate ADVIA Centaur Total hCG k925277
Intended use	The Access Total βhCG (5th IS) assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of total βhCG levels in human serum and plasma using the Access Immunoassay Systems. This assay is intended for use as an aid in the early detection of pregnancy. The Access Total βhCG (5th IS) Calibrators are intended to calibrate the Access Total βhCG (5th IS) assay for the quantitative determination of total βhCG levels in human serum and plasma using the Access Immunoassay Systems	Same
Technology	Sandwich Immunoassay	Same
Format	Chemiluminescent	Same
Method	Automated	Same
Measurand	Total βhCG (intact hCG and free beta-subunit)	same
Calibration	Utilizes a stored calibration curve	Same
Calibration Interval	28 days	Same
Differences		
Item	Access Total βhCG (5th IS) Assay and Access Total βhCG (5th IS) Calibrators	Predicate ADVIA Centaur Total hCG k925277
Measuring Range	0.6 – 1350 mIU/mL	2.0 – 1000 mIU/mL
Standardization	WHO 5th International	WHO 4th International

Similarities		
Item	Access Total βhCG (5th IS) Assay and Access Total βhCG (5th IS) Calibrators	Predicate ADVIA Centaur Total hCG k925277
	Reference Standard, 07/364	Reference Standard, 75/589
Sample Type	Serum or plasma	Serum
Calibrator Levels	6 levels (0, 6, 35, 195, 620, and 1350 mIU/mL)	2 levels (7 and 300 mIU/mL)
Matrix	Bovine serum albumin	Equine serum

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

- CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Method
- CLSI EP6-A: Evaluation of Linearity of Quantitative Measurement Procedure: A Statistical Approach
- CLSI EP7-A2: Interference Testing in a Clinical Laboratory
- CLSI EP9-A2: Method Comparison and Bias Estimation using Patient Samples
- CLSI EP14-A2: Evaluation of Matrix Effects
- CLSI EP17-A2: A Protocol for Determining Limit of Detection and Limit of Quantitation
- CLSI EPC28-A3: Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory

L. Test Principle:

The Access Total βhCG (5th IS) assay is a sequential two-step immunoenzymatic ("sandwich") assay. A sample is added to a reaction vessel along with a citrate buffer. After an initial incubation, a rabbit anti-βhCG alkaline phosphatase conjugate and paramagnetic particles coated with goat anti-mouse IgG: mouse monoclonal anti-βhCG complexes are added. The hCG binds to the immobilized monoclonal anti-βhCG on the solid phase while, at the same time, the rabbit anti-βhCG alkaline phosphatase conjugate reacts with different antigenic sites on the hCG. 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 proportional to the concentration of hCG 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:

Seven serum and four plasma patient samples were tested following CLSI EP5-A2. One serum sample with a hCG concentration approximating 200,000 mIU/mL was tested using the onboard dilution feature. Three commercial controls were tested with each run. Each patient and control sample was run in duplicate. Testing occurred over twenty days (2 runs per day) for a total of 40 runs and 80 replicates. The study was run at an internal site on three instruments, using three reagent pack lots, one calibrator lot, and one lot of reagent pack per instrument. Results from multiple lots were similar. Results from one representative lot for serum and plasma samples are provided in the tables below.

Precision Study – Serum

Sample ID	Grand Mean (mIU/mL)	Within Run SD (mIU/mL)	Within Run CV (%)	Between Run SD (mIU/mL)	Between Run CV (%)	Total SD (mIU/mL)	Total CV (%)
Serum 1	0.6	0.07	11.7	0.11	18.3	0.13	21.7
Serum2	4.1	0.11	2.7	0.24	6.0	0.27	6.6
Serum 3	24.0	0.68	2.8	0.30	1.2	0.74	3.1
Serum 4	106.7	1.97	1.8	2.60	2.4	3.26	3.1
Serum 5	791.3	24.24	3.1	40.81	5.2	47.46	6.0
Serum 6	1116.1	38.19	3.4	53.29	4.8	65.56	5.9
Serum 7	208,778	7,482	3.6	9,400	4.5	12,014	5.8

Precision Study – Plasma

Sample ID	Grand Mean (mIU/mL)	Within Run SD (mIU/mL)	Within Run CV (%)	Between Run SD (mIU/mL)	Between Run CV (%)	Total SD (mIU/mL)	Total CV (%)
Plasma 1	1.4	0.06	4.3	0.06	4.3	0.08	5.7
Plasma 2	4.7	0.23	4.8	0.2	4.2	0.30	6.4
Plasma 3	675.0	19.81	2.9	21.45	3.2	29.2	4.3
Plasma 4	1112.3	43.35	3.9	58.09	5.2	72.48	6.5

b. Linearity/assay reportable range:

i) Linearity:

Fifteen sample dilutions at concentrations across the measuring range of the Total βhCG (5th IS) assay (0.6 to approximately 1350 mIU/mL) were prepared by mixing different proportions of a high and a low serum sample. The hCG concentration of the low serum sample was determined using three lots of Total βhCG (5th IS) assay and two lots of Total βhCG (5th IS) Calibrators. The low sample was tested in replicates of eight and concentration was determined from the mean of the eight results to be approximately 0.03

mIU/mL. The hCG concentration of the high sample was tested in eight replicates with three lots of Total βhCG (5th IS) assay and one lot of Total βhCG (5th IS) secondary calibrators, where the highest calibrator level was approximately 1700 mIU/mL. The high sample concentrations were determined from the mean of the eight Total βhCG (5th IS) replicates for each reagent lot.

Linearity results from one representative lot are presented below in terms of expected versus observed. Results from other lots were similar. The results support linearity across the claimed measuring range of 0.6 – 1350 mIU/mL.

Sample ID	Expected (mIU/mL)	Observed (mIU/mL)	% Recovery
Sample 1	0.03	0	0
Sample 2	0.71	0.73	102.8
Sample 3	1.43	1.77	123.8
Sample 4	14.25	14.38	100.9
Sample 5	42.74	41.62	97.4
Sample 6	85.49	81.85	95.7
Sample 7	128.23	127.56	99.5
Sample 8	178.1	171.97	96.6
Sample 9	356.19	337.26	94.7
Sample 10	534.29	508.57	95.2
Sample 11	712.38	664.39	93.3
Sample 12	890.48	836.4	93.9
Sample 13	1068.58	1003.22	93.9
Sample 14	1246.67	1188.67	95.3
Sample 15	1432	1424.77	99.5

ii) Dilution Recovery

(a) Manual Dilution:

Dilution recovery was tested on five serum samples and five plasma samples at hCG concentrations between 100 and 120% of 1350 mIU/mL. Six independent dilutions for each sample were made with wash buffer as the diluent at dilutions of 1/5, 1/10, 1/101, 1/200 and 1/250, resulting in sample concentrations of approximately 6 – 1500 mIU/mL.

This study was run on two instruments, using one reagent pack lot, two calibrator lots, and two lots of wash buffer. Neat samples were run in replicates of eight. Each sample dilution was measured in replicates of four. Three quality controls were run in replicates of two on each day of each run.

A 1/200 dilution is recommended in the Instructions for Use for over-range samples (> 1350 mIU/mL). When both sample matrices were diluted 1/200, recovery ranged from 97% to 115%. The mean recoveries from manual dilution at 1/200 were 110% for serum samples and 104% for plasma samples.

(b) On-board Dilution:

Verification studies were performed to determine the sample dilution recovery of the Access Total βhCG (5th IS) assay. Sample dilution recovery was tested on five serum samples and five plasma samples at hCG concentrations between 100 and 120% of 1350 mIU/mL. Each sample was diluted with wash buffer as the diluent at a dilution of 1/200 on the instrument using two different assay protocol files: dil-hCG or on-board dilution (OBD).

This study was run on three instruments, using one reagent pack lot, two calibrator lots, and two lots of wash buffer. All samples were run in replicates of eight. Three quality controls were run in replicates of two on each day of each run. The results are summarized in the tables below.

Sample	Dil-hCG Average % Recovery	Dil-hCG Recovery Range (%)	OBD Average % Recovery	OBD Recovery Range (%)
Serum 1	109	99-118	114	106-123
Serum 2	109	96-119	112	106-119
Serum 3	97	88-108	100	92-108
Serum 4	95	84-102	100	93-107
Serum 5	96	84-103	102	93-108

Sample	Dil-hCG Average % Recovery	Dil-hCG Recovery Range (%)	OBD Average % Recovery	OBD Recovery Range (%)
Plasma 1	106	94-113	109	104-123
Plasma 2	104	96-124	107	101-113
Plasma 3	101	88-114	106	97-116
Plasma 4	99	87-109	106	98-113
Plasma 5	104	86-110	108	102-113

Individual percent recoveries for serum and plasma generally demonstrated a bias of ≤ 15%. The sponsor has a limitation in the labeling that states that there is potential for end-users to obtain dilution results with a bias >15% from dil-hCG or OBD assay protocol dilution of serum samples (but not plasma).

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

Traceability:

The measurand in the Access Total βhCG (5th IS) Calibrators is traceable to the WHO 5th International Standard (NIBSC Code 07/364). Traceability process is based on EN ISO17511.

Stability:

All stability study protocols were based on CLSI EP25-A. Stability protocols and acceptance criteria for real-time, opened and closed calibrators were reviewed and found to be acceptable.

Value Assignment:

Concentrations of calibrators are assigned through an internal procedure. Primary working calibrators are prepared from WHO 5th IS and secondary working calibrators are value assigned using the primary working calibrators and the Access Immunoassay System. Primary working calibrators and the secondary working calibrators are run together on the Access Immunoassay System in a matched, multi-replicate process. Product (commercial) calibrators are value assigned using the secondary working calibrators on the Access Immunoassay System following a similar value assignment process. The product calibrators are prepared at six levels; 0, 6, 35, 195, 620, and 1,350 mIU/mL.

d. Detection limit:

LoB, LoD, and LoQ below were determined according to CLSI EP17-A2.

i) Limit of Blank (LoB) and Limit of Detection (LoD)

One hundred fifty six (156) replicates of the S0 (zero) calibrator were measured for the LoB determination. Six very low hCG concentration serum samples between the anticipated LoB and ten times the anticipated LoB were measured in triplicates over 12 runs (36 replicates in total) to determine the LoD. The study was run on three instruments, using three reagent pack lots and two calibrator lots. Three quality controls were run in replicates of two on each day of each run. The sponsor claims that LoB and LoD is ≤ 0.5 mIU/mL for the Access Total βhCG (5th IS) Assay are supported.

ii) Limit of Quantitation (LoQ)

The WHO 5th IS was diluted with hCG-negative serum at nominal concentrations of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 and 6.0 mIU/mL to determine

the LoQ. The study was run on three instruments, using three reagent pack lots and one calibrator lot. Each sample dilution was run in replicates of ten. Three quality controls were run in duplicates on each day. The sponsor claimed that the LoQ is 0.6 mIU/mL and demonstrated that recovery is within ± 0.1mIU/mL and %CV is < 20% at the LoQ.

e. Analytical specificity:

i) Cross-reactivity:

A cross-reactivity study was performed on pituitary hormones FSH, LH, TSH and α-subunit of hCG. The potential cross-reactants were added to the Access Total βhCG (5th IS) Calibrator S0 (zero calibrator) and one patient sample with an hCG concentration of ~2.9 mIU/mL. Results from these cross-reactant spiked samples were evaluated against that of the unspiked sample alone, defined as the control. No cross-reactivity was observed at the concentrations that were tested for LH, FSH, TSH and hCG α-subunit as shown below.

Substance	**concentration (mIU/mL)**
FSH	1
LH	103
TSH	1000
hCG α-subunit	500

ii) Isoform Recognition:

Known concentrations of commercially available WHO reference standards (isoforms) were diluted in normal human serum (negative for hCG) for isoform recognition studies. The results (as shown in the table below) demonstrated that the Access Total βhCG (5th IS) assay measures four hCG isoforms: intact hCG, β subunit of hCG, nicked intact hCG and nicked βhCG, while free α subunit and β-core fragment yield no detectable response.

hCG Isoform	**WHO Code**	**Mean % Recovery**
Intact hCG	99/688	109%
hCG-β, subunit, highly purified	99/650	193%
hCGn, nicked dimer	99/642	127%
hCG-βn, nicked β subunit	99/692	127%
hCG-β, subunit, less pure	75/551	140%
hCG-βcf, beta core fragment*	99/708	0.5%
hCG-α, subunit, highly purified	99/720	0.1%

*Note: high levels of β-core fragment were not tested.

iii) Interference Study:

An interference study was performed by assessing common sample abnormalities (including hemolysis, icterus, and lipemia), common prescription drugs, over-the-counter drugs, and medications most often prescribed in the patient population for which the test is ordered. Two patient serum samples at hCG concentrations of approximately 2.9 mIU/mL and 500 mIU/mL were used for the study. The potential interferents were spiked individually into each patient sample with a low and high concentration of potential interferent. Results from these spiked patient samples were compared to results of the unspiked sample alone, defined as the control. No interference was observed at the concentrations tested as shown below.

Potential Interferent	Low Level	High Level
Acetaminophen	3 mg/dL	20 mg/dL
Acetylsalicylic acid	39 mg/dL	65 mg/dL
Bilirubin (conjugated)	0.3 mg/dL	40 mg/dL
Bilirubin (unconjugated)	1.22 mg/dL	40 mg/dL
Hemoglobin	200 mg/dL	500 mg/dL
Heparin (low molecular weight)	100 U/dL	7200 U/dL
Human serum albumin	3.5 g/dL	6 g/dL
Ibuprofen	7 mg/dL	50 mg/dL
Intralipid	0.3 g/dL	3 g/dL
Multi-vitamin	0.3% (v/v) dilution	0.9% (v/v) dilution

iv) High Dose Hook Effect:

To determine any hook effect, 1,000,000 mIU/mL of commercially available WHO reference standards (intact hCG, highly purified hCG β-subunit, nicked hCG dimer, and nicked hCG β-subunit) were spiked into one human serum patient sample (negative for hCG). The difference between the different concentrations of hCG isoforms and the Access Total βhCG (5^{th} IS) S5 calibrator (~1350 mIU/mL) was calculated. No high dose hook effect was observed at the concentrations tested.

f. Assay cut-off:

See detection limit above.

2. Comparison studies:

a. Method comparison with predicate device:

Patient serum samples with hCG concentrations falling within the measuring range of the ADVIA Centaur Total hCG Assay (2-1000 mIU/mL) were evaluated. A total of 224 serum samples, including 85 fresh and 139 frozen samples were used in the analysis.

The study was run at two external sites, one instrument per site, with two reagent pack lots and one calibrator lot for the Total βhCG (5th IS) assay. All samples were run in singleton.

The correlations between paired measurements for fresh, frozen, and all samples were analyzed. Each analysis was completed by fitting the observed Access Total βhCG (5th IS) values (dependant variable, y) into a Passing-Bablok linear regression model, with the observed ADVIA Centaur Total hCG values as the only independent variable (x, standard).

Method Comparison vs. ADVIA Centaur Total hCG (Fresh Samples)

N	Concentration Range (mIU/mL)	Intercept (95% CI)	Slope (95% CI)	Correlation Coefficient (r)
85	4.9 – 1000	1.37 (0.56 – 2.67)	1.00 (0.97 – 1.02)	0.99

Method Comparison vs. ADVIA Centaur Total hCG (Frozen Samples)

N	Concentration Range (mIU/mL)	Intercept (95% CI)	Slope (95% CI)	Correlation Coefficient (r)
139	3.2 – 1000	3.42 (2.65 – 4.42)	1.09 (1.07 – 1.12)	0.99

Method Comparison vs. ADVIA Centaur Total hCG (All Samples)

N	Concentration Range (mIU/mL)	Intercept (95% CI)	Slope (95% CI)	Correlation Coefficient (r)
224	3.2 – 1000	2.87 (2.25 – 3.79)	1.04 (1.02 – 1.06)	0.99

b. Matrix comparison:

The sample types for the study were serum (no gel), serum (gel) and lithium-heparin plasma. 42 matched sets of serum and plasma (lithium-heparin) samples were used. The sample (neat or spiked) concentrations spanned the reportable range of the assay (0.5 -1350 mIU/mL). Spiked samples were prepared using commercially available recombinant hCG antigen spiked into the Total βhCG (5th IS) calibrator S0 (zero) matrix (BSA).

The study was run on one instrument using one reagent pack lot and one calibrator lot. One replicate per sample type was analyzed. Linear regression results are shown below.

Sample Type	N	Intercept		Slope	
		Estimate	95% CI	Estimate	95% CI
Serum (no gel) vs. serum (gel)	42	-0.05	-0.15 – 0.10	0.99	0.98 – 1.01
Serum (no gel) vs. lithium heparin plasma	42	-0.08	-0.28 – 0.05	1.05	1.02 - 1.07

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:

   Expected values for apparently healthy non-pregnant adult females, from the U.S. population, with no known serious chronic or underlying conditions were used in the study. Separate upper 95th percentiles were determined for: a) women ≥ 18 and < 40 years of age; b) women ≥ 40 years of age; and, c) post-menopausal women (female subjects who had not had a menstrual period for 12 months or more). Samples for the study were prospectively procured at one internal site and one external site. The study was run at one external site on one instrument, using one reagent pack lot and one calibrator lot.

   The data was analyzed separately for each population using the non-parametric method and following CLSI C28-A3. Additionally, a two-sided non-parametric 95% confidence interval (CI) was used for the analysis. The upper 95^{th} percentile is reported as an estimate of the upper reference limit (URL) of serum hCG levels in apparently healthy non-pregnant female populations.

Subject Category (non-pregnant females)	N	Median (mIU/mL)	Range (mIU/mL)	95th Percentile (mIU/mL), 95% CI
≥ 18 and < 40 years	132	0	0 – 0.6	0.3, [0.2 – 0.4]
≥ 40 years	141	0	0 – 3.1	1.5, [1.1 – 2.9]
Post-menopausal	134	2.8	0.1 – 11.6	7.7, [6.4 – 10.4]

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.