Total Human Chorionic Gonadotropin (TβhCG 5th IS) - Serum
Beckman UniCel DXI Systems

Technical Procedure 3203T

For In Vitro Diagnostic Use Only

Principle

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 using the Beckman UniCel DxI Immunoassay Systems. This assay is intended for use as an aid in the early detection of pregnancy.

Summary and Explanation

Human chorionic gonadotropin (hCG) is a glycoprotein hormone, produced by the placenta, with structural similarity to the pituitary hormones FSH, TSH, and LH. The alpha subunit (MW 15,000–20,000 daltons) is common to all of these hormones but the beta subunits differ, and confer immunological and biological specificity. βhCG (MW 25,000–30,000 daltons) shares several peptide sequences with βLH, but has a unique carboxyl terminal region.(1) Shortly after implantation of a fertilized ovum into the uterine wall, the trophoblast begins to produce hCG, which-maintains steroid secretions of the corpus luteum until the placenta can do so.(2) hCG can be detected after implantation; concentrations double approximately every 1.5 to 3 days for the first six weeks and then continue to rise until the end of the first trimester, gradually falling to a lower level for the remainder of the pregnancy.(3,4) After delivery, hCG returns to < 5 mIU/mL (IU/L) and is usually undetectable several days postpartum.
The hormone is an excellent marker for pregnancy. Healthy, non-pregnant individuals have low (< 5 mIU/mL [IU/L]) to undetectable hCG levels; however, hCG, originating from the pituitary gland, can be found at detectable levels in peri- and post-menopausal women.(5) During pregnancy, unusually low or rapidly declining levels may indicate an abnormal condition such as an ectopic pregnancy or impending spontaneous abortion.(6)

Methodology

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, 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 total βhCG in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

Acceptable Sample Containers

13 x 75 SST and Red Top BD tubes
13 x 75 PST Lithium Heparin Plasma BD tubes
SST and Red Top BD microtainers
PST Lithium Heparin Plasma BD microtainers
Optimum volume: 0.5 mL, Minimum volume: 0.25 mL

Specimen Collection and Preparation

Type of Sample

1. Serum (Red Top & SST) or Lithium Heparin plasma are acceptable sample types.
   Whole blood or urine are not recommended for use as a sample.

2. The role of preanalytical factors has been described in a variety of published literature.(9,10,11) To minimize the effect of preanalytical factors observe the following recommendations for handling, processing, and storing blood samples:(10)
   - Collect all blood samples observing routine precautions for venipuncture.

- Allow serum samples to clot completely in an upright position before centrifugation. Clotting may be slowed at cooler temperatures or if the patient is on anticoagulant therapy.
- Keep tubes stoppered at all times.
- Physically separate sample from contact with cells as soon as possible.
- Store samples tightly stoppered at room temperature (15°C to 30°C) for no longer than eight hours.
- If the assay will not be completed within eight hours, refrigerate the samples at 2°C to 8°C.
- If the assay will not be completed within 48 hours, or for shipment of samples, freeze at -20°C or colder. Frozen specimens can be stored up to six months before testing.(12)
- Thaw samples only once. Do not thaw in water bath. Mix gently by inversion and centrifuge after thawing prior to sample analysis.

2. Use the following guidelines when preparing specimens:
   - Ensure residual fibrin and cellular matter has been removed prior to analysis.
   - Use laboratory posted settings for sample centrifugation of sample tubes.

Reagents

Access Total βhCG (5th IS) Reagent Pack

Cat. No. A85264: 100 determinations, 2 packs, 50 tests/pack

- Provided ready to use.
- Store upright and refrigerate at 2°C to 10°C.
- Refrigerate at 2°C to 10°C for a minimum of two hours before use on the instrument.
- Stable until the expiration date stated on the label when stored at 2°C to 10°C.
- Stable at 2°C to 10°C for 28 days after initial use.
- Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range.
- If the reagent pack is damaged (i.e., broken elastomer), discard the pack.
- All antisera are polyclonal unless otherwise indicated.
- A fine suspension of particulate may be observed in the R1b well of the reagent pack. The presence of this particulate has demonstrated no effect on assay performance.

R1a	Paramagnetic particles coated with goat anti-mouse IgG: mouse monoclonal anti-βhCG complexes suspended in TRIS buffered saline, with surfactant, bovine serum albumin (BSA), < 0.1% sodium azide, and 0.1% ProClin** 300.
R1b	Protein (goat, murine, and recombinant) diluted in citrate buffered saline, with surfactant, < 0.1% sodium azide, and 0.1% ProClin** 300.
R1c	Rabbit anti-βhCG alkaline phosphatase (recombinant) conjugate diluted in MES buffered saline, with surfactant, BSA, protein (rabbit), < 0.1% sodium azide, and 0.25% ProClin** 300.

Warnings and precautions

- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
- Human source material used in the preparation of the reagent has been tested and found negative or non-reactive for Hepatitis B, Hepatitis C (HCV), and Human Immunodeficiency Virus (HIV-1 and HIV-2). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.(7)

- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.(8)
- Xi. Irritant: 0.25% ProClin** 300.

R 43: May cause sensitization by skin contact.
S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- Safety Data Sheet (SDS) is available online.

Access Total βhCG (5th IS) Calibrators

Catalog Reorder No. B11754: S0–S5, 4.0 mL/vial

- **Freeze upon receipt**.
- Provided ready to use.
- Store at -20°C or colder.
- Thaw at room temperature and mix contents by gently inverting before use. Avoid bubble formation.
- **Return calibrators to -20°C or colder after each use**. **Calibrators expires 120 days after date opened or 15 freeze/thaw cycles.**
- Stable until the expiration date stated on the label when stored at -20°C or colder.
- Signs of possible deterioration are control values out of range. Discard the vials if there is evidence of microbial contamination or excessive turbidity in the calibrators.
- Refer to calibration card for exact concentrations.

S0	Buffered bovine serum albumin (BSA) matrix with surfactant, < 0.1% sodium azide, and 0.5% ProClin** 300. Contains 0.0 mIU/mL (IU/L) hCG.
S1, S2, S3, S4, S5:	hCG at levels of approximately 6, 35, 195, 620 and 1350 mIU/mL (IU/L), respectively, in buffered BSA matrix with surfactant, < 0.1% sodium azide, and 0.5% ProClin** 300.
Calibration Card:	1

The Access Total βhCG Calibrators are intended to calibrate the Access Total βhCG (5th IS) assay for the quantitative determination of total βhCG levels in human serum. The calibrators are provided at six levels – zero and approximately 6, 35, 195, 620 and 1350 mIU/mL – prepared gravimetrically from purified hCG and buffered BSA matrix. Assay calibration is required every 28 days. Calibrators run in duplicate.

Quantitative assay calibration is the process by which samples with known analyte concentrations (i.e., assay calibrators) are tested like patient samples to measure the response. The mathematical relationship between the measured responses and the known analyte concentrations establishes the calibration curve. This mathematical relationship, or calibration curve, is used to convert RLU (Relative Light Unit) measurements of patient samples to specific quantitative analyte concentrations.

Traceability

The measurand (analyte) in the Access Total βhCG (5th IS) Calibrators is traceable to the WHO 5th International Standard for Chorionic Gonadotropin (NIBSC Code 07/364.(23,24) Traceability process is based on EN ISO 17511.

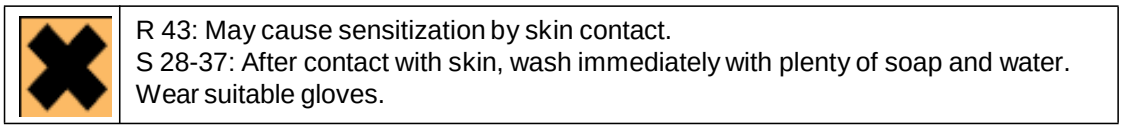
The assigned values were established using representative samples from this lot of calibrator and are specific to the assay methodologies of the Access reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Warnings and precautions

- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and

good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.

- Human source material used in the preparation of the reagent has been tested and found negative or non-reactive for Hepatitis B, Hepatitis C (HCV), and Human Immunodeficiency Virus (HIV-1 and HIV-2). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.(7)
- Human source material was purified from the urine of pregnant women. Treat as potentially infectious.(7)
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.(8)
- Xi. Irritant: 0.5% ProClin** 300.



- Safety Data Sheet (SDS) is available online.

Access Substrate

Catalog Reorder No. 81906: 4 x 130 mL

Provided ready to use. Refer to the following chart for storage conditions and stability. An increase in substrate background measurements may indicate instability.

Condition	Storage	Stability
Unopened	2°C to 8°C	Until expiration date stated on the label
Equilibration prior to use (unopened)	15°C to 30°C (room temperature)	Minimum 18 hours Maximum 14 days
In use (opened)	Internal substrate supply position	Maximum 14 days

1. R2 Access Substrate: 4 x 130 mL.
   - Lumi-Phos* 530 (buffered solution containing dioxetane Lumigen* PPD, fluorescer, and surfactant).

Warnings and precautions

- Substrate is sensitive to air exposure. Keep tightly closed at all times. **Do not pool bottles of substrate**.

Access Wash Buffer II

UniCel DxI Wash Buffer II, Cat. No. A16793: 1 x 10 L

Provided ready to use. Stable until the expiration date stated on the label when stored at room temperature (15° to 30°C). An increase in substrate background measurements or increased relative light units for the zero calibrators in “sandwich”-type assays may indicate instability.

1. R3 Wash Buffer II: 1 x 10 L.
   - TRIS buffered saline, surfactant, < 0.1% sodium azide, and 0.1% ProClin* 300.

Warnings and precautions

- For in vitro diagnostic use.
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.(9)
- ProClin** 300 is a potential skin sensitizer. Avoid spilling or splashing this reagent on skin or clothing. In case of contact with the reagent, flush thoroughly with soap and water.

DxI Access Wash Buffer II Diluent Pack

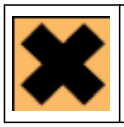
Cat. No. A79784: 2 diluent packs, 32.9 mL/pack

- Provided ready to use.
- Store upright and refrigerate at 2° to 10°C.
- Refrigerate at 2° to 10°C for a minimum of two hours before use on the instrument.
- Stable until the expiration date stated on the label when stored at 2° to 10°C.
- Stable at 2° to 10°C for 56 days after initial use of each well.
- Signs of possible deterioration are a broken elastomeric layer on the pack.
- If the diluent pack is damaged (i.e., broken elastomer), discard the pack.

R1a - R1e	TRIS buffered saline, surfactant, < 0.1% sodium azide, and 0.5% ProClin** 300.

Warnings and precautions

- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.(9)
- Xi. Irritant: 0.25% ProClin** 300.



R 43: May cause sensitization by skin contact.
S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- Safety Data Sheet (SDS) is available online.

Equipment

This test is performed on the Beckman UniCel DxI800 systems; Beckman-Coulter , Brea, California. For technical assistance, call the Beckman-Coulter hotline: 1-800-854-3633.

Refer to the Beckman UniCel DxI systems *Instructions for Use* manual, *Reference Manual* and/or *Help System* for detailed instructions.

Calibration

An active calibration curve is required. For the Access Total βhCG (5th IS) assay, calibration is required every 28 days. Refer to the UniCel DxI System *Instructions for Use* manual and/or *Help System* for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

Quality Control

Quality control materials simulate the characteristics of patient samples and are essential for monitoring the system performance of immunochemical assays. Because samples can be processed at any time in a “random access” format rather than a “batch” format, quality control materials should be included in each 24-hour time period.(13)

At least three levels of control material should be analyzed daily. In addition, these controls should be run with each new calibration, with each new reagent lot, and after specific maintenance or troubleshooting procedures as detailed in the UniCel DxI *Instructions for Use* manual. More frequent use of controls or the use of additional controls is left to the discretion of the operator based on work load and work flow.

The following controls should be used in accordance with the package instructions for use inserts. Copies of these inserts can be found in the *Control IFUs* folder on the S drive (*S:\APS\ClinLab\PoliciesandProcedures\1000-8999CLINICALPATHOLOGY\3000-3999Chemistry\3000-3499AutomatedChemistry\Control IFUs*). Quality control results should be evaluated and handled with respect to the Clinical Chemistry Quality Control Procedure #3000.T. Controls are compiled statistically in the LIS and reagent lot changes are documented on the UniCel DxI 800 Reagent Log sheets. Quality control results that do not fall within acceptable ranges may indicate invalid test results.

Examine all test results generated since obtaining the last acceptable quality control test point for this analyte. Refer to the UniCel DxI *Instructions for Use* manual and/or *Help System* for information about reviewing quality control results.

Quality Control Material

Control	Storage
MAS OmniImmune 1	until the expiration date at -20°C or colder /30 days at +2°C to +8°C
MAS OmniImmune 2	until the expiration date at -20°C or colder /30 days at +2°C to +8°C
MAS OmniImmune 3	until the expiration date at -20°C or colder /30 days at +2°C to +8°C

Controls are received frozen and stored at –15°C to –20°C.
Bottles of controls in use are thawed and stored at +2°C to +8°C and are good for 30 days.

Thaw controls at room temperature (18° to 25°C) on a rocker until liquid and then immediately store at 2° to 8°C. Thoroughly mix the control bottles before each use by mixing on a rocker for five minutes. Use immediately and return bottles to 2° to 8°C after use.

Testing Procedure

Refer to the UniCel DxI *Instructions for Use* manual and/or *Help System* for information on managing samples, configuring tests, requesting tests, and reviewing test results.

1. If necessary, load the reagent onto the system. Refer to the Beckman UniCel DxI systems *Instructions for Use* manual and/or *Help System* for detailed instructions. Date, initial cartridge and document in reagent log before loading each new cartridge.
2. After reagent load is completed, calibration may be required. Refer to the Beckman UniCel DxI systems *Instructions for Use* manual and/or *Help System* for detailed instructions.
3. Program samples and controls for analysis. Refer to the Beckman UniCel DxI systems *Instructions for Use* manual and/or *Help System* for detailed instructions.
4. For assaying samples containing total βhCG concentrations up to the concentration of the Access Total βhCG (5th IS) S5 calibrator, select HCG5 as the test name.
5. Select d-CG5 as the test name for assaying samples containing total βhCG concentrations greater than the concentration of the Access Total βhCG (5th IS) S5 calibrator. The same reagent pack is used for both assays.
6. After loading samples and controls onto the system, follow the protocols for system operation. Refer to the Beckman UniCel DxI systems *Instructions for Use* manual and/or *Help System* for detailed instructions.

Procedural Comments

1. Refer to the UniCel DxI *Instructions for Use* manual, *Reference Manual* and/or *Help System* for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.
2. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs. Document lot number in reagent log, date and initial pack prior to loading.
3. Use **twenty-five (25) µL** of sample for each determination in addition to the sample container and system dead volumes when requesting the **HCG5** assay. Use **twenty (20) µL** of sample in addition to the sample container and system dead volumes for each determination run with the DxI system onboard dilution feature (test name:**d-CG5**). Refer to the UniCel DxI *Instructions for Use* manual and/or *Help system* for the minimum sample volume required.
4. The system default unit of measure for sample results is mIU/mL. To change sample reporting units to the International System of Units (SI units), IU/L, refer to the UniCel DxI *Instructions for Use* manual and/or *Help system*. To manually convert concentrations to the International System, multiply mIU/mL by multiplication factor 1.

Reporting Results

Patient Results

Patient test results are determined automatically by the system software. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data. Patient test results can be reviewed using the appropriate screen. Refer to the UniCel DxI *Instructions for Use* manual and/or *Help System* for complete instructions on reviewing sample results.

Expected Values

Total βhCG concentrations were measured in human serum samples collected from apparently healthy non-pregnant females using the Access Total βhCG (5th IS) assay. Concentrations of total βhCG measured in 100% of samples were determined to be ≤ 11.6 mIU/mL (IU/L). The observed ranges and 95th percentile of total βhCG concentrations are shown in the table below.

Reference Population (non-Pregnant Females)	n	Median (mIU/mL)	Range (mIU/mL)	95% 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]

***Post-menopausal status confirmed using circulating FSH and estradiol levels.

Limitations of the Procedure

1. This assay is only intended for use as an aid in the early detection of pregnancy.
2. Samples can be accurately measured within the analytical range of the lower limit of quantitation and the highest calibrator value (0.6 to approximately 1350 mIU/mL [IU/L]).
   - If a sample contains less than the lower limit of quantitation for the assay, report the results as less than that value (i.e., < 0.6 mIU/mL [IU/L]).
   - If a sample contains more than the stated value of the highest Access Total βhCG (5th IS) Calibrator (S5), the Onboard Dilution Feature will be used with assay test **d-CG5**.

   Onboard Dilution Feature for use on UniCel DxI
   Samples containing hCG concentrations greater than the concentration of the Access Total βhCG (5th IS) S5 calibrator can be processed using the DxI onboard dilution feature. The DxI system onboard dilution feature automates the dilution process, using one volume of sample with 199 volumes of Wash Buffer II from the UniCel DxI Access Immunoassay Systems Diluent Pack (**Cat. No. A79784**) allowing samples to be quantitated up to approximately 270,000 mIU/mL.

Test Name	Reportable Range (mIU/mL)	Sample Volume Required)
d-CG5	1050 to ~270,000	20µL

   Note: The system reports the results adjusted for the dilution. Any neat sample reading < 1050 mIU/mL in the **d-CG5** assay should be retested in the **HCG5** assay.
   - For short samples not enough for dilution, use the regular 0.5 mL cup rack without flexible volume.
   - Samples with sufficient volume requiring a dilution, but front loaded using the non-flexible volume rack, must be reloaded for the on-board dilution.
   - Short samples QNS for dilution will be reported as **>1350.00 mIU/mL, QNS for dilution**.
3. **DO NOT** reuse small sample volumes that have been resident on the analyzer for more than 1 hour.
4. The Access Total βhCG (5th IS) assay has no discernible “hook effect” at 1,000,000 mIU/mL.
5. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may

be present in patient samples.(14,15) Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.

6. Other potential interferences in the patient sample could be present and may cause erroneous results in immunoassays. Some examples that have been documented in literature include rheumatoid factor, endogenous alkaline phosphatase, fibrin, and proteins capable of binding to alkaline phosphatase.(16) Carefully evaluate the results of patients suspected of having these types of interferences.
7. The role of preanalytical factors in laboratory testing has been described in a variety of published literature. (9,10,11) Following blood collection tube manufacturers' specimen collection and handling recommendations will help to reduce preanalytical error.
8. Low levels of hCG β-core fragment do not react with the Access Total βhCG (5th IS) assay. Interference from high levels of hCG β-core fragment has not been tested.
9. Automatic dilutions of serum samples by the on board dilution method has the potential of generating individual results with bias > 15%. For representative data see Dilution Recovery section.
10. The Access Total βhCG (5th IS) results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests, and other appropriate information.
11. If the total βhCG result is not consistent with clinical presentation, results should be confirmed by an alternate hCG method or a urine-based assay.(17)
12. Trophoblastic or nontrophoblastic neoplastic conditions and post-menopausal status should be considered before interpreting results.
13. The Access Total βhCG (5th IS) assay has not been validated by Beckman Coulter as a tumor marker. Requests for this test will continue to be a send-out test.

Performance Characteristics

Analytical Measurement Range

Samples can be accurately measured within the analytic range of the lower Limit of Quantitation (LoQ) and the highest calibrator value (S5) (**approximately 0.6–1350 mIU/mL [IU/L])**

Clinical Reportable Range

- If a sample contains less than the lower Limit of Quantitation (LoQ) for the assay, report the results as less than that value [i.e. **< 0.6 mIU/mL** (IU/L)].
- Samples containing greater than the concentration of the Access Total βhCG (5th IS) S5 calibrator for the TβhCG assay (~1350 mIU/mL) will reflex the dilution assay, d-CG, to extend the analytical measurable range from 1050 to ~270,000 mIU/mL. If the d-CG5 result is greater than 270,000 mIU/mL, the TβhCG2 result is reported as **> 270,000 mIU/mL**.
- Short samples QNS for dilution will be reported as **>1350.00 mIU/mL, QNS for dilution**.

Linearity

Based on CLSI EP6-A,(19) one high sample (> 1350 mIU/mL) and one low sample (< 0.2 mIU/mL) were mixed to make 13 evenly distributed sample concentrations. Four replicates of the 13 mixed samples, 8 replicates of the low sample and 8 replicates of the high sample were run on a single DxI 800 system. The Access Total βhCG (5th IS) assay was designed to be linear, with a maximum deviation from linearity of ≤ 10.0% for samples > 3.9 mIU/mL, and ≤ 0.39 mIU/mL (1.0 SD) for samples ≤ 3.9 mIU/mL. One study, analyzed using a polynomial regression method demonstrated a maximum deviation from linearity of 7.5% for samples > 3.9 mIU/mL, and 0.09 mIU/mL for samples ≤ 3.9 mIU/mL.

Dilution Recovery

Five serum samples and five lithium heparin plasma samples slightly above the Access Total βhCG (5th IS) S5 calibrator (approximately 1350 mIU/mL) were diluted 1/200 with Wash Buffer II. When run on the UniCel DxI 800 Immunoassay System, the average percent recoveries of the individual plasma samples ranged from 99-109% and the average percent recoveries of the individual serum samples ranged from 95-114%.

Representative data using five individual serum samples with eight replicates for each sample at approximately 1,500 mIU/mL is shown below:

Serum Sample	Dil-Total βhCG (5th IS) Assay Average Recovery (%)	Dil-Total βhCG (5th IS) Assay Recovery Range (%)	Onboard Dilution Assay Average Recovery (%)	Onboard Dilution Assay Recovery Range (%)
Sample 1	109	99 - 118	114	106 - 123
Sample 2	109	96 - 119	112	106 - 119
Sample 3	97	88 - 108	100	92 - 108
Sample 4	95	84 - 102	100	93 - 107
Sample 5	96	84 - 103	102	93 - 108

Limit of Blank

The Access Total βhCG (5th IS) assay is designed to have a Limit of Blank (LoB) of ≤ 0.5 mIU/mL (IU/L). In one study, LoB was tested using a protocol based on CLSI EP17-A2.(22) A total of 156 replicates of a zero analyte sample, the Access Total βhCG (5th IS) Calibrator S0, were measured in 12 runs using multiple reagent packs and calibrator lots on multiple UniCel DxI 800 Immunoassay Systems. This study determined the LoB for Access Total βhCG (5th IS) to be 0.1 mIU/mL (IU/L).

Limit of Detection

The Access Total βhCG (5th IS) assay is designed to have a Limit of Detection (LoD) of ≤ 0.5 mIU/mL (IU/L). In one study, LoD was tested using a protocol based on CLSI EP17-A2.(22) Thirty-six replicates from 6 low-level samples were measured using multiple reagent packs and calibrator lots in 12 runs on multiple UniCel DxI 800 Immunoassay
Systems. This study determined the LoD for Access Total βhCG (5th IS) to be 0.2 mIU/mL (IU/L).

Limit of Quantitation

The Access Total βhCG (5th IS) assay is designed to have a Limit of Quantitation (LoQ) of ≤ 0.6 mIU/mL (IU/L). In one study, LoQ was tested using a protocol based on CLSI EP17-A2.(22) Ten replicates of eight low-level samples were measured using three reagent pack lots and one calibrator lot in six runs on multiple UniCel DxI 800 Immuno- assay Systems. LoQ was determined as the lowest concentration which met the design requirements of 20% CV
and recovery of +/- 0.1 mIU/mL for three reagent lots when compared to the WHO 5th International Standard for Chorionic Gonadotropin (NIBSC Code 07/364). This study determined the LoQ for Access Total BhCG (5th IS) to be 0.6 mIU/mL (IU/L).

Methods Comparison

As determined by Beckman

A comparison of 224 serum values using the Access Total βhCG (5th IS) assay (Cat. No. A85264) on the UniCel DxI 800 Immunoassay System and a commercially available immunoassay system yielded the following statistical data using Passing-Bablok regression:

n	Range of Observations† (mIU/mL)	Intercept (mIU/ml)	Slope (95% CI)	Correlation Coefficient (r)
224	3.2 - 1095.9	2.87	1.04 (1.02 - 1.06)	0.99

†Observed concentration range of the Access Total βhCG (5th IS) assay.

Total Human Chorionic Gonadotropin (TβhCG 5th IS) - Serum
Beckman UniCel DXI Systems

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As determined by UCDMC

DxI-602049
Serum (in the range of 0.00 to 129111.00 mIU/mL)

Y (TβhCG 5th IS)	= M1.2393X - 4.53
N	= 40
Mean (TβhCG 5th IS)	= 154.2
Mean (TβhCG 3rd IS)	= 128.1
Correlation Coefficient (r)	= 0.9979

DxI-602053
Serum (in the range of 0.00 to 129111.00 mIU/mL)

Y (TβhCG 5th IS)	= 1.1057X - 1.1057
N	= 40
Mean (TβhCG 5th IS)	= 140.4
Mean (TβhCG 3rd IS)	= 128.1
Correlation Coefficient (r)	= 0.9984

TβhCG 5th IS
Serum (in the range of 0.24 to 132184.00 mIU/mL)

Y (DxI800-602053)	= 0.8923X + 2.84
N	= 40
Mean (DxI800-602053)	= 140.4
Mean (DxI800-602049)	= 154.2
Correlation Coefficient (r)	= 0.9980

Dilution Recovery (Linearity)

Multiple dilutions of one sample containing elevated hCG levels with Access Wash Buffer II resulted in the following data for DxI800-602049:

Sample	rep1 (mIU/mL)	rep2 (mIU/mL)	Average Expected Concentration (mIU/mL)	Determined Concentration (mIU/mL)	Recovery (%)
Neat	N/A	N/A	N/A	1047.6	N/A
1/2	519.7	523.2	521.5	523.8	99.6
1/4	235.2	239.7	237.5	261.9	90.7
1/8	125.6	123.9	124.8	131.0	95.3
1/16	63.4	63.8	63.6	65.5	97.1
1/32	31.1	31.9	31.5	32.7	96.2
1/64	16.6	17.2	16.9	16.4	103.2
			Mean % Recovery		97.0

Total Human Chorionic Gonadotropin (TβhCG 5th IS) - Serum
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Multiple dilutions of one sample containing elevated hCG levels with Access Wash Buffer II resulted in the following data for DxI800-602053:

Sample	rep1 (mIU/mL)	rep2 (mIU/mL)	Average Expected Concentration (mIU/mL)	Determined Concentration (mIU/mL)	Recovery (%)
Neat	N/A	N/A	N/A	1225.5	N/A
1/2	652.0	637.7	644.9	612.8	105.2
1/4	320.8	326.0	323.4	306.4	105.6
1/8	167.3	169.1	168.2	153.2	109.8
1/16	84.3	86.6	85.5	76.6	111.6
1/32	40.7	40.8	40.8	38.3	106.4
1/64	20.8	20.8	20.8	19.1	108.6
			Mean % Recovery		107.9

Imprecision

The Access Total βhCG (5th IS) assay exhibits total imprecision ≤ 10.0% CV at concentrations greater than 3.9 mIU/mL, and total Standard Deviation (SD) ≤ 0.39 mIU/mL at concentrations ≤ 3.9 mIU/mL. Two separate studies, one using human serum samples and one using human plasma samples, involved a total of 40 runs, with two replicates per run, over 20 days. The following data were calculated based on CLSI EP5-A2(20) guidelines.

Serum Sample	Grand Mean (n=80) (mIU/mL)	Within-run		Total Imprecision			
		SD (mIU/mL)	%CV	SD (mIU/mL)	%CV	SD (mIU/mL)	%CV
Sample 1	0.6	0.07	N/A	0.11	N/A	0.13	N/A
Sample 2	4.1	0.11	2.7	0.24	6.0	0.27	6.6
Sample 3	24.0	0.68	2.8	0.30	1.2	0.74	3.1
Sample 4	106.7	1.97	1.8	2.60	2.4	3.26	3.1
Sample 5	791.3	24.24	3.1	40.81	5.2	47.46	6.0
Sample 6	1116.1	38.19	3.4	53.29	4.8	65.56	5.9
Sample 7	208,778	7,482	3.6	9,400	4.5	12,014	5.8

Plasma Sample	Grand Mean (n=80) (mIU/mL)	Within-run		Total Imprecision			
		SD (mIU/mL)	%CV	SD (mIU/mL)	%CV	SD (mIU/mL)	%CV
Sample 1	1.4	0.06	N/A	0.06	N/A	0.08	N/A
Sample 2	4.7	0.23	4.8	0.20	4.2	0.30	6.4
Sample 3	675.0	19.81	2.9	21.45	3.2	29.20	4.3
Sample 4	1112.3	43.35	3.9	58.09	5.2	72.48	6.5

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Precision established at UCDMC

DxI800-602049

Analyzer	Type of Precision	Sample Type	n	Mean (mIU/mL)	1 SD	%CV
DxI800-602049	Within-run	MAS OmniImmune 1	20	5.5	0.12	2.25
		MAS OmniImmune 2	20	32.2	0.84	2.62
		MAS OmniImmune 3	20	553.4	14.15	2.56
	Between-run	MAS OmniImmune 1	30	6.0	0.17	2.84
		MAS OmniImmune 2	30	34.4	0.91	2.65
		MAS OmniImmune 3	30	566.9	21.64	3.82
DxI800-602053	Within-run	MAS OmniImmune 1	20	5.6	0.17	3.01
		MAS OmniImmune 2	20	33.0	0.89	2.69
		MAS OmniImmune 3	20	510.3	15.97	3.13
	Between-run	MAS OmniImmune 1	30	5.8	0.16	2.68
		MAS OmniImmune 2	30	33.6	0.79	2.36
		MAS OmniImmune 3	30	519.0	22.5	4.29

Analyzer	Type of Precision	Sample Type	%CV
DxI800-602049	Total	MAS OmniImmune 1	3.59
		MAS OmniImmune 2	3.70
		MAS OmniImmune 3	4.60
DxI800-602053	Total	MAS OmniImmune 1	4.03
		MAS OmniImmune 2	3.56
		MAS OmniImmune 3	5.31

Serum vs. Plasma Sample Comparison at UCDMC

	DxI 049 SERUM	DxI049 PLASMA	DxI 053 SERUM	DxI 053 PLASMA
AVERAGE	536.48	561.62	537.64	525.62

N = 41
Range = 0.0 – 3787.8
P=0.995
Mean (SD) bias = 6.6 (88.35)
Linear Regression shows R-squared of 0.9961 with an equation: Y = 1.014X – 1.015

Analytical Specificity/Interferences

Samples containing interferents at the concentrations listed below do not affect the concentration of total βhCG reported.

Substance	Concentration Added
Acetaminophen	20 mg/dL
Acetylsalicylic Acid	65 mg/dL
Bilirubin (conjugated and unconjugated)	40 mg/dL
Hemoglobin	500 mg/dL
Heparin (Low Molecular Weight)	7200 U/dL
Total Protein (Human Serum Albumin)	6 g/dL
Ibuprofen	50 mg/dL
Triglycerides (Intralipid)	3 g/dL
Multi-vitamin	0.9% (v/v)

The following table describes the cross-reactivity of the Access Total βhCG (5th IS) assay when substances that are similar in structure to hCG were added to a patient sample with an approximate hCG concentration of 2.9 mIU/mL. This study was performed on a UniCel DxI 800 Immunoassay System and values were calculated as described in CLSI EP7-A2.(21) When the concentrations of cross-reactants below were added to the Access Total βhCG (5th IS) Calibrator S0 (zero), the result was below the limit of detection.

Substance	Concentration Added (mIU/mL)	hCG Concentration Without Cross-Reactant (mIU/mL)	hCG Concentration With Cross-Reactant (mIU/mL)
hLH	103	2.25	2.10
hFSH	1000	2.30	2.23
hTSH	1	2.44	2.53
hCG α-subunit	500	2.95	2.87

The Access Total βhCG (5th IS) assay recognizes intact hCG, the β subunit of hCG, nicked intact hCG and nicked βhCG isoforms. The free α subunit and β-core fragment yield no detectable response.

*Lumi-Phos is a trademark of Lumigen, Inc., a subsidiary of Beckman Coulter, Inc.
**ProClin is a trademark of Rohm and Haas Company or of its subsidiaries or affiliates.

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Prepared By	Date Adopted	Supersedes Procedure #
Michael Inn	May 28, 2014	TβhCG2 3rd IS #33203T

Revision Date	Type of Revision	Revised by	Review/Annual Review Date	Reviewed By
	Replaces TβhCG2 3rd IS	M. Inn		
			07/23/2014	L. Howell
09/11/2014	reference interval clarification	kdagang	09/11/2014	J. Gregg
03/10/2015	Lithium Heparin added as acceptable sample type	kdagang	03/13/15	J. Gregg