DXI & ACCESS (HCG5) TOTAL BETA HCG

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PURPOSE

To provide instruction on how to perform Total βhCG testing on the DXI and Access instruments.

PRINCIPLE

The Total βhCG reagent, when used in conjunction with the Beckman Access or DXI Systems and Access Calibrators, is intended for quantitative determination of Total βhCG concentration in human serum or plasma.

BACKGROUND

Clinical Significance

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 beta LH, but has a unique carboxyl terminal region.

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. 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. 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. During pregnancy, unusually low or rapidly declining levels may indicate an abnormal condition such as an ectopic pregnancy or impending spontaneous abortion.

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.

RELATED DOCUMENTS

R-PO-CH0810	Quality Control Program General Laboratory
R-PO-CH0809	Quality Control Westgard Rules Statistics
R-PR-AD0540	Specimen Rejection/Cancellation Protocol
J-F-CH0824	DXI & Access Controls
J-F-CH0825	DXI Calibrators
M-F-CH0820	Chemistry Controls
M-F-CH0826	Chemistry Calibrators
R-F-CH2000	Access 2 and DXI Analytical Measurement Range

SPECIMEN

Type of Specimen

Biological fluid samples should be collected in the same manner routinely used for any laboratory test. Freshly drawn serum or plasma (lithium heparin) is the preferred specimen.

Specimen Storage and Stability

1. Tubes of blood are to be kept closed and in a vertical position at all times. It is recommended that the serum or plasma be physically separated from contact with cells within two hours of the time of collection.

2. Separated serum or plasma should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed. Ensure residual fibrin and cellular matter have been removed prior to analysis.

Sample Type	Volume	Sample Stability
Serum/Plasma	0.5mL	• Separate serum from cells within 2 hours. • Room Temp 8 hours • Refrigerated 48 hours • Frozen 6 months. Thaw only once.

Criteria for Unacceptable Specimens

Refer to the PROCEDURAL NOTES section of this chemistry information sheet for information on unacceptable specimens.
See Related Documents: Specimen Rejection/Cancellation Protocol

Sample Volume

The optimum volume, when using a 0.5 mL sample cup, is 0.3 mL of sample. For optimum primary sample tube volumes and minimum volumes, refer to the Primary Tube Sample Template for your system.

REAGENTS

1. R1: 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 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 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 (e.g., 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.

Reactive Ingredients	
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.	R1a
Protein (goat, murine, and recombinant) diluted in citrate buffered saline, with surfactant, < 0.1% sodium azide, and 0.1% ProClin 300.	R1b
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.	R1c

2. Access Total βhCG (5th IS) Calibrators
Cat. No. B11754: S0–S5, 4.0 mL/vial

Quantitative assay calibration is the process by which samples with known analyte concentrations (i.e., assay calibrators) are tested 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 Relative Light Unit (RLU) measurements of samples of unknown concentration to specific quantitative analyte concentrations.

Provided ready to use. Store upright and freeze at -20°C or colder. Stable until the expiration date stated on the label when stored at -20°C or colder. Thaw at room temperature. Mix contents by gently inverting before use. Avoid bubble formation. Thaw calibrators no more than 15 times. Return calibrators to -20°C or colder after each use. Vial is stable at -20°C or colder for 120 days after initial use. Signs of possible deterioration are control values out of range. Refer to calibration card for exact concentrations.

Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

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–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

3. Access Substrate
Cat. 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 to 8°C	Until expiration date stated on the label
Equilibration prior to use (unopened)	15 to 30°C (room temperature)	Minimum 18 hours Maximum 14 days
In use (opened)	External fluids tray substrate position	Maximum 14 days

R2 Substrate: Lumi-Phos 530 (buffered solution containing dioxetane Lumigen* PPD, fluorescer, and surfactant).

Refer to the appropriate system manuals and/or Help system for detailed instructions.

4. Access 2, UniCel DxC **600i:**
   Access Wash Buffer II, Cat. No. A16792, 4 x 1950 mL
   UniCel DxI 600, UniCel DxI 800, UniCel DxC 880i, UniCel DxC 860i, UniCel DxC 680i, UniCel DxC 660i,:
   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.

   Refer to the appropriate system manuals and/or Help system for detailed instructions.

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

5. UniCel DxI Access Immunoassay Systems Wash Buffer II
   Cat. No. A79784, 2 diluent packs, 32.9 mL/pack (diluent pack for use with the UniCel DxI system onboard dilution feature).

   The analyte level in patient samples may exceed the level of the specific highest calibrator. If a quantitative value is required, it will be necessary to dilute the samples in order to determine the analyte concentration.

   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 or control values out of range. If the diluent pack is damaged (i.e., broken elastomer), discard the pack.

   Samples can be accurately measured within the analytical range of the lower limit of detection and the highest calibrator value of the specific assay. If a sample contains more analyte than the stated value of the highest calibrator, dilute the sample following dilution instructions in the specific assay labeling under "Limitations of the Procedure" in the reagent pack section. Refer to the appropriate system manuals and/or Help system for detailed instructions.

   R1a–R1e: TRIS buffered saline, surfactant, < 0.1% sodium azide, and 0.1% ProClin 300.

6. Quality Control (QC) materials: commercial control material

7. Access Immunoassay System and supplies

8. Warnings and Precautions
   - For *in vitro* diagnostic use.
   - 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.

- Human source material was purified from the urine of pregnant women and should be treated as potentially infectious.
- 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.
- Xi. Irritant: 0.5% 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.
- Substrate is sensitive to air exposure. Keep tightly closed at all times. Do not pool bottles of substrate.
- The Material Safety Data Sheet (MSDS) is available upon request.

CALIBRATION

An active calibration curve is required for all tests. For the Access Total βhCG (5^{th} IS) assay, calibration is required every 28 days. Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

The Access Total βhCG (5^{th} IS) Calibrators are provided at 6 levels – zero and approximately 6, 35, 195, 620 and 1350 mIU/mL. Assay calibration is required every 28 days.

Calibrators run in duplicate.

QUALITY CONTROL

See Related Documents J-F-CH0824 DXI & Access Controls and M-F-CH0820 Chemistry Controls

STEPS

1. Instrument Operation: Refer to the appropriate system manuals and/or Help system for preparation and operation.

2. Assay Procedure: Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.
   - Select HCG5 as the test name for assaying samples containing total βhCG concentrations up to the concentration of the Access Total βhCG (5th IS) S5 calibrator.
   - UniCel DxI users may use either the UniCel DxI onboard dilution feature (Test name d-CG5) or the special dilution feature (Test name HCG5d) for assaying samples containing total βhCG concentrations greater than the Access Total βhCG (5th IS) S5 calibrator.
   - The same reagent pack is used for all assays.

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 appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

PERFORMANCE CHARACTERISTICS

Reference Range

Type	Reference Range
Male or non-pregnant female	less than 5 mIU/mL
Pregnant female	See chart below

Approximate Gestational Age in Weeks	Approximate βhCG range (mIU/mL)
0.2 – 1	5 – 50
1 – 2	50 – 500
2 – 3	100 – 5,000
3 – 4	500 – 10,000
4 – 5	1,000 – 50,000
5 – 6	10,000 – 100,000
6 – 8	15,000 – 200,000
8 – 12	10,000 – 1.000,000

Analytic Range

Sample Type	Conventional Units
Serum or Plasma (HCG5)	1-1350 mIU/mL
Serum or Plasma (d-CG5 & HCG5d)	1050-200,000 mIU/mL

Reporting results outside of analytical range

Lower limit of detection	0.6 mIU/mL	Results below 0.6 should be reported as <1 mIU/mL
Upper limit of detection (d-CG5 & HCG5d)	200,000 mIU/mL	Results above 200,000, report as >200,000. See limitations below for dilution instructions.

LIMITATIONS

1. This assay is only intended for use as an aid in the early detection of pregnancy.

2. **Onboard Dilution Feature for use on UniCel DxI systems (SJMC only):**

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

   Note: The system reports the results adjusted for the dilution. If the sample contains less than 1050 mIU/mL, the system will report results as < 1050 mIU/mL. Any neat sample reading < 1050 mIU/mL in the **d-CG5** assay should be retested in the **HCG5** assay.

Dil-Total βhCG (5th IS) Special Dilution Feature for use on Access 2 and UniCel Dxl systems (SFH, SCH, SAH, SEH):

Samples containing hCG concentrations greater than the concentration of the Access Total βhCG (5th IS) S5 calibrator can be processed using the Special Dilution Feature. When HCG5d is requested, the system automatically dilutes the sample using Wash Buffer II and reads the resulting dose off the HCG5 calibration curve.

Test Name	Manufacturer Reportable Range (mIU/mL)	Sample Volume Required
HCG5d	1050 to ~270, 000	6 µL

Note: The system multiplies by the dilution factor defined in the software to calculate final test results. If the sample contains less than 1050 mIU/mL, the system will report results as < 1050 mIU/mL. Any neat sample resulted as < 1050 mIU/mL in the **HCG5d** assay should be retested in the **HCG5** assay.

Manual off-line pre-dilution:

- Dilute one volume of sample with 199 volumes of Wash Buffer II (1/200).
- Type in the pre-dilution factor when entering the test request. Order the **HCG5** test.
- The system will automatically multiply the result by the pre-dilution factor and report that value.
- If the pre-dilution option was not entered when requesting the test, multiply the calculated value by the dilution factor 200 after assaying the diluted sample using the **HCG5** assay.
- Refer to the appropriate system manuals and/or Help system for additional instructions on processing pre-diluted samples.

3. DO NOT reuse small sample volumes that have been resident on the analyzer for more than one hour.

4. 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. Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.

5. 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. Carefully evaluate the results of patients suspected of having these types of interferences.

6. The role of preanalytical factors in laboratory testing has been described in a variety of published literature. Following blood collection tube manufacturers' specimen collection and handling recommendations will help to reduce preanalytical error.

7. 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.

8. Automatic dilutions of serum samples (by Dil-Total βhCG (5th IS) or onboard dilution) have the potential of generating individual results with bias > 15%. For representative data see Dilution Recovery section of the Access Total BhCG (5th IS) product insert.

9. 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.

10. The Access Total βhCG (5th IS) assay has no discernible “hook” effect up to 1,000,000 mIU/mL.

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.

12. Trophoblastic or nontrophoblastic neoplastic conditions and post-menopausal status should be considered before interpreting results.

PROCEDURAL NOTES

1. Refer to the appropriate system manuals 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.

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**). Use six (6) µL of sample for each determination in addition to the sample container and system dead volumes when requesting the **HCG5d** assay. Refer to the appropriate system manuals 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 appropriate system manuals and/or Help system. To manually convert concentrations to the International System, multiply mIU/mL by multiplication factor 1.

REFERENCES

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DOCUMENT APPROVAL **Purpose of Document / Reason for Change:**			
Major changes were made due to change in reagent to the new HCG5. Used new Beckman HCG5 (5th IS) procedure as template. Upper AMR to remain at 200,000 mIU/mL.			
Committee Approval Date	☒ Date: 1/8/2015 ☐ NA – revision of department-specific document which is used at only one facility	**Medical Director Approval** *(Electronic Signature)*	Katie Wilkinson, MD 6/23/15