KAISER PERMANENTE®
COLORADO LABORATORY

TOTAL BhCG and Tumor Marker TEST FOR BECKMAN / COULTER ACCESS II

PRINCIPLE

The Access Total BhCG assay is a two-site immunoenzymatic ("sandwich") assay. A sample is added to a reaction vessel with rabbit anti- hCG-alkaline phosphatase conjugate, and paramagnetic particles coated with goat anti-mouse IgG: mouse monoclonal anti-BhCG complexes. The BhCG binds to the immobilized monoclonal anti- hCG on the solid phase while, at the same time, the rabbit anti-BhCG-alkaline phosphatase conjugate reacts with different antigenic sites on the BhCG. 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.

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. Beta hCG (MW 25,000–30,000 daltons) shares several peptide sequences with beta LH, but has a unique carboxyl terminal region 1,2,3.

Shortly after implantation of a fertilized ovum into the uterine wall, the trophoblast begins to produce hCG. The hormone maintains steroid secretions of the corpus luteum until the placenta can do so. During a normal pregnancy, hCG is generally approximately 50 mIU/mL (IU/L) in the week after conception, and doubles every 1.5–3 days for the first six weeks

Levels continue to rise until the end of the first trimester, then gradually fall 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. During pregnancy, hCG concentrations increase as noted above and then show a gradual decrease after the first trimester. Unusually low or rapidly declining levels may indicate an abnormal condition such as an ectopic pregnancy or impending spontaneous abortion

SCOPE

All Medical Technologists and Medical Laboratory Technicians working at the Franklin Laboratory.

SPECIMEN REQUIREMENTS

1. Serum and plasma (heparin) are the recommended samples.
2. Observe the following recommendations for handling, processing, and storing blood samples
   - Collect all blood samples observing routine precautions for venipuncture.
   - Allow serum samples to clot completely before centrifugation.
   - Keep tubes stoppered at all times.
   - Store samples tightly stoppered at room temperature (15 to 30°C) for no longer than eight hours.
3. If the assay will not be completed within eight hours, refrigerate the samples at 2 to 8°C.
4. If the assay will not be completed within 48 hours, or for shipment of samples, freeze at -20°C or colder.
5. Thaw samples only once.
6. Use the following guidelines when preparing specimens, unless instructed otherwise in the product insert:
   - Ensure residual fibrin and cellular matter has been removed prior to analysis.
   - Follow blood collection tube manufacturer's recommendations for centrifugation.

Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot-to-lot.

7. Frozen specimens can be stored up to six months before testing

Order Codes

Cerner Code	Cerner Description	Health Connect Code	Health Connect Description
BHCG Qn	BHCG Qn	84702C	HCG, SERUM, QUANT

EQUIPMENT AND MATERIALS

1. Beckman Coulter Access II Analyzer(Franklin Facility ID# 982972/SN #504760)
2. Beckman Coulter Access reaction Vessels Ref#81901
3. Beckman Coulter Access Waste Bags Ref#81904
4. Transfer Pipettes Item # 9990 5580
5. Fiber-free polyester swabs
6. 2.0 mL sample cups(3 for maintenance)

REAGENTS

1. R1 Access Total βhCG Reagent Packs 100 determinations, 2 packs, 50 tests/pack
   a. Store upright and refrigerate at 2 to 10°C.
   b. Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
   c. Stable until the expiration date stated on the label when stored at 2 to 10°C.
   d. Stable at 2 to 10°C for 28 days after initial use.
   e. Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range.

f. If the reagent pack is damaged (i.e., broken elastomer), discard the pack.
g. All antisera are polyclonal unless otherwise indicated

2. Access Total βhCG Calibrators
Provided at zero and approximately 5, 25, 150, 500 and 1000 mIU/mL (IU/L).
Cat. No. 33505

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

4. Access Substrate Cat. No. 81906

5. Access Wash Buffer Cat. No. 81907 (Access, Access 2, SYNCHRON LX
Cat. No. 8547197 (UniCel™ DxI)

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:	Rabbit anti-βhCG alkaline phosphatase (bovine) conjugate diluted in TRIS buffered saline, with surfactant, BSA, protein (goat, rabbit, mouse), < 0.1% sodium azide, and 0.25% ProClin 300.

CALIBRATION- See Additional Access Calibration Procedure

QUALITY CONTROL- See Additional Access QC procedure.

PROCEDURE

- **Loading patient samples – Step by step instructions**

1. Place SST tube in appropriate Rack
2. Load Rack and press run.
3. For Manual Entry:
    - Go to “sample manager” and barcode the rack.
    - Select “test request”
    - Use the barcode scanner and read the barcode on the plasma cup.
    - Select the test “BhCG” and press ENTER.
    - Turn on batch mode to program the rest of the samples on the same rack for the same test.
    - Use the barcode scanner and read the barcode of all the samples you have on the rack.
4. Load the rack on the instrument – remember to tell, wait, do, done – then press RUN.
5. Check QC on log to make sure it is OK before reporting patient results.
6. The completed report will automatically print
7. Enter results in CERNER – see below
8. Cap and save samples in the back refrigerator and discard after 24 hours.

REPORTING RESULTS

1. Patient results are entered in the “ARE” application of CERNER – accession or instrument queue mode.

2. Enter the accession # (by typing or scanning the barcode) in the accession # field.

3. PERFORM then VERIFY.
4. Patient test results are determined automatically by the system software using a smoothing spline math model. 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.

- **Additional Information**

1. This assay is intended for early detection of pregnancy.
2. Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value [approximately 0.5–1000 mIU/mL (IU/L)].
3. To accurately measure samples containing 1000–200,000 mIU/mL, select the Dil-hCG2 test. This test uses the T βhCG2 pack. When Dil-hCG2 is requested, the system autodilutes the sample and reads the resulting dose off the T βhCG2 calibration curve. The system multiplies by the dilution factor defined in the software (200) to calculate final test results.
4. Alternatively, samples containing > 1000 mIU/mL can also be processed via **off-linepre-dilution** following these steps:

• Dilute one volume of sample with 199 volumes of Wash Buffer (1/200) or dilute per laboratory dilution protocol. NOTE: Dilution with alternate buffers may cause erroneous results.

• Type in the pre-dilution factor when entering the test request. Order the T BhCG orDil-hCG2 test.

• The system will automatically multiply the result by the pre-dilution factor and report that value.

NOTE: If the system reports a pre-diluted Dil-hCG2 result as < 1000 mIU/mL (IU/L), redilute the sample such that it will read between 1,000 and 200,000 mIU/mL (IU/L). A neat sample reading < 1000 mIU/mL (IU/L) in the Dil-hCG2 assay should be retested in the T βhCG2 assay.

• If the pre-dilution option is not selected, multiply the calculated value by the dilution factor 200 (or by another selected factor) after assaying the diluted sample using the Access Total βhCG assay.

• If the calculated value of the diluted sample using the Access Total B hCG assay is< 5 mIU/mL (IU/L), re-dilute one volume of the neat sample with 99 volumes of Wash Buffer (1/100) and re-assay, remembering to multiply the calculated value by the dilution factor 100.

• Refer to the appropriate system manuals and/or Help system for additional instructions on processing pre-diluted samples.

5. DO NOT reuse small sample volumes that have been resident on the analyzer for more than 1 hour.
6. The Access Total βhCG assay has no discernible “hook effect” at 1,000,000 mIU/mL.
7. For assays employing mouse antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) in the sample. Human anti-mouse antibodies may be present in samples from patients who have received immunotherapy or diagnostic procedures utilizing monoclonal antibodies14,15or in individuals who have been regularly exposed to animals. Additionally, other heterophile antibodies, such as human anti-goat antibodies, may be present in patient samples.

8. The Access Total βhCG 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.
9. If the total βhCG level is inconsistent with clinical presentation, results should be confirmed by an alternate hCG method or a urine-based assay16.
10. Trophoblastic or nontrophoblastic neoplastic conditions should be ruled out before reporting results.

REFERENCE RANGES:

	Median (mIU/mL)	95% Range (mIU/mL)
Males	<0.5	<0.5 – 2.67
Non-pregnant females	<0.5	<0.5 - 2.90

- Representative hCG ranges during normal pregnancy.

Approximate Gestational Age (weeks)	Approximate hCG Range (mIU/mL, IU/L)	Approximate Gestational Age (Weeks)	Approximate hCG Range (mIU/mL, IU/L)
0.2-1	5- 50	4-5	1000-50,000
1-2	50-500	5-6	10,000-100,000
2-3	100-5000	6-8	15,000-200,000
3-4	500-10000	8-12	10,000-100,000

Analytical Measurement Range	Clinical Reportable Range
2 - 1000	2 – 200,000 (Instrument performed dilution)

LIMITATIONS OF THE PROCEDURE

- **Imprecision**

This assay exhibits total imprecision of less than 10% across the assay range. One study, using commercially available human serum based control material generating a total of 20 assays, 3 replicates per assay, over 14 days provided the following data, analyzed via analysis of variance (ANOVA).

- **Analytical Specificity / Interferences**

Samples containing up to 10 mg/dL (171µmol/L) bilirubin, lipemic samples containing the equivalent of 1800 mg/dL (20.32 mmol/L) triglycerides, and hemolyzed samples containing up to 500 mg/dL (5 g/L) hemoglobin do not affect the concentration of total βhCG assayed. In addition, samples with 3 g/dL (30 g/L) of human albumin added to the endogenous albumin in the samples do not affect the concentration of total βhCG assayed. No significant cross reactivity [< 0.5 mIU/mL (IU/L)] was observed when hLH, hFSH, or hTSHwere added to the Access Total βhCG Calibrator S0 (zero) at 1000 mIU/mL (IU/L),1000 mIU/mL (IU/L), 1 mIU/mL (IU/L), respectively.

The molar percent specificity when10 mIU/mL (IU/L) of the WHO 75/551 free BhCG subunit is added to Access Total βhCGCalibrator S0 (zero) is approximately 200%.

- **Analytical Sensitivity**

The lowest detectable level of hCG distinguishable from zero (Access TotalβhCG Calibrator S0) with 95% confidence is 0.5 mIU/mL (IU/L). This value is determined by processing a complete six point calibration curve, controls, and 10 replicates of the zero calibrator in multiple assays. The analytical sensitivity value is interpolated from the curve at the point that is two standard deviations from the mean measured zero calibrator signal.

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