BHCG – TOTAL BHCG

Intended Use

The Alinity i Total β-hCG assay is a chemiluminescent microparticle immunoassay (CMIA) used for the quantitative and qualitative determination of beta-human chorionic gonadotropin (β-hCG) in human serum and plasma for the early detection of pregnancy on the Alinity i analyzer.

Clinical Significance

Human chorionic gonadotropin (hCG) is a sialoglycoprotein with a molecular weight of approximately 46,000 daltons.1 HCG is initially secreted by the trophoblastic cells of the placenta shortly after implantation of the fertilized ovum into the uterine wall.2, 3 The rapid rise in hCG serum levels after conception makes it an excellent marker for early confirmation of pregnancy. As uncomplicated pregnancies progress, the placenta assumes the production of these hormones. The serum hCG levels increase to a peak concentration, then decrease and plateau. HCG circulates as the intact molecule in the serum of normal women who have an uncomplicated pregnancy. The subunits are cleaved rapidly and cleared by the kidney.

Methodology

This assay is a two-step immunoassay for the quantitative and qualitative determination of β-hCG in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology. Sample and anti-β-hCG coated paramagnetic microparticles are combined and incubated. The β-hCG present in the sample binds to the anti-β-hCG coated microparticles. The mixture is washed. Anti-β-hCG acridinium-labeled conjugate is added to create a reaction mixture and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added.
The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of β-hCG in the sample and the RLUs detected by the system optics.

Specimen

Continued on next page

BHCG – TOTAL BHCG, *Continued*

Type of Specimen

Specimen Type	Collection Vessel
Serum	Serum tubes (with or without gel barrier)
Plasma	Dipotassium EDTA Tripotassium EDTA Lithium heparin Lithium heparin plasma separator Sodium heparin

Specimen Storage and Stability

1. Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum or plasma be physically separated from contact with cells within two hours from the time of collection.
2. For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
3. To ensure consistency in results, recentrifuge specimens prior to testing if they contain fibrin, red blood cells, or other particulate matter. NOTE: If fibrin, red blood cells, or other particulate matter are observed, mix by low speed vortex or by inverting 10 times prior to recentrifugation.
4. Prepare frozen specimens as follows: Frozen specimens must be completely thawed before mixing. Mix thawed specimens thoroughly by low speed vortex or by inverting 10 times. Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous. If specimens are not mixed thoroughly, inconsistent results may be obtained. Recentrifuge specimens.
5. **Recentrifugation of Specimens**: Transfer mixed specimens to a centrifuge tube and centrifuge. Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.

Continued on next page

BHCG – TOTAL BHCG, *Continued*

Specimen Type	Temperature	Maximum Storage Time	Special Instructions
Serum/ Plasma	2 to 8°C	7 days	If testing will be delayed more than 7 days, specimens should be frozen at -10°C or colder.
	-10°C or colder	12 months	Specimens stored frozen at -10°C or colder for 12 months showed no performance difference.

Sample Dilution Procedures

Serum/Plasma

Samples with a β-hCG value exceeding 15 000.00 IU/L are flagged with the code “15 000.00 IU/L " and may be diluted with either the Automated Dilution Protocol or the Manual Dilution Procedure.

Automated Dilution Protocol

The system performs a 1:15 dilution of the sample and automatically calculates the concentration by multiplying the result by the dilution factor.

Manual Dilution Procedure

Suggested dilution: 1:15

It is recommended that dilutions not exceed 1:75.

Add 20 µL of the sample to 280 µL of Alinity i Multi-Assay Manual Diluent. The operator must enter the dilution factor in the Specimen or Control tab of the Create Order screen. The system will use this dilution factor to automatically calculate the concentration of the sample and report the result.

Continued on next page

BHCG – TOTAL BHCG, *Continued*

Reagents

Reagent Handling

- Upon receipt, gently invert the unopened reagent kit by rotating it over and back for a full 180 degrees, 5 times with green label stripe facing up and then 5 times with green label stripe facing down. This ensures that liquid covers all sides of the bottles within the cartridges. During reagent shipment, microparticles can settle on the reagent septum.
  – Place a check in the square on the reagent kit to indicate to others that the inversions have been completed.
  • After mixing, place reagent cartridges in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- If a reagent cartridge is dropped, place in an upright position for 8 hours before use to allow bubbles that may have formed to dissipate.
- Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results.

Reagent Storage and Stability

	Storage Temperature	**Maximum Storage Time**	**Additional Storage Instructions**
Unopened	2 to 8°C	Until expiration date	Store in upright position. If cartridge does not remain upright, gently invert the cartridge 10 times and place in an upright position for 1 hour before use.
Onboard	System Temperature	30 days	

BHCG – TOTAL BHCG, *Continued*

Opened	2 to 8°C	Until expiration date	Store in upright position. Do not reuse original reagent caps or replacement caps due to the risk of contamination and the potential to compromise reagent performance.

Calibration

Calibration Required

For instructions on performing a calibration, refer to the Alinity ci-series Operations Manual, Section 5.
Each assay control must be tested to evaluate the assay calibration. Once a calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
• A reagent kit with a new lot number is used.
• Daily quality control results are outside of statistically-based quality control limits used to monitor and control system performance, as described in the Quality Control Procedures section of this package insert.
– If statistically-based quality control limits are not available, then the calibration should not exceed a 30-day limit for recalibration frequency.

Calibration Preparation

Calibration material is the 07P5101 Alinity i Total β-hCG Calibrators. Allow temperature before use. Prior to each use, mix by gentle inversion.

Calibration Storage and Stability

	Calibrator Storage	Stability Once OPEN
Total β-hCG Cal (CAL A to Cal F)	Unopened: 2 to 8 (until expiration date) Protect from light.	**2 to 8** °C**:** until expiration date

Continued on next page

BHCG – TOTAL BHCG, *Continued*

Calibration Information

1. Calibrator lots may be configured using the bar code label on the calibrator carton.
2. Verify that the correct calibrator values have been entered into the calibration file.
3. Calibration is performed by running Alinity i Total β-hCG Calibrators.
4. For information on configuring calibrator data, refer to the Alinity ci-series Operations Manual, Section 5.

Quality Control

See Policy Chemistry Quality Control Policy

Sample Processing

See Policy RIV-PPP-1199

Reference Range

Test unit= IU/L for all ages and both sexes.

Test	Age Year Low	Age Year High	Ref Low	Ref High
BHCG_FU	0	250	3510	
BHCG_INT	0	250	3510	
BHCG	0	56		5
BHCG	56	250		7.6

Analytic Range

AMR Low	AMR High	CRR Low	CRR High
3	15000.0	3	225000.0

Author

Melanie Beermann, CLS

Distributions

Kaiser Permanente Riverside Service Area Laboratory

End