

Quantitation of Beta-Human Chorionic Gonadotropin by LOCI™ Technology

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Abstract

Human chorionic gonadotropin is a dimeric glycoprotein hormone produced by the placenta and used as a marker for the early detection of pregnancy. The alpha subunit is similar to other anterior pituitary glycoprotein hormones while the beta subunit imparts the biological and immunological specificity. We describe the development and initial analytical performance of a homogeneous sandwich immunoassay* for measurement of beta human chorionic gonadotropin (BHCG) using LOCI™ technology on a new instrument system (Dimension Vista™ system).

The method is based on oxygen channeling luminescence technology. The LOCI™ reagents include two latex bead reagents and a biotinylated anti-BHCG monoclonal antibody fragment. The first bead reagent (sensibead) is coated with streptavidin and contains a photosensitizer dye. The second bead reagent (chemibead) is coated with a second anti-BHCG monoclonal antibody and contains chemiluminescent dye. A 2 µL sample of serum or plasma is incubated with chemibeats and biotinylated antibody to form chemibead-BHCG-biotinylated antibody sandwiches. Sensibeats are added and bind to the biotin to form bead-pair immunocomplexes. Illumination of these complexes at 680 nm generates singlet oxygen from sensibeats which diffuses into the chemibeats, triggering a chemiluminescent reaction. The resulting signal is measured at 612 nm and is directly related to the sample BHCG concentration.

The method detects both intact hCG and free beta subunits, including their nicked forms. The analytical range of 0.5-1000 mIU/mL spans the interval from the limit of detection (mean plus 2 SDs of an analyte-free sample) to the upper calibration standard. No high-dose hook effect was observed to at least 3,000,000 mIU/mL. The time to first result is 10 minutes. Within-run precision <3% CV with total precision <5% CV were observed over a 20 day testing interval, using serum pools and commercial quality control materials over the range of 20-500 mIU/mL. No significant interference (<10 % bias) was seen from lipemia (3000 mg/dL triglycerides), hemolysis (500 mg/dL hemoglobin), icterus (60 mg/dL conjugated bilirubin or 20 mg/dL unconjugated bilirubin), or rheumatoid factors (500 IU/mL). No cross-reactivity was observed from LH, FSH, or TSH. Comparison of results from 50 patient samples processed by the new method (Y) and the HCG method on the Dimension® clinical chemistry system (X) showed good agreement by linear regression analysis: $Y = 0.91 (\pm 0.01)X - 0.76 (\pm 5.8)$, $r = 0.99$, range = 1-1,000 mIU/mL.

We conclude that use of LOCI™ technology provides excellent sensitivity, precision, turnaround time, and dynamic range suitable for measurement of βhCG and the early detection of pregnancy.

Reaction Steps

The β-human chorionic gonadotropin (βhCG) method (BHCG*) uses a homogeneous sandwich immunoassay format. Sample is reacted simultaneously with biotinylated tag monoclonal antibody and Chemibeats, coated with a second monoclonal antibody. After an incubation period, Sensibeats are added to form bead immunocomplexes. Following a final incubation, the reaction solution is flashed with a light source to stimulate generation of singlet oxygen. The resulting chemiluminescent signal is directly proportional to the analyte concentration in the sample.

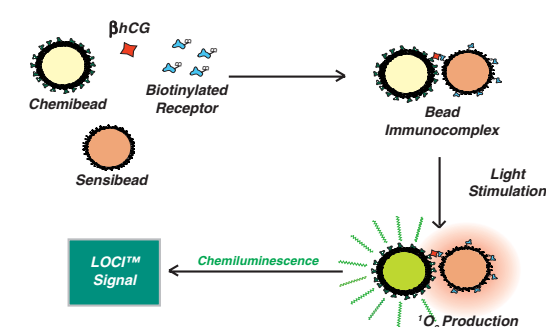


where: CB-Ab = Chemibead particle coated with capture monoclonal antibody
Ab-Biotin = Monoclonal tag antibody covalently bound to biotin
SA-SB = Sensibead particle coated with streptavidin

*Product under development - Not available for sale

LOCI™ Technology and Assay Format

Luminescent oxygen channeling immunoassay (LOCI™) technology enables high sensitivity homogenous immunoassays in either a sandwich or a competitive format. LOCI™ reagents include two latex bead reagents and a biotinylated analyte-specific receptor. The chemibead is coated with a binding partner specific for βhCG and contains chemiluminescent dye. A generic bead reagent (Sensibead) is coated with streptavidin and contains photosensitive dye. During an assay, the three reactants combine with analyte to form a bead-aggregated immunocomplex. Illumination of the complex releases singlet oxygen from the sensibead, which channels into the chemibead and triggers chemiluminescence.

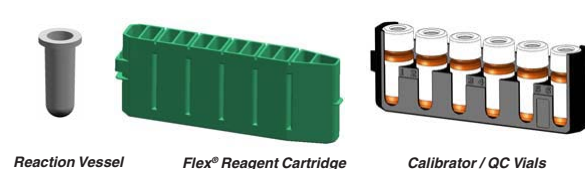


Chemibeats Latex beads (200 nm) contain an olefin dye that reacts with singlet oxygen to form a ¹O₂ adduct, which decays and generates the chemiluminescent signal. The beads also contain a fluorescent energy acceptor that shifts the emission wavelength to 612 nm. A blocking layer surrounds the beads to isolate the label and minimize potential nonspecific binding. In the sandwich format, capture antibodies are bound to the chemibead surface.

Sensibeats Latex beads (200 nm) contain a photosensitive dye that absorbs light at 680 nm and generates singlet oxygen (¹O₂). A blocking layer surrounds the beads to minimize potential nonspecific binding. Streptavidin, conjugated to the bead surface, binds to the biotinylated receptor reagent. This brings the singlet oxygen source (Sensibead) in close proximity of the singlet oxygen receptor (Chemibead) to form the bead pair immunocomplex for LOCI™ signal generation.

Biotinylated Receptors Analyte specific reagents typically a biotinylated antibody. They serve as part of the bridge between Chemibead and Sensibead in the bead pair immunocomplex.

Reaction Components



Method Specifications

Assay Time: 10 min
Assay Range: 0.5 - 1000.0 mIU/mL
Analytical Sensitivity*: 0.5 mIU/mL
Sample Volume: 2 µL
Specimen Types: serum, heparinized plasma

* Computed as two standard deviations above the mean (N = 20) of the zero calibrator.

Recovery

Samples of purified hCG were spiked into aliquots of pooled human serum and processed in triplicate by the LOCI™ BHCG method on a Dimension Vista™ System.

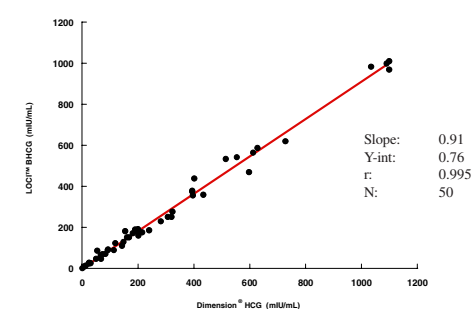
Spiked [BHCG] (mIU/mL)	Recovery (%)
23.6	97.3
48.9	98.0
98.0	96.6
200.9	100.3
398.8	98.1
811.4	96.0

Mean Recovery 97.7
Range 96.6 - 100.3

Samples of the 1st WHO Reference Reagent were tested for recovery by the BHCG method on a Dimension Vista™ system. The materials tested were: hCG-nicked (99/642), hCG-β-nicked (99/650) and hCG-β-core fragment (99/708).

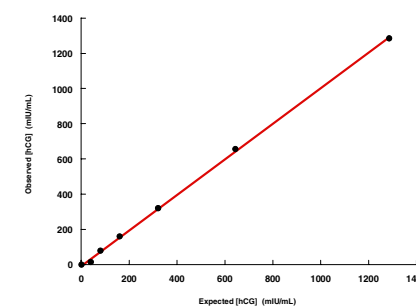
Form	Recovery (%)
hCG-nicked	100.3
hCG-β-nicked	98.7
hCG-β-core fragment	Not Detected

Method Comparison



The LOCI™ BHCG method processed on a prototype Dimension Vista™ system was compared with the Dimension® HCG method in a patient correlation study. The results were analyzed by standard linear regression.

Dilution Linearity



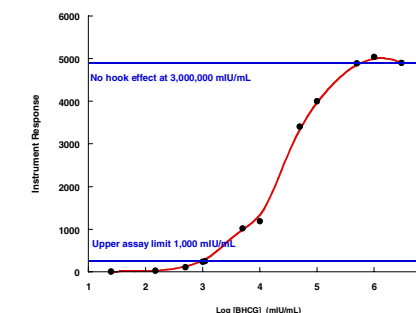
Samples of purified human chorionic gonadotropin were spiked into aliquots of pooled human serum and processed in triplicate by the LOCI™ BHCG method on a prototype Dimension Vista™ system. Linear regression of observed means versus theoretical values gave a slope of 1.00. The Y-int of 3.4 was not statistically significant (p = 0.40).

Precision

Testing was based on the NCCLS EP5-A2 protocol. Aliquots of commercial QC materials (Bio-Rad Liquichek™ Immunoassay Plus Control) were tested in duplicate twice a day for 20 days. The data were treated by analysis of variance to determine the following precision estimates:

	Mean (mIU/mL)	Precision (% CV)	
		Repeatability	Within-Lab
QC Level 1	8.7	2.2	3.6
QC Level 2	27.4	1.2	2.9
QC Level 3	432.5	1.7	4.6

Hook Effect



Samples of purified hCG were spiked into aliquots of pooled human serum, then processed in triplicate with the method calibrators using the LOCI™ BHCG method on a prototype Dimension Vista™ system. The observed signal for all spiked samples exceeded that of the upper calibrator level, showing no high dose hook effect up to at least 3,000,000 mIU/mL.

Interferences

Potential cross-reactants and interferences were tested with the BHCG method. Cross-reactants were tested by spiking the indicated concentrations into aliquots of a human serum pool with less than 5 mIU/mL hCG. Interferences were tested by spiking the test concentrations indicated into aliquots of a human serum pool with 25 mIU/mL hCG. None of these test samples showed a significant bias (> 10%).

Compound	Concentration	Bias (%)
TSH	100 mIU/mL	-2.3
FSH	1000 mIU/mL	4.0
LH	1000 mIU/mL	-3.6
Lipemia (as triglycerides)	3000 mg/dL	3.2
Hemoglobin	500 mg/dL	-8.3
Icterus (as conjugated bilirubin)	60 mg/dL	9.7
Rheumatoid Factors	500 IU/mL	-7.9

Conclusions

- A prototype method to measure total β-human chorionic gonadotropin (BHCG) was developed using LOCI™ technology implemented on a prototype Dimension Vista™ system.
- The prototype method demonstrated acceptable assay range, reproducibility, and turnaround time.
- BHCG for the Dimension Vista™ system showed good agreement with the Dade Behring Dimension® Clinical Chemistry System HCG method.
- No hook effect was observed up to at least 3,000,000 mIU/mL βhCG.
- Method sensitivity was at or below 0.5 mIU/mL.

Note: Dimension, Dimension Vista, and LOCI are trademarks of Dade Behring Inc.