

## Quantitation of Beta-Human Chorionic Gonadotropin (BHCG) by LOCI™ Technology

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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™ reagents and technology on a new instrument system under development (Dimension Vista™ system\*).

The method is based on Luminescent Oxygen Channeling Immunoassay (LOCI™) 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 chemibeads and biotinylated antibody to form chemibead-BHCG-biotinylated antibody sandwiches. Sensibeads are added and bind to the biotin to form bead-pair immunocomplexes. Illumination of these complexes at 680 nm generates singlet oxygen from sensibeads which diffuses into the chemibeads, 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:  $Y = 0.90(\pm 0.01)X - 1.6(\pm 5.8)$ ,  $r = 0.99$ , range = 1–1,000 mIU/mL.

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

\* Product under development—not available for sale.