Human choriogonadotropin (hCG), a 36.7-kDa glycoprotein hormone secreted by the human placenta throughout gestation, is an established indicator of pregnancy. hCG is composed of two disimilar subunits, alpha and beta, that are closely homologous with the subunits of the other glycoprotein hormones: lutropin (LH), follitropin (FSH), and thyrotropin (TSH). Screening blood or urine with a sensitive and specific qualitative hCG test provides rapid, accurate information that allows early detection of pregnancy (1). Most current commercially available qualitative assays of hCG in urine are two-site immunometric assays with solid-phase "capture" antibodies and enzyme-labeled conjugate antibodies. These tests are generally sensitive, specific, and rapid, but are complex, requiring as many as four timed manual steps.

We have developed a rapid, one-step immunochromatographic assay for the qualitative detection of hCG in urine with use of a direct colloidal label. This Abbott TestPack Plus™ hCG Urine assay (Abbott Laboratories, Abbott Park, IL) utilizes both monoclonal and polyclonal antibodies to selectively identify hCG in urine with high sensitivity and specificity.

A single molded-plastic disposable container (5.6 × 5.6 cm) houses a diagonally oriented strip of laminated nitrocellulose attached to a glass fiber pad containing the colloidal selenium conjugate. In the test procedure, three drops of urine are transferred with a disposable pipette into the sample well. The sample well directs the urine into the glass fiber pad containing colloidal selenium particles (160 nm diameter) conjugated to monoclonal anti-alpha-hCG antibody. The rehydrated conjugate then migrates through the nitrocellulose membrane until it reaches an end-of-assay window (in about 5 min). As the rehydrated conjugate migrates along the membrane strip, it passes through a capture region consisting of two bars in the form of a plus/minus (+/−). The horizontal bar (control bar) contains an immobilized polyclonal anti-beta-hCG/hCG complex, which captures any conjugate. The vertical bar (patient's result bar) contains immobilized polyclonal anti-beta-hCG antibody and captures conjugate only in the presence of hCG in the sample. Accumulated conjugate produces a colored signal at the capture site in the form of a plus sign (+) for positive specimens or minus sign (−) for negative specimens. This signal is visible in the result window and can be read when a red color in the end-of-assay window indicates that the test is complete. The entire process, from start to finish, takes ~5 min. Results should be read within 30 min after application of sample. The test is calibrated such that hCG at 50 int. units/L (WHO Third International Standard) is visibly detectable, whereas concentrations <5 int. units/L are undetectable. A gradual increase in assay sensitivity is observed with time. Specimens containing hCG <50 int. units/L may test positive after 30 min. Positive results are consistently observed with specimens containing hCG concentrations as high as 10^6 int. units/L.

Addition of LH or FSH at 1000 int. units/L, TSH at 1000 milli-int. units/L, or free beta-hCG subunit at 100 µg/L to hCG-negative (0 int. unit/L) and hCG-positive (50 int. units/L) urine specimens showed no cross-reactivity or interference in the assay. A wide range of potentially interfering substances were tested and did not interfere (2).

We compared the performance of the TestPack Plus assay with that of two sensitive qualitative urine hCG assays: Abbott TestPack™ hCG Urine and Hybritech ICON® II hCG (Hybritech, La Jolla, CA). Among 796 samples (501 positives, 295 negatives) tested, including urine specimens from 96 post-menopausal women, 99.7% concordance (795/796) was obtained between TestPack Plus and TestPack. One urine specimen that tested positive by the TestPack hCG Urine assay and negative by the TestPack Plus hCG Urine assay had an hCG concentration of 12 int. units/L [determined by use of a whole-molecule (intact hCG) quantitative assay]. Among 430 samples (150 positives, 280 negatives) tested by both TestPack Plus and ICON II, there was 100% agreement of results.

In summary, this rapid qualitative pregnancy test combines excellent performance with a one-step procedure, eliminating several of the manual steps characteristic of current qualitative methodologies.

References