Evaluation of Five Kits for Detecting Choriogonadotropin in Urine

Catherine Sheehan and Eileen Carreiro

We report an evaluation of the performance characteristics, accuracy, and sensitivity of five test kits designed to detect human choriogonadotropin (hCG) in urine: "Sensitex" (latex agglutination inhibition), "β-Neocept" (hemagglutination inhibition), "Duoclone" (direct agglutination), "Tandem-Visual HCG" (enzyme immunoassay), and "Sensichrome" (enzyme immunoassay). For comparison, all specimens and dilutions were also quantified by a radioimmunoassay. The accuracy of these kits was excellent and the manufacturers' stated sensitivity was confirmed. We conclude that the enzyme immunoassay method is the most sensitive procedure for qualitatively detecting hCG.

Additional Keyphrases: "kit" methods · pregnancy testing · radioimmunoassay compared · enzyme immunoassay · agglutination inhibition

Human choriogonadotropin (hCG) is produced during uterine pregnancy, ectopic pregnancy, and by tumors of chorionic origin. Because of the seriousness of the latter two conditions, hCG testing is now often used to detect the presence of hCG rather than simply as a means for detecting pregnancy (1, 2). The most commonly used methods for detecting hCG—latex agglutination inhibition (LAI) and hemagglutination inhibition (HAI)—are based on the principle that hCG neutralizes the agglutination of anti-hCG with indicator particles or cells already coated with hCG; a urine sample that does not turn opaque or does not cause the formation of a mat thus indicates a positive test result. An alternative to these more traditional methods is the direct hemagglutination assay (DA) in which erythrocytes coated with two different monoclonal antibodies to hCG form a lattice in the presence of hCG. More recently, immunoassays involving enzymes have been developed as qualitative tests for pregnancy. More sensitive than earlier methods, these are designed to detect pregnancy sooner by being able to detect less hCG in urine.

We have examined the performance of five currently available kits for the determination of pregnancy, based on these principles, and have evaluated the manufacturers' claims of analytical sensitivity for each. We compared the results for each test with those by quantitative radioimmunoassay (RIA) for the β-subunit of hCG in urine.

Materials and Methods

_Urine specimens_. Urine specimens were collected, aliquoted, and stored frozen at −20 °C. Urine specimens collected from male and female university students contained no measurable hCG as confirmed by RIA. hCG-positive urine specimens were collected from pregnant women in their 14th to 40th week of gestation, during their routine visit to their obstetrician. The pregnancies were normal uterine pregnancies without any indication of ensuing difficulty.

*Expected hCG concentrations were 23 to 68 kilo-int. units/L. Each urine was screened for protein with "Albusix" (Ames Co., Division Miles Laboratories, Ind., Elkhart, IN.).*_

_Radioimmunoassay_. The hCG in each urine specimen was quantified with a modified RIA kit: "β-HCG RIA" (Roche Diagnostics, Inc., Nutley, NJ) and "β-HCG" (Becton-Dickinson Immunodiagnostics, Orangeburg, NY). Each procedure was modified so that there was sequential addition of the antibodies. The control material was Reference Urine Standard (Roche Diagnostics).

_Qualitative tests evaluated_. We evaluated each of the following tests for hCG, performed according to the directions of the manufacturers: "Sensitex" (Roche Diagnostics), an LAI; "β-Neocept" (Organon Diagnostics, West Orange, NJ), an HAI; "Duoclone" (Organon Diagnostics), a DA; "Tandem-Visual HCG" (Hybritech, Inc., San Diego, CA), a two-site immunoenzymetric "sandwich" assay; and "Sensichrome" (Roche Diagnostics), an enzyme immunoassay assay. The latter two both involve a solid-phase reaction matrix and a colorimetric endpoint.

_Dilution studies_. To mimic samples with low hCG concentrations, we diluted the hCG-positive urine specimens with isotonic saline or "β-HCG Diluent" (Roche Diagnostics; a phosphate-buffered saline solution containing disodium EDTA, bovine serum albumin, thimerosal, and sodium azide). This obviated the problem of obtaining early-pregnancy samples. β-hCG in each dilution was quantified by RIA, then by the qualitative urine tests.

Results and Discussion

The results of the clinical portion of the study demonstrated that each kit was able to confirm pregnancy by detecting hCG in urine. Sensitex performed accurately 100% of the time; the other kits performed almost as well, detecting hCG in 98% to 99% of the samples. Table 1 summarizes the performance characteristics of each kit. In the dilution studies, we examined the ability of each kit to detect decreasing amounts of hCG. Considering only those specimens having more hCG than the manufacturer's stated sensitivity (see Table 1), we found that the Sensitex kit detected 20/23 expected hCG-positive samples (87%); β-Neocept, 17/26 (65%); Duoclone, 17/21 (81%); Tandem-Visual, 37/38 (97%); and Sensichrome, 21/22 (95%).

The enzymic methods were more sensitive than the DA, HAI, and LAI methods for detecting hCG. Sensichrome appeared to detect the smallest amount of hCG, but was somewhat unreliable, giving positive results for six urines that by RIA contained less than 50 int. units of hCG per liter. Because Sensichrome does not include any control or reference against which to compare specimens with a low hCG concentration, interpretation is difficult and, at times, subjective. Tandem-Visual provides a clear endpoint, because the reference preparation in the test kit contains hCG at 50 int. units/L, the kit's stated sensitivity limit; therefore, each patient's result could be compared with the color generated by the positive reference control, thus providing a reproducible endpoint. The most critical step in the enzymic assays is the washing step; incomplete washing can result in a false-positive result.
The other methods demonstrated the sensitivity specified by the manufacturer. The increased diameter of the tube in the Duoclone kit facilitates the reading of the mat or ring. The endpoint of flocculation in the Sensitex kit is usually easy to read.

Protein interferes in the DA, LAI, and HAI methods; it causes the cells to form a mat and the latex particles to remain evenly suspended whether or not hCG is present. This interference became apparent when the samples were diluted with β-HCG Diluent. The observed results were ascribable to the protein in the diluent (about 9.0 g/L by the biuret method) instead of hCG. Consequently, there were false-positive results with Duoclone and false-negative results with Sensitex and β-Neocept; therefore, any results generated with use of this diluent were invalid for these three methods and were excluded from our statistics. To eliminate this interference, we used isotonic saline for all dilutions to be assayed by the DA, LAI, and HAI methods. There was no apparent protein interference in the undiluted urines, the maximum quantity of protein being about 1.00 g/L as measured with the reagent strips. A second interference in any hemagglutination method is vibration: if the agglutinated "mat" is shaken loose, the Duoclone test will give a false-negative result and the β-Neocept a false-positive result.

In summary, Sensitex (LAI) and β-Neocept (HAI) are the easiest tests to perform but are the least sensitive. Duoclone (DA) is as easy to perform as the LAI and HAI, but has greater sensitivity, but requires the longest incubation. The most sensitive of the methods we studied were the enzymic immunosassays, the sensitivity of which approaches that of RIA with none of the drawbacks of radioisotopic methods.

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References


Concentrations of Protease and Anti-Protease in Serum of Patients with Pancreatic Cancer

Paul K. Buamah and Andrew W. Skillen

We measured the concentrations of trypsin, elastase, and three anti-proteases—α₁-macroglobulin, α₁-antitrypsin, and α₁-antichymotrypsin—in serum from 10 patients with pancreatic carcinoma. All 10 showed increased elastase and decreased α₂-macroglobulin concentrations, nine had increased α₁-antichymotrypsin, and eight had increases in α₁-antitrypsin and trypsin. Serial studies during chemotherapy of one patient showed that the protease concentrations decreased during treatment but the concentrations of the anti-proteases remained abnormal.

Additional Keyphrases: α₂-macroglobulin · elastase · trypsin · acute-phase protein · enzyme activity · pancreatic carcinoma · chemotherapy

The release of proteolytic enzymes, including trypsin, during experimental bile-duct-injured pancreatitis in dogs (1) and during human pancreatitis (2, 3) is consistent with the concept of autodigestion of the pancreatic gland by the pancreatic proteases (4). Ohlsson et al. (4) have shown that acut pancreatitis is characterized by low concentrations of α₂-macroglobulin, C₃, and kininogen in plasma, indicating a protease/anti-protease imbalance. We have studied protease and anti-protease concentrations in serum from patients with pancreatic carcinoma.

Patients and Methods

We collected blood from 10 patients with pancreatic carcinoma, ages 48 to 83 years (mean 62.6), who were being investigated at Freeman Hospital before surgery. The samples were stored at −20 °C until assay. The diagnosis was established by histological examination of biopsy and laparotomy specimens.

We measured anti-proteases—α₁-antitrypsin, α₁-antichymotrypsin, and α₂-macroglobulin—in the serum by single radial immunodiffusion, using antisera and standards from Hoechst U.K., Ltd., Hounslow, TW4 6JH, U.K. Serum elastase concentrations were estimated with the Dainabot Elastase I RIA kit (Abbott Diagnostic Div., Basingstoke Hampshire, RG22 4EH, U.K.), which involves second-antibody precipitation and has a dynamic range of 0.1-50.0 μg/L. We measured the concentrations of trypsin in serum by using an "RIA-gnost" trypsin assay kit (Behringwerks AG, Marburg, F.R.G.), another second-antibody precipitation RIA with an interassay CV of 5.5%.

Table 1. Performance Characteristics of Qualitative Tests for hCG in Urine

<table>
<thead>
<tr>
<th>Sensitex</th>
<th>β-Neocept</th>
<th>Duoclone</th>
<th>Tandem-Visual</th>
<th>Sensitochrome</th>
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