New Immunochemical Method for Rapid Detection of Human Chorionic Gonadotropin in Urine

Zeev Shoham (Schwartz), Emil Katz, Isaac Bliskstein, Zvi Katz, Ariel Zoerner, Ram Dgani, and Moshe Lancet

We describe a visual assay for rapid detection of chorionic gonadotropin (hGC) in human urine, and evaluate a pregnancy test kit that is based on this assay and designed for use by the general public. The assay involves the formation of an antibody-antigen complex between the anti-hGC antibodies coated on the membrane protein of Staphylococcus aureus bacteria, prestained with hematoxylin, and the hGC concentrated on a column of Sepharose-concanavalin A. The test was calibrated to detect as little hGC as 0.9 (SD 0.15) int. unit/mL. The kit, clinically tested with 448 urine samples, was 99.6% accurate. Simple to perform, the test gives highly reliable results as early as five days after the missed menstrual period.

Additional Keyphrases: pregnancy test · "kit" methods · immobilized antibodies on S. aureus membrane protein

Diagnosis of both normal and abnormal pregnancies has been improved with the advent of immunological methods for detection of pregnancy (1, 2). Concentrations of human chorionic gonadotropin (hGC) in urine increase from undetectable at the beginning of pregnancy to about 100 int. units/mL at 10 weeks of gestation. Thereafter they decline and remain at approximately 10 to 20 int. units/mL (3). Rapid measurement of hGC to detect pregnancy and to investigate gynecological emergencies is now a common procedure. Procedures in current use are based on immunoassay with use of radioisotopic, fluorescent, or enzyme tracers (4, 5). Although accurate and sensitive, these methods are usually time consuming, because the sample must be brought to the laboratory and the techniques and equipment ordinarily are available only during working hours. A test of equivalent sensitivity and accuracy that could be performed at any time by untrained individuals would therefore be of great value.

We describe here the immunochemical method and clinical studies of a pregnancy kit based on this method and designed for home use by the general public. The method is sensitive enough to detect as little as 1 int. unit of hGC per milliliter of urine, so the kit can be expected to give reliable results as early as five days after the missed period.

Materials and Methods

Kit procedure. The pregnancy test kit (TPK, Home Use Pregnancy Test; TEVA Pharmaceutical Industries Ltd., Tel-Aviv, Israel) consists of the following components: a column containing concanavalin A (Con A)-Sepharose, a blue-colored reagent, and a buffer solution (pH 7.0 ± 0.1). The test takes 10 min. The patient is instructed to pour 3 mL of urine onto the column and add 0.5 mL of the color reagent, which turns the test area of the column blue. Finally, she adds 2 mL of the buffer solution. If the column remains blue, the test is positive; if it loses color, the result is negative.

Immunochemistry bases of the kit. The kit is based on an antigen-antibody reaction between the hGC in the urine of a pregnant patient and a specific antibody to hGC. The latter is present in the blue color reagent, bound to Protein A of killed Staphylococcus aureus (Cowen 1, ATCC no. 12598 strain); the reagent itself is blue because the bacteria are stained with hematoxylin. The bacteria are grown, as described by Kessler, on Penassay broth containing betaglycerophosphate (6); they are killed by fixation in 15 g/L formaldehyde solution, followed by heating at 80 °C for 20 min, a procedure that is uniformly lethal.

The hGC in the urine is concentrated by binding to Con A-Sepharose column. The column packing is prepared by mixing one volume of 250 g/L Con A-Sepharose 4-B with 1.5 volumes of 250 g/L suspension of Sepharose 4-B and 1.25 volumes of acetate buffer, pH 6.0 ± 0.1 (CH3COONa 0.3 mol/L, NaCl 3.77 mol/L, CaCl2 2H2O 2.99 mmol/L, MgCl2 6H2O 3.0 mol/L, and MnCl2 4H2O 3.0 mmol/L, plus glacial acetic acid or NaOH until the required pH is reached. Each gram of lyophilized material gives about 3.5 mL of final gel volume. Once the colored suspension is poured onto the column, the Protein A-anti-hGC antibody will be bound to the Sepharose-Con A-hGC by forming a complex between the anti-hGC antibodies and any hGC already bound (Figure 1). The column will thus appear blue. In the absence of hGC, no such complex is formed; thus the blue-colored reagent will be washed away when the next reagent, acetate-Tween buffer (sodium acetate buffer supplemented with Tween 20, pH 7.0 ± 0.1), is added, and the column will become white.

The test was calibrated to be able to detect 0.9 (SD 0.15) int. unit/mL. The correct desired calibration was achieved by mixing a fixed amount of bacteria with various quantities of anti-hGC, and testing the mixtures with a standard hemagglutination inhibition test for hGC ("UCG-Quiktube"; Wampole Laboratories, Division of Carter-Wallace Inc., Cranbury, NJ) with a sensitivity of 1 int. unit/mL and with a standard dilution of hGC (Second International Reference Preparation, obtained from WHO). The production

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Department of Obstetrics & Gynecology, Kaplan Hospital, Rehovot 76100, Israel (affiliated with the Hebrew University-Hadassah Medical School, Jerusalem).

1 Diagnostic Laboratories, Teva Pharmaceutical Industries, Tel-Aviv, Israel.

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batch was prepared according to the proportions determined from these tests.

**Evaluative studies.** First morning urines were collected from 448 women in the Gynecology Department and outpatient clinic and tested for hCG. Criteria for subjects' inclusion in the study were good general health and age between 16 and 45 years; their age, date of last period, previous pregnancies, and current medication were recorded. In a double-blind study we analyzed each urine sample for hCG by two different methods: the immunochemical method described above, and the UCG-Quiktube test, which reportedly detects hCG at 1 int. unit/mL. When there were discrepancies between the two tests, we also used a specific beta-hCG enzyme immunoassay (Abbott Labs., N. Chicago, IL). The tests were done by experienced clinical laboratory technicians, in strict conformity with the manufacturers' directions. In addition, 25 pregnant women (with ultrasound confirmation of pregnancy) and 25 controls (negative for hCG in urine) performed the test in the presence of an investigator. All the women found the test simple to perform and interpreted the result correctly.

To evaluate the specificity of the method, we tested 16 samples of urine containing 1 int. unit of hCG per milliliter, as determined by hemagglutination inhibition test. Adding 0.5 to 8.0 g of human albumin per liter to each of the samples did not affect the results; no false-positive or false-negative results were obtained. Similarly, adding glucose (5 to 100 mmol/L) or erythrocytes (5 x 10^6 to 20 x 10^6 per milliliter) did not yield false results.

We also tested 40 specimens of urine collected from women admitted to the Internal Medicine Department with various pathological conditions between the fifth to tenth days after the first day of the last menstrual period. By both the immunochemical method and the UCG-Quiktube test, all the results were negative. For 20 urine samples taken from women with pathological pregnancies (10 with proven ectopic pregnancy and 10 samples with missed abortion), four specimens gave positive results and 16 gave a negative result by both methods.

**Results**

Change of color on the blue test area of the column is quite clear. The color intensity on the column increases with increasing hCG concentration, reaching a plateau at about 2 int. units/mL. hCG <0.5 int. unit/mL gives a negative result. Because the claimed sensitivity (detection limit) of the present method for hCG is 1 int. unit/mL, a negative result obtained with a urine containing <1 int. unit/mL (as measured by the specific beta-hCG test) was not regarded as a false negative.

Of the 448 urine samples tested, the conventional methods of pregnancy detection (serum beta-hCG, hemagglutination inhibition test, or ultrasound examination) yielded "true-positive" results in 243 cases and "true-negative" in 205. In Table 1, the results for the 243 true-positive samples are arranged according to the number of days after the expected beginning of a missed menstrual period. The immunochemical method of detection had an accuracy rate of 99.2% (241 of 243). The corresponding accuracy rate for the 205 true-negative samples was 100%. The average overall accuracy for the 448 specimens tested was 99.6%.

**Discussion**

A new method for early pregnancy detection is described. Advantages of the kit include its low detection limit (1 int. unit/mL) and the short time it takes to perform (10 min). The widely used agglutination inhibition pregnancy tests have the following detection limits and time requirements, as listed by their manufacturer: Pregnoticon Dri-Dot slide 1.5 int. units/mL, 2 min; Wampole's UCG-Quiktube, 1 int. unit/mL, 2 h; and Pregnoticon tube, 0.75 int. unit/mL, 2 h (7, 8).

In an evaluative study of five kits for detecting chorionic gonadotropin in urine (9), protein was found to interfere with direct hemagglutination inhibition methods. In the presence of protein, the cells form a "mat," causing the latex particles to remain even suspended whether or not hCG is present. Vibration of the test tube also caused interference in any hemagglutination method: shaking the agglutinated "mat" produced a false test result (9). In the pregnancy kit we discuss, the results were not affected by vibration or by the addition of human albumin in various concentrations.

A novel advantage of the present method is the effective concentration of relatively small amounts of hCG through binding and accumulation on the Sepharose-Con A column. The amount of hCG available for the formation of complexes with the hCG antibodies is thus optimized, improving sensitivity of detection several-fold over that obtained with commercially available non-isotopic techniques.

Another significant innovation is the use of a prestained color reagent and the absence of any in situ color reaction. Because of their content of Protein A, which interacts with IgG, *Staphylococci* are widely used as carriers in immunoassays. The binding of Protein A to IgG occurs rapidly, being completed in a matter of seconds (10), and displays very high affinity (5 x 10^12 to 8 x 10^13 L/mol) for >90% of the IgG from rabbits, humans, and guinea pigs (and to a lesser extent for the IgG from mice, rats, and other species). Advantage has been taken of these properties by using *Staphylococci* instead of the second antibody for direct binding of primary immune complexes (11). The pregnancy kit presented here makes use of the immunochemical properties of *Staphylococci* in a way not previously described. The test is sensitive and simple to perform, and gives highly reliable results as early as five days after the missed menses.

**Table 1. Comparison between True-Positive Results Obtained by Conventional Pregnancy Tests and the Present Kit**

<table>
<thead>
<tr>
<th>No. of days after</th>
<th>Present Kit</th>
<th>Conventional test*</th>
</tr>
</thead>
<tbody>
<tr>
<td>missed menses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-10</td>
<td>42</td>
<td>43</td>
</tr>
<tr>
<td>11-15</td>
<td>56</td>
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<td>61</td>
</tr>
<tr>
<td>Total</td>
<td>241</td>
<td>243</td>
</tr>
</tbody>
</table>

*Ultrasound, hemagglutination inhibition, or enzyme immunoassay for beta-hCG in serum.*

**References**